Discussion
5. DISCUSSION

5.1. Transfer of rust resistance genes into Indian wheat cultivars

5.1.1. Importance of gene transfers for rust resistance in wheat

Breeding for disease resistance through genetic means in crop plants have greatly been emphasized as the most economical and preferable method of reducing recurrent crop losses due to various diseases including rusts. The development of resistant cultivars is an important and most effective method of biological control of rusts as it reduces the cost of production, is environmentally safe and for some situations is the only applicable method for combating stress. Therefore, hybridization has become an increasingly important tool for the genetic enrichment of cultivated species. It has been widely used in the transfer of desirable genes into cultivated plants from their wild relatives.

The gene pool present in the taxons closely/distantly related to the genus *Triticum* offers a valuable source of genetic variability for wheat breeding (Bothmer *et al.*, 1992). Exploitation of genetic diversity for resistance is vital for a resistance breeding programme to be effective. The interaction between wheat lines with specific gene for resistance and a series of local virulence in the rust pathogen provides essential information for the planned utilization of diverse resistance in the development of cultivars. The need for additional sources of resistance to the rusts of common bread wheat (*Triticum aestivum*) arises from the well-known observations that new races of the pathogen evolve periodically that are virulent on previously resistant cultivars (Reddy, 2002). To counter a new race, effective resistance gene(s) must be available for incorporation into the breeding programme. Furthermore, it has become apparent that to reduce the vulnerability of this
crop to rusts, the genetic base for resistance among cultivars must be broadened and diversified.

The common experience that resistance is often neutralized with the evolution of new pathogenic races soon after the new disease resistant cultivar is extensively grown makes resistance breeding an ever-continuous process. The success of breeding is therefore dependent upon the availability of new resistance sources, and their effectiveness to the pathogen population in the area of cultivation. Varietal breakdown due to the evolution of the matching pathotypes reduces the field life of cultivars (Bayles et al., 1989). Further, wide spread cultivation of resistant wheat cultivars possessing near similar gene combinations trigger the 'boom' and 'bust' cycle (Vanderplank, 1984), unplanned large-scale usage of seedling resistance genes has rendered them ineffective to many pathotypes. Using the virulence survey data, lines with different genes or gene combinations can be developed and recommended to suit various agro-ecological zones. Thus genetic diversity will also reduce to frequent evolution of new variants and will promote a stable pathogen population leading to the extended field life of wheat cultivars.

5.1.2. Status of the recurrent Indian wheat parents against wheat rust

The three Indian wheat cultivars, namely HW 2084, PDSN-32, and K 9107 used in the present study as recurrent parents are highly popular and are largely grown in Nilgiri Hills of Tamil Nadu in South India and several other parts in Central and North-Eastern Plain Zones of India. These cultivars, after few years of their cultivation became highly susceptible to respective rust races prevailing in India, more particularly in the Nilgiri Hills. Many of the seedling rust resistance genes like Sr2, Sr5, Sr8, Sr9b, Sr11, Lr13, Lr14a, and Yr2 are present in many Indian wheats, including two of the
3 wheat parents (PDSN-32, K 9107) of the present study (Agarwal, 1986; Nayar et al., 1991a, b, 1994), however the adult plants became highly susceptible to virulent rust races. Moreover, due to evolution of new rust races, most of the seedling rust resistance genes also become ineffective in Nilgiri Hills and in other parts of India. All the three wheat cultivars were highly susceptible to stripe rust, while HW 2084 was free from leaf rust and K 9107 was highly resistant to stem rust, due to presence of either known or unknown respective rust resistance genes in the genetic background of these two wheats.

5.1.3. Methods employed for gene transfer

Since the recipient wheat cultivars were having all the desirable attributes except they are deficient in rust resistance, backcross method, which provides a precise way of improving them, was adopted. Specific rust resistance gene(s) from ten hexaploid wheat stocks were transferred into three wheat cultivars by simple backcrossing, where every backcross progeny was screened for rust resistance. Except two stripe rust resistance genes Yr15, Yr17, all other genes were found dominant under Wellington conditions. These two stripe rust resistance genes Yr15, Yr17, behaved as recessive genes, therefore, for transferring of these genes, after the first backcross, and after every two backcrosses selfed progenies were screened for rust resistance. Backcross breeding method is widely used method for transferring desirable characters either from related or distantly related alien sources into cultivated wheats (Islam and Shepherd, 1991; Cox et al., 1992, 1994). Simple backcross breeding method is generally used when genomes of the parents involved in the cross are completely or partially similar.

5.1.4. Morphology of F₁ hybrids and identification of rust resistant seedlings and plants

The seed setting in F₁ and the germination of F₁ seeds were quite good.
The morphology of F<sub>1</sub> hybrid plants and their spikes derived from the crosses between Indian wheat cultivars X stocks carrying various rust resistance genes were intermediate of the two parents. In some cases the spike morphology of the hybrids resembled to the donor parent. In general F<sub>1</sub> hybrids were tall and many of them tillered profusely.

F<sub>1</sub> hybrids derived from Indian wheats X wheat stocks carrying dominant rust resistance genes showed immune (0) to highly resistant reaction (0; -1) at seedling stage and highly resistant reaction (F-type) at adult plant stage in field conditions. Some of the seedlings, particularly those obtained from crosses between Indian wheats and stocks carrying \textit{Lr24+Sr24, Sr27, Lr28+Sr34+Yr8,} and \textit{Sr38+Lr37+Yr17} even produced reaction of highly resistant type accompanied by very minute pustules, particularly when inoculated seedlings were kept for longer period. However, adult plants in the field were completely free from infection indicating that these genes are adult resistance genes. Hybrids involving recessive stripe rust resistance genes \textit{Yr15} and \textit{Yr17} showed susceptibility in F<sub>1</sub> generation both at seedling stage and adult plant stage. Thus resistance nature of F<sub>1</sub> hybrids clearly established the dominant and/or recessive nature of resistance genes that are under transfer.

5.1.5. Selection of rust resistant plants in different generations

In the present study two to five backcrosses were made to transfer rust resistance genes, followed by five generations of continuous selfing was given for restoring the recurrent genotype. Thus final selections were made either in BC<sub>2</sub>F<sub>5</sub> / BC<sub>3</sub>F<sub>5</sub> or BC<sub>5</sub>F<sub>5</sub> generations. From BC<sub>2</sub>F<sub>5</sub> / BC<sub>3</sub>F<sub>5</sub> generation selected plants it is expected that transgressive segregants could be obtained.
5.1.6. Performance of the constituted near-isogenic lines against wheat rusts

The successes of wheat rust resistance breeding programmes have been aided by the use of resistance sources that have proved durable. It is generally accepted view that wheat cultivars carrying field resistance have remained effective over many years despite their cultivation over large areas. All the constituted lines derived from the crosses between Indian wheats X stocks contributing various stem, leaf and stripe rust genes were tested against the most predominant and virulent races of respective rusts.

$Lr19+Sr25 +Sr36$

The rust resistance linked gene complex $Lr19+Sr25$, originally derived from wheat–Agropyron elongatum translocation (Sharma and Knott, 1966), is one of the highly effective rust resistance gene complex used world wide. Reynolds et al. (2001) noticed an increase in grain yield and biomass when this gene complex was introgressed from Agropyron elongatum into wheat cultivars. Allard and Shands (1954), and Nyquist (1957) transferred stem rust resistance gene $Sr36$ from Triticum timopheevi to common wheat chromosome 2BS. The gene complex $Lr19+Sr25$ and the stem rust resistance gene $Sr36$ were successfully incorporated into hexaploid wheat stock Cook*6/C 80-1 (Tomar and Menon, 1999), which was used as the donor parent of the present study. The combination of these genes $(Lr19+Sr25+Sr36)$ shown to provide high degree of resistance to stem rust pathotypes in many Indian wheats (Tomar and Menon, 2001a,b). All the constituted lines carrying the stem rust resistance genes $Sr25$ and $Sr36$ from the donor parent Cook*6/C 80-1 were completely free from both leaf rust and stem rust at both seedling stage and adult plant stage. It is noted that the transfer of specific genes for resistance from alien sources, often associated with other undesirable genes located in the alien segment,
restricts usability of alien gene transfers. Undesirable linkage of \( Lr19 \) (Agatha) with a gene for yellow flour colour (Sharma and Knott, 1966) had restricted its utility in wheat improvement programmes. Marias et al. (2001) reported the reduction of yellow pigment in a wheat line \( Lr19 - 149 \), a recombinant form of the \( Lr19 \) translocation of wheat. Knott (1980) developed two mutant lines with reduced yellow pigment viz., Sunstar*6/C 80-1 and Cook *6/C 80-1, both carry \( Lr19 \) and \( Sr25 \).

The leaf rust resistance gene \( Lr19 \) has conferred total immunity to all Indian leaf rust races both at seedling stage and adult plant stage (Sawhney et al., 1977; Sawhney and Goel, 1981, 1983), and has been included in the differentials to detect new virulence in India (Nagarajan et al., 1981). Tomar and Menon (1998b) have reported that \( Lr19 \) provided adult plant resistance for over 20 years at Wellington. A wide spectrum of pathotypes of leaf rust prevalent in USA, Canada, Australia and other countries are avirulent on \( Lr19 \). However, Huerta-Espino and Singh (1994) detected for the first time virulence (caused by CBJ / QQ) on \( Lr19 \) in Mexico. Subsequently, Sibikeev et al. (1996) had also identified a pathotype of leaf rust virulent on \( Lr19 \) and \( Lr19d \) in Saratov and Qrenbhurg districts of Russia.

By virtue of close linkage, lines carrying leaf rust resistance gene \( Lr19 \) also carry stem rust resistance gene \( Sr25 \). Stem rust resistance gene \( Sr25 \) alone did not seem to provide adequate resistance, it showed 40MS type of moderate susceptibility as seen in one of the Indian recipient wheat parent HW 2084. Since, the donor parent Cook*6/C 80-1 also carried another stem rust resistance gene \( Sr36 \), the constituted near-isogenic lines carrying these stem rust resistance genes, \( Sr25 \) and \( Sr36 \) from the donor parent Cook* 6/C 80-1, is completely free from stem rust both at seedling stage and at adult plant stage. Therefore, combination of \( Sr25 \) and \( Sr36 \) might have provided high degree of stem rust resistance in the two Indian wheats, namely HW
2084 and PDSN-32. It has been shown that Sr25 in combination with Sr36 and Sr6 has exhibited a high degree of resistance in many Australian wheat cultivars (Luig, 1983; McIntosh et al., 1977) and many wheat cultivars in USA (Roelfs and McVey, 1979). In India, Sr25 reported to be effective at seedling stage against 19 stem rust cultures (Patil and Deokar, 1996). Sr25 seems to be moderately effective individually (McIntosh et al., 1977) but provided durable resistance in combination in several Australian wheats. Knott (1980) recorded low infection types with the Canadian pathotypes of stem rust on Sr25 as compared to the Australian pathotypes. Gough and Merkle (1971) suggested that lines with Sr25 may become more susceptible at high temperature. Huerta-Espino (1992) identified one virulent culture from Ethiopia and two virulent cultures from Nepal. The use of Sr25 in cultivars can be justified by its linkage with Lr19. Very few cultivars carrying these linked genes have been released for commercial use in the world. In the present study the line Cook*6/C 80-1 and its derivatives exhibited immune reaction to stem rust pathotypes prevailing in the Nilgiris indicating the useful combination Sr25+ Sr36.

Lr24+Sr24

The rust resistant donor parent Darf*6/3Ag/Kite, a hexaploid wheat stock, also carry closely linked leaf–stem rust resistance gene complex, Lr24+Sr24. This linked complex was originally transferred from Agropyron elongatum (Sears, 1973). The leaf rust resistance gene Lr24 was highly effective both in seedlings and in adult plants in India and many other parts of the world. Pathotypes virulent on Lr24 have been reported from Australia (Park et al., 2002; North America (Browder, 1973), Canada (Kolmer, 1991), South America (Tomar and Menon, 2001a, b), Russia (Sibileev et al., 1996), and South Africa (Pretorious et al., 1990). Isolates of P. graminis virulent to Sr24 appeared first in South Africa (Le Roux and Rijikenburg, 1987) and later
However, Huerta - Espino (1992) did not find virulence for $Sr24$ among a wide range of international collections and this gene is still effective in North American and Australian wheat cultivars (McIntosh et al., 1995b). Nevertheless, stem rust resistance gene $Sr24$ found to be highly effective and has been widely used in Australian wheat cultivars (McIntosh et al., 1995 a, b), indicating its specific expressivity in certain geographical areas. However, this linked gene complex still found to be effective in India for both stem and leaf rust races either individually or in combination (Patil and Deokar, 1996).

In the present study all the backcross improved lines carrying $Lr24+Sr24$ showed high degree of resistance to leaf rust both at seedling stage and adult plant stage. Constituted line carrying leaf rust resistance gene $Lr24$ gave immune to moderately resistant reaction at seedling stage and high degree of resistance type of reaction at adult plant stage. On the other hand, at seedling stage, the constituted lines carrying this gene complex, showed susceptible reaction against stem rust race 40-1, however, at adult plant stage highly resistance ('$F$') reaction was noticed in the constituted lines in the genetic background of K 9107 and moderate degree of resistance (10RMR) in PDSN-32 (as that of donor parent). Three cultivars namely Vaishali, DL 788-2 and HW 2004, all carrying this linked gene complex ($Sr24+Lr24$) have been released in recent years in India for commercial cultivation and are highly resistant to stem rust and leaf rusts (Sawhney, 1994; Tomar and Menon, 2001a). It has been suggested that high magnitude of adult plant resistance to stem rust in these cultivars is attributed to presence of some additional stem rust resistance gene(s) effective to stem rust race 40-1.

$Lr26+Sr31+Yr9$

In the present study, constituted lines in three Indian wheat genetic backgrounds carrying the linked gene complex $Sr31+Lr26+Yr9$, derived from
hexaploid wheat stock Veery’S’, were developed. This gene complex was originally transferred to wheat through a wheat–rye translocation involving 1R of rye chromosomes and 1B of wheat chromosome (Driscoll and Jenson, 1964; Zeller, 1973) and is present in hundreds of wheat cultivars world wide (McIntosh et al., 1995a). This translocation confers resistance to several foliar diseases, including genes for resistance to powdery mildew (Pm8 and Pm 17), yellow rust (Yr9), stem rust (Sr31), and leaf rust (Lr26) (Friebe et al., 1996; McIntosh, 1988; Heun et al., 1990). All the three rust resistance genes are highly effective at seedling stage to many rust races (Sawheny and Goel, 1983). Leaf rust resistance gene Lr26 is highly susceptible at adult stage to several leaf rust races belonging to 77 group (Nayar et al., 1987, 1991 a, b). Stripe rust resistance gene Yr9 is completely effective both at seedling stage and at adult plant stage (Macer, 1975), and it is fully exploited either individually or in combination with Yr2 or Yr6 in several wheats (Nayar, 1989; Nayar et al., 1991a, b).

Among the three linked genes stripe rust resistance gene Yr9 is completely effective against stripe rust races, while the stem rust resistance gene Sr31 and leaf rust resistance gene Lr26 were not completely effective against respective rusts, since Sr31 showed ‘40MR’ type of resistant reaction, while Lr26 showed ‘70S’ type of highly susceptible reaction. Constituted lines carrying this linked gene complex were found highly resistant to stripe rust and stem rust, while the leaf rust resistance gene Lr26 is found effective only in the genetic background of K 9107, where the constituted lines were completely free from leaf rust, while in the variety PDSN–32 this gene produced (10MS - type) moderate susceptible reaction. It is suggested that rust resistance provided by Sr31 and Lr26 could be due to their combination effect or interactive effect with already existing respective rust resistance genes in the genetic background of recipient wheat. As it is evident that stem rust resistance gene Sr31 provided ‘F’ type reaction in HW 2084 and K 9101,
and the same gene produced '10R' type of resistant reaction in PDSN-32. Similarly, leaf rust resistance gene *Lr26* showed 'F' type of highly resistant reaction in K 9107, and (10MS-type) moderate susceptible reaction in PDSN-32. Several examples of resistance genes that do not act independently of each other were also reported; where genes promoting extreme resistance are usually expressed independently of other genes that may provide lower levels of resistance (Jhonson, 1992).

There are other examples where genes conditioning intermediate level of resistance act together to give a higher level of resistance, which was attributed to either non-additive genes (Singh and McIntosh, 1984; Davoyan and Ternovskaya, 1996; Tsomin et al., 1990) or additive genes (Dyck and Kerber, 1985). Kerber and Green (1980) have shown that resistance genes may be suppressed by inhibitors in some wheat genetic backgrounds. The stem rust resistance gene *Sr31* has been profitably exploited in CIMMYT wheat breeding programmes and continues to occur at high frequencies. This gene is present in many European, Chinese and American wheats. Wide spread occurrence of this gene complex (*Sr31+Lr26+Yr9*) in Indian subcontinent can be noticed from the list of recently released cultivars, namely Pak 81, Sarhad 82, CPAN 1922, HUW 206, CPAN 3004, UP 2338, WH 542, PBW 343, HD 2643 and HD 2687. *Sr31*, despite its susceptibility to stem rust race 117, is well exploited in several Indian wheats because of its durability (Reddy *et al.*, 1993a; Asir *et al.*, 1994). In the present study clear cut difference has been observed in the effectiveness of the stem rust resistance gene. The donor parent although not completely free from stem rust but showed 40MR type resistant reactions. At seedling stage constituted lines carrying *Sr31* showed resistant reaction (0-1) in HW 2084 and PDSN-32. While at adult plant stage this gene showed 'F' type of high degree resistance in HW 2084 to 10R type of resistance reaction in PDSN-32. The gene *Sr31* in combination with the genes *Sr25* and *Sr24* has shown enhanced
resistance to stem rust in some Indian wheat cultivars (Tomar and Menon, 2001a; Reddy, 2002).

Constituted lines, in the backgrounds of PDSN-32 and K 9107, carrying the leaf rust resistance gene $Lr26$ showed susceptible reaction (type 2-3) in the seedlings against leaf rust races 77-1 and 77-5. However adult plants showed moderate susceptibility (in PDSN-32) to high degree of resistance (in K 9107). In the present study, rust resistance provided by leaf rust resistance gene $Lr26$ in Indian wheat cultivar (K 9107) is most probably due to some combination or interactive effect of this gene with other $Lr$ genes already present in its genetic background. Leaf rust resistance genes $Lr1$, $Lr3$, $Lr10$, $Lr13$, $Lr14a$ and $Lr15$ are present in many Indian wheat varieties. Among these genes $Lr13$ and $Lr14a$ are found to be highly interactive with other leaf rust resistance genes to produce durable resistance in adult plants (Dyck et al., 1966; Sawhney, 1995; Rajaram et al., 1988). Resistance genes that produced intermediate infection types of 0; 1 - 2 generally interacted with $Lr13$ to produce lower infection type than either of the single gene parental lines. Genes $Lr13$ and/or $Lr34$ are present in wheats occurring in the pedigree of cultivars that have been described as having partial resistance (Singh et al., 1991). It is evident that the Indian wheat varieties PDSN-32 and K 9107 also possess both of these leaf rust resistance genes in their genetic backgrounds. Leaf rust resistance gene interacted for enhanced resistance with other resistance gene(s) that conditioned resistance/partial resistance when present singly. Resistant gene(s) that did not contain effective resistance did not interact with $Lr13$ for enhanced resistance (Roelfs, 1988a,b). Therefore, interactions for enhanced resistance between $Lr13$ and other resistance genes may contribute to the durability of leaf rust resistance in cultivars with $Lr13$. Dyck and Samborski (1979) and Samborski and Dyck (1982) observed that $Lr13$ is highly Interactive gene and its resistance was enhanced by $Lr16$ in wheat cultivar Neepawa and $Lr30$ in
cultivar Columbus. There are several examples showing combination of two or more genes in providing more durable resistance than provided by a single gene (Reddy et al., 1993b, 1995). Gene combinations involving \( Lr13 \) with other leaf rust resistance genes also provided durable resistance in many wheat cultivars (Gupta and Saini, 1987; Kolmer, 1992, 1996). Effectiveness of gene combinations involving \( Lr13 \) include \( Lr13+Lr23 \) in WL 711 and \( Lr13+Lr14a \) in Sonalika (Sharma and Sawhney, 1991), \( Lr13+Lr22b \) in Manitou and \( Lr13+Lr17+Lr14a \) in Inia 60 (McIntosh, 1992), \( Lr13+Lr33 \) in V 745 (Dyck, 1994), \( Lr13+Lr16 \) in several Canadian wheats (Samborski and Dyck, 1982), \( Lr13+Lr34 \) and \( Lr13+Lr24 \) in W 3751 and W 3760 (Roelfs, 1988a, b; Singh and McIntosh, 1990). Studies on wheats with low coefficients of rust infections internationally, have indicated that resistance was based on oligogenic combinations (Rajaram and Luig, 1972; Singh and McIntosh, 1986, 1987). Some more Indian wheats which carry combination genes include \( Lr13+Lr26 \) in HD 2380, \( Lr14a+Lr26 \) in VL 616 and \( Lr13+Lr14a+Lr26 \) in MACS 2496 (Brahma et al., 1996; Nayar, 1996). All these results support the interactive nature of leaf rust resistance gene \( Lr26 \) in providing high degree of leaf rust resistance in the present study.

\[ Lr28+Sr34+Yr8 \]

The \textit{Aegilops speitoides} derived resistance gene \( Lr28 \) (CS 2A/2M 4/2) (McIntosh et al., 1982) was very effective against leaf rust. The gene \( Lr28 \) confers a high degree of resistance at seedling stage to as many as ten most prevalent Indian races of leaf rust (Sawhney et al., 1977). In the present study, all the constituted lines carrying this gene were found completely free from leaf rust. The genetic stocks carrying this gene have been screened thoroughly against leaf rust races at Wellington, the Nilgiris under field conditions over several years and has been found that they are completely free from leaf rust (Brahma et al., 1999; Tomar and Menon, 2001a, b). This
gene has been incorporated into wheat cultivar Sunland, and is reported to be highly effective in several wheat cultivars in Australia, South Asia and Europe. However, this gene seems not completely effective against North American leaf rust races (McIntosh et al., 1995b). In India, the leaf rust resistance gene Lr28 is very effective, but this gene is not yet exploited for commercial use so far, and therefore, this is the first attempt made in this direction. The stem rust resistance gene Sr34, which is linked along with Lr28, however, seems not effective in any Indian wheat, as evident that constituted lines carrying this linked gene complex showed no improvement in the stem rust resistance as compared to the recurrent wheat parents. Another rust resistance gene linked with this gene complex is the stripe rust resistance gene Yr8. The donor parent CS 2A/2M#4/2 exhibited adequate degree of stripe rust resistance (10RMR). The constituted lines carrying this gene also showed either similar type of rust resistance (10RMR in HW 2084) or higher degree of stripe rust resistance (5RMR in PDSN-32 and TMR in K 9107). The results also suggesting the involvement of seedling stripe rust resistance gene Yr2 in enhancing stripe rust resistance in K 9107.

**Lr37+Sr38+Yr17**

This gene complex was derived from *Aegilops ventricosa* (McIntosh et al., 1991). The stem rust resistance gene Sr38 provided moderate susceptible to moderate resistance against stem rust pathotypes prevailing in the Nilgiris. This gene complex has been successfully incorporated into Australian wheat cultivars Trident, Sunstate and Sunberi (McIntosh et al., 1995a). Similarly, Seah et al. (2001) also reported the introgression of this gene complex into wheat cultivars. Ambrozkova et al. (2002) verified the translocation of Lr37+Sr38+Yr17 from *Aegilops ventricosa* into the wheat cultivars Hussar, Eureka, Torfrida, Renan, Rendezvous, Rapier and Brigadier. Both Lr37 and Sr38 were found highly effective both in seedlings and in adult
McIntosh et al. (1991) and Kloppers and Pretorius (1994) found that leaf rust resistance gene \( Lr37 \) provides adult plant resistance. Though both \( Sr38 \) and \( Lr37 \) were reported to prove effective resistance both in the seedlings and in adult plants, however, in the present study, at seedling stage, the constituted lines carrying these gene complex showed susceptible reaction '2' to stem rust races 40A and 40-1 and '2- 3' to leaf rust races 77-5 and 12-2 respectively. Labuschagne and Pretorius (2002) reported that in South Africa the genes \( Lr29, \) \( Lr34, \) \( Lr35 \) and \( Lr37 \) conferred effective resistance to leaf rust qualifying them for use in cultivar improvement. Stem rust resistance gene \( Sr38 \) provided TR-5RMR type at adult stage in HW 2084 and PDSN-32. The result suggesting that stem rust resistance gene \( Sr38 \) providing resistance in adult plants either by interacting with other stem rust resistance gene(s) already present in recurrent parents. Roelfs (1988b) has shown that the effectiveness of stem rust resistance genes may vary depending on the presence or absence of minor seedling resistance genes in the recurrent wheat genetic background. Involvement of other background rust resistance gene in providing complete rust resistance along with \( Lr37 \) was reported in some South African wheats (Kloppers and Pretorius, 1994).

\( Yr17, \) is the other lined stripe rust resistance gene that was transferred into three Indian wheat cultivars. The stock (RL 6081) carrying stripe rust resistance gene \( Yr17 \) is not completely free from stripe rust under field condition, it showed '15MS' type resistant reaction. However, in the present study when this gene was incorporated into the three Indian wheat cultivars it showed variable results. In K 9107 background, the constituted were completely free from stripe rust, while the same gene provided '15MS' type reaction (as that of donor parent) in the other two Indian wheat cultivars. Stripe rust resistance gene \( Yr2 \) was reportedly present in many Indian wheat
cultivars (including K 9107 in the present study), and it confers only seedling rust resistance and is not effect at adult plant stage (Nagarajan et al., 1984). Gene combination involving Yr2 with other Yr genes (Yr17 in present case) were suggested to provide effect resistance to stripe rusts in several Indian wheats (Nagarajan et al., 1987) and wheats derived from CIMMYT material (Milus and Line, 1986; Badebo et al., 1990; McIntosh, 1992; Sawhney, 1998 a,b). Moderately effective stripe rust resistance gene Yr17 provided complete rust resistance in combination with some other stripe rust resistance genes in several European wheat varieties (Robert et al., 2000).

**Sr27**

Sr27, is one of the most effective stem rust resistance gene transferred, by using irradiation treatment from Secale cereale (Imperial rye) chromosome 3R to chromosome 3A of Chinese Spring wheat (Acosta, 1962; Rao, 1978). Sawhney and Goel (1981) reported that Sr27 is effective in seedling stage against as many as 19 pathotypes of stem rust in India which included the pathotypes commonly occurring in the Nilgiris. In the present study, the near-isogenic lines carrying Sr27 exhibited very high degree of resistance at adult plant stage in Wellington. Though virulence for Sr27 is rare, Harder et al. (1972) isolated an East African culture virulent on a Pembina wheat line with Sr27. Initially Sr27 very effective in Australia but later on isolates of stem rust from triticale variety Coorong were virulent on wheat seedlings with Sr27 (McIntosh et al., 1983). Virulence against Sr27 was also reported in some South African wheats (Smith and Le Roux, 1992). This gene has not been fully exploited in commercial wheat cultivars; however, it is present in a complete rye chromosome 3R in many triticale lines produced by CIMMYT (McIntosh, 1983; McIntosh et al., 1995b). In the present study, the constituted near-isogenic lines derived from the crosses between Indian wheat X donor parent carrying stem rust resistance genes Sr27 were immune (0-1) and completely free (F) from stem rust both at
seedling stage and at adult plant stage respectively. Suggesting the highly usefulness of this gene against a broad spectrum of stem rust races of the Nilgiri Hills.

**Yr11 and Yr13**

Stripe rust resistance genes *Yr11* and *Yr13* utilized in the present study are derived from hexaploid winter wheat sources (Priestley, 1978; McIntosh, 1988). Donor parents carrying these stripe rust resistance genes showed high degree of stripe rust resistance under field condition. Constituted lines carrying these stripe rust resistance genes, in the genetic background of all the three Indian hexaploid wheats showed immune (0-1) at seedling stage and complete resistance at (F) at adult plant stage thereby suggesting that these genes are adult plant rust resistance genes. Stripe rust resistance gene *Yr11* was suggested to be race specific and found to be a slow rusting gene (Jhonson and Taylor, 1972), this gene was not fully exploited any where in the world for commercial purpose. On the other hand the stripe rust resistance gene *Yr13* is available in a few wheat cultivars at Plant Breeding Institute, Cambridge, UK (Jhonson, 1992a,b).

**Yr15**

Stripe rust resistance gene *Yr15*, another highly effective gene used in the present study, was originally transferred from *T. dicoccoides* (Gerechter-Amitai *et al.*, 1989; Roelfs *et al.*, 1992). Constituted lines carrying this highly effective stripe rust resistance gene were immune to stripe both at seedling stage (0-1) and in adult plants. Commercially the gene has not been utilized, although, breeders have introduced *Yr15* into some of the Indian wheat cultivars (Tomar and Menon, 2001a). Resistance due to *Yr15* is highly effective throughout the world; however, it showed partial susceptibility to one of the stripe rust race in Afghanistan (van Slifhour *et al.*, 1989).
Yr16

Yr16, another stripe rust resistance gene is derived from hexaploid wheat source namely Cab 5BL-7BL (Worland and Law, 1988). Constituted lines carrying this highly effective stripe rust resistance gene showed resistance both at seedling stage and adult plant stage suggesting that this gene is also an adult plant resistance gene. This gene so far not utilized in commercial wheats.

Constituted lines carrying linked gene complexes like Lr19+Sr25+Sr36 also carry moderate to high degree of stripe rust resistance due to unknown stripe rust resistance gene(s). This gene complex either alone or in combination with some other stripe rust resistance genes (example Yr2 in K9107) might be responsible for high degree of stripe rust resistance in the recurrent parents. Field rust reaction of the some constituted lines also revealed that severity of particular rust in a constituted line varied (decreased or increased) when it carried a different type of known or unknown rust resistance gene(s). For example lines carrying stripe rust resistance genes Yr11 and Yr13 showed slight enhancement in the susceptibility to stem rust, probably this is due to lack of competition from the other rusts.

Overall, in the present study, all the rust resistance genes used provided moderate to high degree of resistance to respective rusts in the field condition. Specific rust resistance genes, namely Lr19, Lr24, Lr28, Sr27, Yr9, Yr11, Yr13, Yr15, and Yr16 provided their resistance in singly, while other rust resistance genes, namely Lr26, Sr24, Sr25, Sr31, Sr38 and Yr17 provided their resistance in combination with other resistance genes already present in the recurrent parents. A resistance gene when overcome by a virulent pathogen, the resistance of the particular wheat cultivar was not completely neutralized, a residual resistance, ranging from moderate to fairly
high exists (Daniel et al., 1994), and such a gene provide durable resistance in combination with other genes. Susceptible reaction of Lr26, Lr37 and Sr24 for certain rust races at seedling stage, and their resistance reaction at adult plant stage clearly established such an interaction of adult plant resistance.

Flor (1971) pointed out that cultivars with two or more genes for rust resistance should be less likely to succumb to new races than those cultivars carrying a single resistance gene. Saione et al. (1993) suggested that durable resistance in some cultivars might be related to many specific disease reaction genes. Additive interaction between resistance genes has been defined as the combination of two or more genes results in increased resistance relative to that conferred by the individual genes (Dyck, 1977; Samborski and Dyck, 1982; Schafer et al., 1963; Sawhney and Sharma, 1998; Singh and Rajaram, 1994; Ma and Singh 1996; Reddy, 2000b). Interactive effects of Lr10, Lr13 and Lr34 with other leaf rust resistance genes have been shown in wheat cultivar Era (Pretorius and Roelfs, 1996).

In wheat lines that have combination of resistance genes, the genes usually act independently, producing the infection type of the gene that conditions the lowest infection type when present singly (Dyck and Kerber, 1985; Roelfs 1988b). Knott and Weller (1988) described instances where the adult plant resistance could be the consequences of modifying genes or a 'ghost' effect of defeated genes for seedling and adult plant resistance. Roelfs et al. (1992) suggested that one of a specific rust resistance gene is introduced into a wheat cultivar, to prevent its breakdown, the gene should be used in combination with other rust resistance genes. Their combination effect may be additive or interactive. Since, the resistance genes already present in the recurrent parents are ineffective, the combined action must be interactive in nature. A number of cases have been reported where the combination of two or more genes results in a lower infection type. In some cases the interaction involves identified genes (Saini et al., 1998) but often
the genes are unknown (Kaur and Saini, 2001). Kruipinsky and Sharp (1978) showed that minor genes for resistance to yellow rust could be combined to give high level of resistance. Moderately effective stripe rust resistance gene \(Yr17\) provided complete rust resistance in combination with some other stripe rust resistance genes in several European wheat varieties (Robert et al., 2000).

5.1.7. Agronomic performance of the constituted near-isogenic lines

Near-isogenic lines for rust resistance with desirable agronomic characters were constituted in BC\(_2\)F\(_5\) / BC\(_3\)F\(_5\) and BC\(_5\)F\(_5\) generations. The early generation (BC\(_2\)F\(_5\)/BC\(_3\)F\(_5\)) selected plants, as expected, had little morphological similarity to the recurrent parents; whereas plants that are selected in BC\(_5\)F\(_5\) had more similarity with recurrent parents for many characters. Some of the lines particularly those derived genes from the donor parents CS 2A/2M#4/2 (\(Lr28+Sr34+Yr8\)), Darf* 6/3Ag/Kite (\(Lr24+Sr24\)), and RL 6081 (\(Sr38+Lr37+Yr17\)) even in BC\(_5\)F\(_5\) did not show much similarity to the recurrent parents, perhaps such lines needed few more backcrosses to achieve this state. Nevertheless, the plants in both BC\(_2\)F\(_5\) and BC\(_5\)F\(_5\) generations possessed reasonably good agronomical characters that were the basis for selection in the present study.

The results obtained in the present study further revealed that BC\(_2\)F\(_5\) selected plants had superior yield potential and this potential tended to be on decline as more number of backcrosses were made. This was in the expected lines, since after repeated backcrosses the lines regain the original genotype with all the agronomical traits of the given recurrent parent. Thus the values recorded for several yield components in BC\(_5\)F\(_5\) plants were almost comparable to those in recurrent parents. BC\(_2\)F\(_5\) / BC\(_3\)F\(_5\) selected plants were more vigorous than BC\(_5\)F\(_5\) plants and the recurrent parents, and all the lines gave higher grain yield than the recurrent parents. These plants being tall
and vigorous naturally acquired more biological product, which ultimately might have contributed to higher yield in BC2F5 / BC3F5. Early generation selections in backcross progenies were also followed in recent years for resistance to diseases and yield improvement. For e.g. Friebe et al. (1990) selected Hessian fly resistant wheat lines with good heterotic characters derived from radiation-induced translocations involving wheat and rye chromosomes, in BC2F3 generation. Li and Dong (1991) isolated agronomically desirable derivatives from wheat - A. mihnoi crosses in BC1F2 itself. Reddy et al. (1993a,b) and Asir et al. (1994) transferred several specific stripe rust resistance genes to several Indian hexaploid wheats and highly desirable genotypes were constituted in BC2F5 / BC3F5.

In general, many constituted lines exhibited superior performance in yield components both in BC2F5 / BC3F5 and BC5F5 generations compared to the recurrent parents. This could be due to the fact that constituted near-isogenic lines were resistant to all the three types of rusts. It is seen that in certain cross combinations, the number of rust resistant plants with good agronomical characteristics were much limited, while in others, the number of such plants was fairly large. The differences could be due to presence or absence of linkage drag. Constituted near-isogenic lines carrying resistance genes Sr36+Sr25+Lr19, Sr31+Lr26+Yr9 and Sr38+Lr37+Yr17 exhibited superior agronomic performance for all the quantitative characters under study in all three Indian wheat genetic backgrounds.

It is now established that the most important yield components in wheat are the number of effective tillers, number of seeds per spike, and seed size/weight. Improvement in any of these characters would cause more yield per unit area. In the present study, many of the constituted near-isogenic lines, either in BC2F5 or BC5F5, had more number of tillers, being slightly higher in BC2F5 / BC3F5. Studies have indicated that, in India, the
highest yielders like MP 195, Mukta, NI 5439, HW 1085, HW 2004, HW 2045 had higher number of productive tillers in multilocation tests (Joshi et al., 1985; Bagga, 1986; Tomar and Menon, 2001a). High yielding Indian wheat varieties WL 711 and HW 2044 have two yield components i.e. tiller number and number of seeds per spike, accounted for its higher grain yield (Gill, 1979; Tomar and Menon, 2001a). Plants in the constituted lines, in general, had longer spikes and more number of spikelets than the respective recurrent parents, and due to same reason BC2F5 derived plants had more grain yield per plant. In a number of constituted lines, spike length was comparatively shorter or comparative in length to recurrent parents, however, due to compact arrangement of the spikelets, the grain yield was increased. In other cases, generally the constituted lines carrying the linked gene complex \textit{Lr24+Sr24} and \textit{Yr15} spikelets were widely arranged and a reduction in grain yield was noticed. These morphological features, though singly cannot by itself be considered as an index of grain yield, however, may express the inherent capacity of the plants for higher yield. In the present study, the longer spike with more number of spikelets and having number of seeds per spike observed could be considered as some of the expression of the constituted near-isogenic lines for higher yield capability. Many of the constituted near-isogenic lines, both in BC2F5 and BC3F5, exhibited higher values of 1000-grain weight. Among the various yield components responsible for higher yield in wheat, more number of seeds per spike and higher seed weight were suggested as the most desirable characters (Bagga, 1986). A gradual reduction in the seed weight was noticed with increase in the degree of susceptibility to various type of rusts (Csosz et al., 1995). Lobachev (1992) had clearly shown that wheat isogenic lines carrying \textit{Lr19} had higher grain yield due to increase in grain weight. Similarly, Knott (1989c) used weight per 100-kernels as one of the criteria for identifying promising lines.
5.1.8. Grain yield performance of the constituted near-isogenic lines

Grain yield of the constituted near-isogenic lines was compared with their respective recurrent parents under rust free condition. The chemical "Contaf" (Hexaconazole) (Rallis India Ltd., Mumbai, India) used in the present study has successfully controlled all the three wheat rusts. The effect of rust severity on grain yield is evident that chemical treated control had more grain yield over untreated control. Significant yield increase was noticed in all the constituted near-isogenic lines over untreated control. However, when compared to chemical treated control, both increase and decrease in grain yield was noticed. The yield depression was in the range of -0.05% (HW 2084 X Darf*6/3Ag/Kite) to -14.63% (PDSN-32 X G-25). Yield reduction was noticed in the constituted near-isogenic lines that carried rust resistance genes \( Lr24+Sr24 \) and \( Yr15 \). In all the constituted near-isogenic lines, the yield increased with a range of 0.05% (PDSN-32 X WRT 238-5) to 18.54% (K 9107 X Veery'S').

There are a few earlier reports of yield variation associated with transfer of rust resistance gene(s) (Knott, 1989 a,b,c). Islam and Shepherd (1991) noticed a 7 to 9% yield reduction associated with stem rust resistance genes \( Sr21 \) and \( Sr26 \), respectively. Reports of positive effect of rust resistance gene on grain yield were also available. Reddy (2001) observed an increase of 0.05% and 11.61% yield enhancement due to \( Lr36 \) and \( Sr25+Lr19 \) respectively. Wheat stocks developed by Sawhney and Sharma (1996), carrying diverse rust resistance genes in the genetic background of Indian wheat cultivars Kalyansona and Sonalika, also out yielded their recurrent parents by 5-10%. Dyck (1992) did not observe any deleterious effect of stem rust resistance gene \( Sr40 \) when transferred from \textit{Triticum araraticum} to bread wheat. Four of the eleven rust resistant lines developed by Zeven \textit{et al.} (1983) exhibited superior agronomic performance. Drijepondt \textit{et al.} (1990) observed that Thatcher backcross derivative RL 6058, carrying leaf rust
resistance gene \textit{Lr34}, out yielded the parent by 0.3%. In spring wheats, \textit{Lr9} incorporation is associated with improvement in yield and yield contributing characters (Ortelli \textit{et al.}, 1996a, b). Cox \textit{et al.} (1997) observed that incorporation of leaf rust resistance gene \textit{Lr41} increased the grain yield and milling quality. Ma and Singh (1996) observed that stripe rust resistance gene \textit{Yr18} enhanced yield by 36 to 58\% over untreated control. Ortelli \textit{et al.} (1996a) observed that yield reduction in resistant wheat cultivars was due to limitation of assimilate supply as well as to a reduced sink capacity of the grain. Ortelli \textit{et al.} (1996b) further observed that lower root activity coupled with reduced CO$_2$ assimilation are responsible for reduction in grain number and grain weight of resistant wheat cultivars. The absence of yield depressive effect of leaf rust resistance genes in wheat cultivar Agent (Knott, 1989a), stem resistance genes in wheat cultivar LMPO (Knott, 1993), inconsistency (low or high) in the effect of the same gene on yield at different years (Knott, 1993) and locations (Knott, 1989b,c), and variation in the yield (low or high) due to various resistance genes in a particular wheat genetic background (Knott, 1989c), it is evident that the effect of resistance gene(s) on yield may vary with different genotypic backgrounds, different genes, different sources of rust resistance (donor parents) and the environment.

5.1.9. \textbf{Constituted near-isogenic lines selected for commercial purpose}

In addition to good agronomic characteristics, grain characters ultimately play an important role in the selection of desirable lines. Bold, hard, lustrous and amber coloured grains are more preferred than others. In the present study the constituted near-isogenic lines in BC$_2$F$_5$ produced seeds of inferior quality in most of the cases. Hence no lines were selected from this generation. Except a few lines (nine lines) all other BC$_3$F$_5$ near-isogenic
lines (eighteen lines) produced seeds of superior quality with desirable characters were selected.

5.1.10. Confirmation of gene transfers into Indian wheats

(A) Confirmation through morphological markers

'Awnless spike,' a simple and easily identifiable morphological character has been frequently used to confirm gene transfers in wheat. In the present study, all the F₁ hybrids of the crosses between Indian wheat cultivars and donor parents viz. Joss Chambier, Longbow, Cap-5BL-7BL and Darf*6/3Ag/Kite were awnless. Clubby tip, waxy coloured spike, lax spike and reduced yellow pigment in the grain flour are the other morphological characters which are used for confirmation of gene transfer. The donor parent G - 25 \( (Yr15) \) was characterized by clubby tip. Similarly the donor parent Veery'S' \( (Sr31+Lr26+Yr9) \) is characterized by waxy coloured leaves and spikes. The donor parents Cook*6/C80-1 \( (Sr36+Sr25+Lr19) \) is characterized by reduced level of yellowish pigment in the seed flour, while the donor parent RL 6081 \( (Sr38+Lr37+Yr17) \) is characterized by lax spike. All these characters were noticed in the progenies of initial crosses. These progenies were only finally used to develop constituted lines carrying various rust resistance genes. Obviously these characters were inherited from the donor parents along with respective rust resistance genes.

In the present study, gene transfers were also confirmed on the basis of effectiveness of other disease resistance genes. For instance, all the three recipient Indian wheat parents were susceptible to powdery mildew (showed a score of '4' on the scale of 0 - 4). The near-isogenic lines that carry linked gene complex \( Sr31+Lr26+Yr9+Pm8 \) showed a severity of score '3-4' for powdery mildew resistance (which is susceptible) as that of the donor parent Veery'S'. On the other hand, near-isogenic lines carrying the linked gene
complex \( Lr19+Sr36+Sr25+Pm6 \) showed high degree of powdery mildew resistance (score 1) as that of the donor parent Cook*6/C 80-1. These results support the successful incorporation of respective rust resistance genes into Indian wheat cultivars during backcrossing.

(B) Confirmation through genetical studies

(i) Inheritance of rust resistance

Genetic confirmation of the identity of resistance genes, by evaluating resistance and/or the segregation pattern of resistance in the progeny derived from crosses, are commonly used methods in wheat breeding. The transfer of rust resistance genes into wheat cultivars has also been confirmed through a study on rust inheritance (Chen and Line, 1993; Borner et al., 2000). Saini et al. (1995) identified additional undesignated resistance gene in the rust resistant near-isogenic line carrying \( Lr3 \) through rust inheritance studies. In the present study, when representative constituted rust resistant near-isogenic lines were crossed with the universal susceptible wheat variety Agra Local, all the \( F_1 \) hybrids were resistant to respective rusts indicating the dominant nature of rust resistance genes under transfer. The \( F_2 \) plants further segregated in 3:1 ratio for resistant to susceptible plants. Further, the \( BC_1 \) hybrids obtained from crosses between \( F_1 \) and recurrent parent, segregated in a 1:1 fashion for resistance to susceptibility. These results also clearly established that resistance in the constituted near-isogenic lines was due to a single dominant rust resistance gene. Since, the parent Indian wheats were susceptible to respective rusts, the rust resistance in the constituted near-isogenic lines developed through the crossing programme involving Indian wheats and various donor parents were only due to the rust resistance gene that was transferred in the present study. Similar approach has been followed to confirm stem rust resistance gene \( Sr36 \) in several Hungarian wheat cultivars recently (Csosz et al., 2001).
(ii) Monosomic analysis

Rust resistance as observed through monosomic analysis also provided clues regarding the presence of the transferred gene in the wheat genetic background (Mahmoud et al., 1987; Hussein et al., 1997). Monosomic analysis also helped in the identification of location of genes on a particular chromosome or chromosome arm. Many, Sr, Lr, and Yr genes have been isolated and mapped to specific chromosomes and given the gene symbols on the basis of monosomic analysis (McIntosh et al., 1995b, McIntosh and Lagudah, 2000). In the present study, the F₂ plants derived from the F₁ monosomic and disomic hybrid between the monosomic Chinese Spring and the rust resistant near-isogenic lines Sr27 (HW 2084), Lr24 (PDSN-32), and Lr19 (K 9107) segregated into 3:1 ratio for resistant to susceptibility for respective rusts except for the lines 3A, 3D and 7D, respectively. These studies revealed the location of the transferred rust resistance gene(s) on the particular chromosomes in the recipient wheat genetic background.

(C) Confirmation through biochemical studies

Gene transfers can also be confirmed indirectly through a study of certain biochemical parameters, as the expression of every gene(s) introduced is invariably regulated through the production of certain biochemical substances, which in turn confer host resistance. The interaction between the host and the pathogen is complex and the resultant resistant or susceptibility is regulated by the altered biochemistry of the host. An understanding of these resistance-triggering chemicals is thus of primary importance to confirm the incorporation of resistance genes in the host plants. The resistance inducing biochemical’s are governed by genetic constitution of the host. The inter allelic interactions for these resistances associated character(s) are frequent (Dey, 1990). As the use of isogenic or near-isogenic lines with dominant gene(s) conferring resistance can be an
ideal alternative to overcome these interactions in plants. The confirmation of gene transfer can also be inferred indirectly through a study of certain biochemical parameters, as the expression of every gene(s) introduced is invariably regulated through the production of certain biochemical substances, which in turn confer host resistance. For e.g. in maize, all the loci \( (rp^1-rp^5) \) for resistance to \( P. sorghi \) has been found to produce catechol oxidase except \( rp^2 \) (Pryor, 1976). This interaction between the host and the pathogen is complex and the resultant resistance or susceptibility is regulated by the altered biochemistry of the host. An understanding of these resistance triggering chemicals is thus of primary importance to confirm the incorporation of resistance genes in the host plants. The resistance inducing biochemical's are governed by genetic constitution of the host. The inter-allelic interaction for these resistance associated characters is frequent (Dey, 1990). As the use of near-isogenic lines with dominant gene(s) conferring resistance can be an ideal alternative to overcome this interaction in plants.

In the present study near-isogenic lines for rust resistance genes were used for studying changes in the enzymatic activities of polyphenol-oxidase, catalase, lipoxygenase, peroxidase, esterase, superoxide-dismutase, specific activities of ribonuclease, combined ribonuclease activity and nuclease, soluble protein, lipid content, total chlorophyll content, total free amino acid content, phenols and tannin content. In addition, chlorophyll content, nuclear DNA and respiration rate was also studied to differentiate susceptible wheat parents and their rust resistant near-isogenic lines.

In the present study, the total soluble protein content showed an increase in the resistant near-isogenic lines after 24 hours of post inoculation with rust pathogen. Such quantitative enhancements in protein metabolism appears to occur in plants which show a resistant reaction to infecting pathogen during early stages of infection. (Von Broembsen and Hadwiger, 1972; Yamamoto et al., 1975). Six gene-for-gene interactions between Flax...
and *Melampsora lini* were examined by Von Broembsen and Hadwiger (1972) and they observed that in general the net rate of protein synthesis increased in four incompatible (resistant) interactions but did not increase in the two compatible (susceptible) interactions. This characteristic increase in protein synthesis is one of the first detectable expressions of disease resistance in the Flax-*Melampsora lini* interactions. However, during later stages of infection the protein content decreased more in the susceptible parents than their resistant near-isogenic lines. Such quantitative reduction in proteins may be due to increased respiration of diseased tissue (Uritani, 1971). During rust infection such reductions in soluble proteins has been observed in the common wheat (Bushuk and Wringley, 1971). Changes in soluble protein constitution of leaves of near-isogenic lines of tomato, carrying resistance genes *Cf*₄ or *Cf*₅ to *Cladosporium fulvum* were investigated using PAGE by Dewit and Baker (1980) after inoculating with race 4 or 5 of this fungus. A protein having relative mobility 29 on 7% polyacrylamide gels appeared more rapidly in the incompatible interaction (*Cf*₄+race 5; *Cf*₅+race 4) than in compatible ones (*Cf*₄ + race 4; *Cf*₅ + race 5). The appearance of this protein was strongly associated with the hypersensitive response in incompatible interactions. In the present study, SDS-PAGE analysis of soluble proteins did not show any major qualitative differences in the protein profiles in the leaves of healthy and its resistant near-isogenic lines, most of the protein bands were common in all lanes. However, quantitative changes were observed as seen from the intensity of banding pattern. The intensity of protein bands were increased more in resistant than susceptible plants. Since uniform quantity of protein was loaded in all the lanes, the quantitative difference could not be due to sampling error. The increase in intensity of protein in resistant near-isogenic lines could be that these proteins possibly were involved in imparting resistance to the plants. Infection by pathogen induces the production of enhanced levels of certain metabolites and these
metabolic transitions have often been correlated with the appearance of new proteins (Gianinazzi et al., 1980). New proteins may be synthesized by host as defence factors (Hadwiger and Wagoner, 1983; White and Brakke, 1983). The intensity of major protein bands decreased in the leaves of susceptible than resistant plants. The possible explanation for more decrease in the susceptible plants could be that intense pathogen growth could be a cause of reduction in host proteins. However, Gabriel and Ellingboe (1982) performed two-dimensional electrophoresis of proteins from wheat lines differing by single resistance genes. Over 300 polypeptides were observed when visualized with Coomassie Brilliant Blue stain. Comparisons of 12 different isogenic host lines, each differing by a single gene for resistance to *Erysiphe graminis* (*Pm* isolines) failed to reveal any differences in polyploids mobility which could be attributed to or associated with a gene for resistance. Heinz et al. (1990) studied specific relationship between host and pathogen using a criss-cross genetic system. The same were well characterized by near-isogenic mutants of *Triticum aestivum* which differed in their compatibility reaction to two genetically related mutant clones of wheat leaf rust *Puccinia recondita tritici* (Fo1 and Fo2). They studied specific and non-specific pathogenesis related changes in protein by pulse labeling with [S35] methionine in rust inoculated and mock inoculated wheat leaves. SDS-PAGE and flurography were subsequently used to detect changes in the patterns or polypeptide synthesis “de novo”. Changes were noted as early as 2 days after infection. Synthesis of 39 KD and 15 KD polypeptide was enhanced in incompatible interactions, while synthesis of an 83 KD and 15 KD polypeptides were enhanced in compatible reactions. Synthesis of polypeptides present in the mock inoculated leaves was suppressed in both the compatible (52 KD band) and incompatible interaction system (37 KD band). Comparisons with the third mutant clone of rust Fo3 which interacts in a manner opposite to interaction of Fo1, elicited an inverse criss-cross
relationship with the same wheat lines indicating that most of the detectable changes were associated with gene for gene relationship.

Javornik et al. (1991) compared four methods (N-banding of mitotic metaphase chromosomes, APAGE of Gliadins, SDS-PAGE of unreduced proteins and Isoelectric focussing of subtilisin inhibitor for detecting 1B-1RS translocations in 59 Yugoslav winter wheat cultivars and recommended the use of Isoelectric focussing of subtilisin inhibitor. As the results of Isoelectric focussing of subtilisin inhibitor and SDS-PAGE of unreduced protein gave the same results in identifying 1BL-1RS translocation in 29 cultivars, in the present study SDS-PAGE of unreduced protein was carried out to confirm the translocation (incorporation) of rye segment (1RS) carrying the gene complex \(Sr31+Lr26+Yr9\) in the F\(_1\) hybrid between the Indian wheat cultivar and Veery'S'. Gupta and Shepherd (1992) in a separate study after screening vast collection of wheat cultivars from as many as 31 countries recommended the use of SDS-PAGE of unreduced proteins for identifying 1BL-1RS translocations in wheat. The F\(_1\) hybrid between K 8962 X Veery'S' showed a similar Sec-R1 banding (Rf 0.56) pattern of Veery'S' and Kavakaz, a known standard for 1BL/1RS translocation. As the Sec-R1 banding pattern of Veery'S' derivatives, Veery'S' and Kavakaz are similar, it can be inferred that the translocation of rye segment carrying the gene complex \(Sr31+Lr26+Yr9\) would have taken place. Their moderate to high resistance reaction for all the three rust confirms the incorporation of the 1RS rye segment carrying the gene complex \(Sr31+Lr26+Yr9\).

Esterases are the hydrolytic enzymes which catalyse the addition or removal of water in biological reactions (Melillo et al., 1992). Esterases are widespread in nature and occur frequently in plants. In the present study, the esterase isoenzyme bands observed in rust resistant near-isogenic lines were stronger when compared with the susceptible wheat parents. The
increase in band intensity of esterase in resistant near-isogenic lines could be due to increase in enzyme activity. In pea roots, infected with *Heterodera goettingiana* (Melillo *et al.*, 1992) and pearlmillet infected with downy mildew (Gupta *et al.*, 1980), the esterase activity revealed high and less intense band for resistant and susceptible cultivars. Isoenzyme activity of superoxide-dismutase in wheats and their relatives were indicating the location and grouping of superoxide-dismutase radicals in wheat cultivars (Jasska, 1982).

In the present study, the resistant response to rust pathogen in all the rust resistant near-isogenic lines was characterized by an increase in peroxidase activity compared to the susceptible response of Indian wheat parents. Enhancement in peroxidase activity is quite common following infections of plants with potential pathogens (Reuveni and Ferreira, 1985; Reuveni and Bothmar, 1985; Srivastava 1987; Sharma and Sharma, 1977; Reddy, 2000a). The increase in peroxidase activity in several host plants following rust infection has been well documented (Macko *et al.*, 1968; Daly *et al.*, 1970; Seevers and Daly, 1970; Daly *et al.*, 1971; Seevers *et al.*, 1971; Johnson and Cunningham, 1972). The increase in peroxidase activity has been suggested as being directly responsible for increase in disease resistance through the inhibition of mycelial growth of the stem rust fungus (Macko *et al.*, 1968). Similarly papillae formation was implicated in resistance in maize mesocotyles upon attempted fungal infection is associated with increased peroxidase activity (Cadena-Gomez and Nicholson, 1987). Peroxidase contributes to resistance by oxidation of phenolic compounds (Sempio *et al.*, 1975) and is also believed to be involved in yielding lignin-like substance via polymerization of phenylpropane compounds by oxidative hydrogen peroxidase dependent system (Vance *et al.*, 1980). The lowered activity of polyphenol oxidase (PPO) and an increased activity of catalase in the susceptible interaction and higher activity
of PPO in resistant interaction towards later stages of progressive rust infection characterizes the general natural infection/resistance in this system. Positive correlation between PPO activity and resistance to different plant pathogens such as *Helminthosporium* (Jennings *et al.*, 1969) and *Verticillum* (Reuveni and Ferreira, 1985) has been reported earlier. Johnson and Lee (1978) conducted similar study and found that PPO activity in *Lr10* infected with wheat leaf rust was higher in infected plants compared to that in healthy controls.

The higher activity of catalase in susceptible plants is due to the virulent races have been shown to reduce the natural defence of the bean plants against *Pseudomonas phaseolicola* through suppression of peroxidase activity by destroying its substrate hydrogen peroxidase and maintaining phenolics in their reduced state which are considered as less active as antimicrobial substances than their quinone forms (Rudolph and Stahman, 1964).

The enzyme lipoxygenase has been proposed to damage the integrity of biological membranes through the production of free radicals (Galliard, 1975) and is also known to catalyse oxidation of fatty acids like linoleic and linolenic acid more commonly found in plants as the major constituents of membranes. The increase of lipoxygenase activity upto two days following inoculation with rust pathogen in both the susceptible parents and their resistant near-isogenic lines may be due to protein denaturation or by formation of antioxidant enzyme complex (Lupu *et al*., 1980) and the subsequent decrease towards later stages of infection particularly in resistant near-isogenic lines may be assumed due to the accumulation of some antifungal compounds of inhibitory nature following interaction (Prusky *et al*., 1985). The consistent increase in the susceptible parents may be assumed as due to the synthesis of new enzymic forms responsible for probable
antifungal compounds and this favouring the host metabolism for easy establish of the pathogen. Multiple molecular forms of lipoxygenase have also been reported in wheat and soyabean (Guss et al., 1968). The decline of lipoxygenase is regulated at the RNA level (Wang et al., 1988).

The total lipid content also showed an increase in the susceptible parents and their resistant near-isogenic lines after 2 days of inoculation but progressively decreased with the advancement of infection. Lipids being important components of the sub-cellular and cellular membranes, any change in membrane lipid components during the process of pathogenesis affect the membrane permeability through enhanced leakage of cell constituents as has been reported in *Phaseolus vulgaris* infection with *Uromyces phaseoli* (Hoppe and Heitefuss, 1974). The changes in host cell permeability during host-parasite interaction have been often reported in the case of fungal pathogens (Cahill et al., 1985; Saini et al., 1988).

Data on total content of free phenols and tannins indicate that rust resistant near isogenic lines contained significantly higher amount of total free phenols and tannins compared to their respective recurrent wheat parents. It has already been established that phenolic and tannin compounds impart resistance in many crop plants against pests and diseases (Sharma et al., 1983). It is believed that polyphenol and tannin accumulation is a defense mechanism adopted by resistant plants. An increase in phenol content in disease resistant plants has been reported by several workers (Jite and David, 1987; Govindarajulu, 1976). Kaur and Mehrotra (1990) compared the phenolic compounds in resistant (ICP 28) and susceptible (ICP 7119) varieties of pigeon pea and found higher phenol activity in resistant line as compared to the susceptible one. Saini et al. (1988) also observed a reduction in phenol content in compatible interactions. Phenolic substances seem confer resistance either directly through its antimicrobial properties or
through their oxidation and polymerization products. Increase in tannin content was also reported in *Sorghum* resistant to shoot fly (Khurana and Verma, 1983; Kumar and Singh, 1998).

A comparison of nuclear DNA amounts in the parents and hybrids often provides useful information whether an alien gene has really been incorporated into the hybrids or not (Furuta *et al*., 1977). In the present study, the constituted rust resistant near-isogenic lines, obtained from the crosses between Indian wheat parents X donor wheat stocks carrying various rust resistance genes had relatively higher DNA contents over their respective recurrent susceptible wheat parents. It has been well documented that in plants both interspecific and intraspecific variation exist in DNA contents (Furuta and Nishikawa, 1991), and the factors leading to such variations include addition or deletion of chromosome / chromosome segment / gene / genes (Rees and Hazarika, 1969) and hybridization (Keyl, 1965; Jones and Rees, 1968). In the present study, significant differences were also observed in mean and range of nuclear DNA contents (2C and 4C) between the derivatives and the Indian wheat parents. Therefore, it is evident that increase in the nuclear DNA content in the constituted lines could only be due to the incorporation of rust resistance genes from the donor parents into Indian wheats during hybridization programme.

Data on Ribonuclease indicate that the specific activities of Ribonuclease-I and combined Ribonuclease-II and Nuclease-I were high at the 15th day stage over the 10th day stage after inoculation. The rust resistant near-isogenic lines had significantly reduced activities of RNase-I and combined RNase-II + Nuc-I at both the stages. Scrubb *et al*.(1972) and Chakravorty *et al*. (1974) have reported higher activity of ribonuclease at later stages of infection. They also have observed that ribonuclease activity during pathogenesis increases in two well-defined phases. In an early phase,
ribonuclease activity increased both in resistant and susceptible plants, while in a second later phase, ribonuclease activity increase only in susceptible plants.

Reduction in chlorophyll content was noticed in both susceptible wheat parents and resistant near-isogenic wheat lines. The reduction was drastic and gradual in susceptible parents, while the level of chlorophyll content was at a steady rate in resistant lines. According to Kruger and Hewitt (1984) the reduction in chlorophyll content of a susceptible plant may be due to disruption of the thylakoid membranes freeing the membrane-bound chlorophyll molecules. Riedell, (1989) and Kruger and Hewitt (1984) found a similar reduction in chlorophyll content in infested susceptible barley and wheat after aphid infection.

Free amino acid content were increased in the initial period in both susceptible and resistant near-isogenic wheat lines, however the degree of increase was relatively higher in resistant wheat lines. Increased level of amino acid content in infested plants is due to stress imposed by rust pathogen. It is well known that a general characteristic feature of plants when subjected to stress is reflected in increased level of total free amino acids (Rabe, 1990). Increased in amino acid content, was also noticed due to stress imposed by abiotic factors like freezing temperature (Dorffling et al., 1990). It is suggested that increased amino acid content may provide nutritional requirements for attacking pathogen (Niraj et al., 1985; Ciepiela, 1989). Consequently the chloroplast of resistant plants remains more intact and photosynthesis can proceed relatively normally as opposed to susceptible plants where chloroplasts are damaged (Fouche et al., 1984).

Inoculation with rust pathogen enhanced the respiration rate both in susceptible parents and in resistant near-isogenic wheat lines. The degree of increase was higher in resistant one. Rust pathogen attack may leads to
mechanical damage consequently increased respiration rate. It is also well documented that increased respiration is a characteristic physiological feature of disease plants. It is generally considered that early rise in respiration are rather directly connected with expression of disease resistance. Respiratory increases are regarded as important to supply the extra necessary carbon units and energy to the synthesis of a spectrum of products normally associated with the development of resistance (Smedegaard-Petersen, 1980).

**(D) Confirmation through molecular markers**

The development of molecular genetics has provided new tools that can aid in the identification of resistance genes. Molecular markers linked to resistance genes are particularly useful when suitable rust isolates are not available, when interactions between resistance genes are anticipated and therefore comparison of reactions with lines possessing single genes is difficult, or when an infection test is time consuming.

In the present study the near-isogenic lines for stripe rust resistance gene *Yr15*, together with their parents were screened for polymorphism at the molecular level using the already identified RAPD primers namely, OPA-19 and OPB-13. The RAPD primers OPB-13 amplified the indicative fragment of 1500 bp and OPA-19 amplified the indicative fragment of 1420 bp resulted in one additional band in resistant near-isogenic lines that carry stripe rust resistance gene *Yr15*, and was absent in susceptible parent. Earlier studies had shown that this RAPD marker showed complete linkage to the *Yr15* gene (Sun et al., 1997).

Identification of molecular markers linked to rust resistance genes gaining momentum during the last few years. Using RAPD analysis, Singh *et al.* (1997) confirmed the presence of stripe rust resistance gene *Yr8* in an
Australian wheat cultivars Avocets. Molecular markers linked to leaf rust resistance gene Lr1 (Feuillet et al., 1995), Lr13 and Lr35 (Seyfarth et al., 1998), Lr24 (Schachermayr et al., 1995), Lr29 (Motawei and Barakat, 2001), Lr37 (Bonhomme et al., 1995), Lr41 (Lottering and Botha, 2002), Lr46 (William et al., 2003), Lr45 (Dedryver et al., 1996), Lr25 and Lr29 (Procunier et al., 1995), Lr34 (William et al., 1997; Nelson et al., 1995; 1997), Yr26 (Ma et al., 2001), Lr13 and Lr35 (Seyfarth et al., 1998), Lr21 (Huang and Gill, 2001), Yr17 (Robert et al., 2000) are some of the rust resistance which were confirmed recently in the recipient wheat-genetic background. The present results are in conformity with their findings, suggesting that these specific primers can be reliably used to confirm the incorporation of Yr15 gene.

5.1.11. Conclusions and commercial usefulness of the constituted near-isogenic lines

The genetic improvement of wheat has received considerable attention over the years from plant breeders with the purpose of increasing the grain yield and to minimize crop loss due to unfavourable environmental conditions, and attack by various pests and pathogens. In the early 60’s, conventional breeding coupled with improved farm management practices led to a significant increase in world wheat production thereby ushering in the green revolution. Subsequently, the targets of genetic improvement shifted to reducing yield variability caused by various biotic and abiotic stresses and increasing the input-use efficiency. With the change in the global food policy in the last few decades, biotechnology offered a possible solution firstly, by lowering the farm level production costs by making plants resistant to various abiotic and biotic stresses, and secondly, by enhancing the product equality. The introduction of foreign genes encoding for useful agronomic traits into commercial cultivars has resulted in saving precious time required for introgression of the desired trait from the wild relatives by conventional
practices and alleviating the degradation of the environment due to the use of hazardous biocides. In recent years, wheat improvement effort have therefore focused on raising the yield potential, quality characteristics, resistance to biotic and tolerance to abiotic stresses depending on the regional requirement of the crop.

Production of wheat cultivars carrying diverse rust resistance genes with a broad spectrum of resistance and their geographical deployment based on the race flora may be prove an effective control of spread of rust disease. This is more important so in countries like India where frequent replacement of wheat cultivars requires abundant economic resources. The common experience that the resistance is often overcome by the evolution of new pathogenic race(s) soon after the extensive cultivation of resistant wheat cultivars makes resistance breeding an ever continuous process. Hence, breeding for rust resistant cultivars should be looked as never ending process. Since durability of resistance cannot be assumed, resistance-breeding strategies are usually supported with the maintenance of genetic diversity to produce buffering against extreme crop losses in the event of significant pathogenic changes. The availability of rust resistance gene(s) in the superior wheat genetic background will enhance the exploitation in wheat breeding in India and elsewhere in the world. The incorporation of these genes either singly or in combination would prevent the rust infection in wheat growing areas of the county. One such backcross line HW 2004 carrying linked gene complex (Sr24+Lr24) has been released for commercial cultivation in the central part of India (Govt. of India, 1996). This gene complex was also incorporated into another Indian wheat cultivar Vaishali (Sawhney and Joshi, 1996).

The wheat lines developed in the present study, by virtue of possessing resistance to one or more type of rusts and accompanied by other
desirable agronomical characteristics have definite advantage over their susceptible recurrent parents. The farmers are still extensively cultivating all the three recurrent parents, despite they are highly susceptible to rust. Therefore, there is an immediate need to replace these susceptible cultivars with resistant ones. However, hitherto, there are no such resistant lines coupled with good agronomic characteristics that can substitute these cultivars.

The constituted near-isogenic lines may also serve as fairly good genetic background genotypes for respective genes transferred and these maybe useful in wheat breeding programme. The backcross improved lines could also be blended to constitute multilines (Tomar and Menon, 2001b). Since newly developed lines differ with the original recurrent cultivars in respect of only one gene for resistance, they will also serve as near-isogenic lines for molecular and genetic mapping of the gene. The lines constituted as genetic stocks could also be used as donor parents and the rust resistance genes could be transferred to other well-adapted wheat genotypes.

As more and more biochemical and / or molecular markers conferring rust resistance are now available, it will become possible to rapidly transfer many of the genes coding for rust resistance from cultivar to cultivar. Molecular and Biochemical marker assisted backcross breeding can be used to select directly the gene of interest and, at the same time, to reduce the size of the introgressed segment around the gene. This type of strategy might be important tool for accumulating more rust resistance genes into a single cultivar.

5.2. Quality analysis of wheat grains and wheat flour in different hexaploid Indian wheat varieties

Food grain production occupies the most dominant position in India's agriculture, covering over 65 percent of the gross cropped area. Since the
beginning of the green revolution in the mid 1960s, the country has shown quite impressive growth in food grain production (Sharma and Gandhi, 1990; Bhalla, et al., 1999). Cereal grains and legumes play an important role in supplying the nutrients, as well as over 70% of the daily energy requirements, of over two-thirds of the world’s population (Edwards et al., 1971). Wheat’s pleasant flavor, long shelf-life, and unique gluten-forming characteristics make it the most popular grain for bread-making (Nelson, 1985). Demand for wheat and other various wheat products is increasing steadily compared to its production rate (Morton, 1988).

Wheat is the primary source of energy for most of the Indian population and is a fair source of protein, B-vitamin and minerals. Wheat has been prized since the oldest time, because of its unique properties that permit an array of leavened and unleavened quality food products to be made from it. Quality refers to the desirability of the product and may include various physical and chemical aspects depending on the intended purpose. The quality of wheat flour is the main raw material for the preparation of various bakery products.

Various physical properties of wheat grains, chemical properties of wheat flour, rheological properties of particular wheat dough, and glutenin quality of seed proteins determines the suitability of a wheat genotype for giving rise to a particular end product, and the quality of the end product(s) derived from it. In the present study about 50 popular commercial Indian wheat varieties were evaluated for their suitability for various end products particularly of bread making, chapatti making and biscuit making. The evaluation is based on various physical properties of the grains, chemical composition of wheat flour, rheological properties of wheat dough, and glutenin sub-unit composition of seed proteins.
5.2.1. Physical properties of grains and chemical properties of flour

Various physico-chemical characteristic features of wheat grains and wheat flour influence to a great extent the quality and quantity of protein and other macromolecules. Ultimately these features influence the quality of end products derived from wheat. Among various physical properties of wheat grains, the grain appearance score, texture of the grain, hardness of the grain, moisture content of the seed, test weight and grain weight of the seeds are all influence the quality and quantity of flour derived from wheat grains. The test weight has been shown highly correlated with flour yield, and has been used as indicator for evaluating the grain quality in cereals like triticale and oats (Douglas et al., 2004). A significant correlation between test weight and flour yield was also earlier established (Shuey, 1960; Aguirre et al., 2002). Chunk et al. (1994) on the other hand studied the relationship between the single kernel characteristics and end use quality of hard wheats. They noticed that the texture of kernels play a significant role in determining the end use product of wheat grain. Hard and semi-hard textured grains yield better quality of flour and can be used for all purposes such as bread making, chapatti making and for blending, whereas soft textured grains produce low quality flour and hence it is suitable only for biscuit making. Moisture content is another important physical character of wheat grains influencing the end product quality. Moisture content determines the length of time that grain can be safely held or stored without spoilage. Higher (above 13%) or lower (below 7%) moisture content in seeds leads to grain spoilage or severely affect the quality of the grain. Therefore a grain with moisture content of 10-11% is more suitable for producing high quality end product. Miller et al. (1982) observed that wheats with hard textured grain, medium moisture content with high grain appearance score produced high quality end products. In the present study, 44 out of 50 wheat varieties possessed higher test weight, 1000-grain weight, and higher grain appearance score,
low pearling index coupled with hard or semi-hard textured seed. These wheat varieties are likely to give high percentage of flour yield and good quality of end products. These wheat varieties were therefore suitable for bread making as well as for chapatti making, and can also be useful in blending. In the remaining six wheat varieties, the test weight, 1000-grain weight and grain appearance score was low and the texture of the grains was soft, and the pearling index was high. Therefore these varieties are likely to produce low percentage of flour yield, and the quality of flour will be inferior, therefore these wheat varieties more suitable for making biscuits.

Among various chemical properties of wheat flour that influence the end use quality of wheat flour include protein, wet gluten, starch, ash and fat per cent (Dexter et al., 1994; He and Hoseney, 1992; Gupta et al., 1994; Orth et al., 1972). Higher percentage of protein, wet gluten, and damaged starch wheat flour is more suitable for bread, chapatti making and for blending purpose, whereas higher percentage of ash and fat in wheat flour were suitable for biscuit making. A significant correlation exists in ash content and brightness of flour. Wheat products which contain higher levels of ash are darker in colour. Damaged starch is related to the degree of hardness of wheat. Excessive damaged starch can harm the handling of dough and impair loaf volume and crumb pliability. Whereas moderate damaged starch is beneficial (Kaur et al., 1998). In the present study, chemical composition of wheat flour in 50 genotypes indicated that the wheat flour possesses weak to very strong strength characteristics. Strong strength wheat flour contains higher percentage of protein, wet gluten, and SDS sedimentation value. Weak strength wheat flour on the other hand contains higher percentage fat, ash with low percentage of protein, wet gluten, and SDS sedimentation value. In the present study, the quality of the flour of 6 wheat varieties is of weak strength in nature; hence these six wheat varieties were suitable only for biscuit making. Flour of the 31 wheat varieties showed
medium strength. This suggests that these varieties can be useful for all purposes (bread, biscuit and chapatti). Remaining 13 wheat varieties had strong strength characteristics in the flour that is they contain higher percentage of wet gluten, protein, sedimentation value and very low percentage of ash and fat therefore these wheat varieties were more suitable for blending purpose (mixing with weak quality flour).

5.2.2. Rheological properties of wheat flour

(A) Farinograph characteristics

Recording dough mixers have been used for over half a century to evaluate wheat quality (Brabender, 1937). Balia et al. (1997) reported that interfacial properties of gluten proteins like dough stability, mixing tolerance index and dough development time are important in determining the bread making quality of flour. The results further revealed that higher the water absorption, the stronger is the flour and vice versa. Farinographic water absorption is the amount of water required to develop dough of desired consistency, which causes the curve to rise to the 500 (BU) consistency line at the peak of mixing (i.e. when the gluten is fully developed). If too much water is added, the consistency line will fall below the 500 BU, which indicates that the flour is over absorbed. If too little water is added, the consistency line will arise above the 500 BU, which indicates that the flour is under absorbed. Dough development time is the time from the first addition of water to the development of dough’s maximum consistency with minimum mobility. Long dough development time indicates strong flour containing high percentage of gluten forming proteins. Dough stability time is major index for dough strength. The flour with longer stability is the-best suited for bread baking. The flour with short stability time can be used for biscuit and chapatti making. Longer dough stability time gives greatest strength to gluten and makes it resistant to mechanical damage. In the present study, 44 out of 50
wheat varieties had longer dough development time and stability, remaining six wheat varieties had shorter stability time coupled with low percentage of gluten forming proteins.

Mixing tolerance index (MTI) was another parameter used for indicating flour mixing requirements. Strong flour had lower MTI value and weak flour had higher MTI value (Kaur et al., 1998). In the present study wheat dough of six wheat varieties had low MTI value indicating that the flour of these wheat varieties are soft in nature with low gluten strength. Softness arises when the gluten strength in the wheat flour is low, which in turn caused by low sedimentation value. The results are in agreement with that of Autran and Felllet (1987).

(B) Extensograph characteristics

Extensibility is the total length of the extensographic curve obtained from extensometer. This parameter is used to estimate the resistance of dough to extension. Longer the curve is an indicator of low resistance of the dough to extension, which means the strength of the dough is weak and with less elastic properties therefore it is useful only for biscuit making. On the other hand shorter the curve indicates high resistance of the dough to extension, which means the strength of the dough, is strong and with more elastic properties therefore it is useful only for bread making. The bread making dough shows a strong resistance to stretching at early stages because of its elastic glutenin. After limited extension the dough breaks. In contrast, the biscuit dough is much less resistant to stretching and also extends further before breaking (Chen and Rasper, 1982). It was suggested that a bread making wheat is quite unsuitable for biscuit making and vice versa. Tested doughs in the present study showed, decreased resistance of dough to extension in 6 wheat varieties hence these varieties were suitable only for biscuit making purpose. Remaining 44 varieties had high-medium
resistance to extension with greater smoothness and hence these varieties were suitable for all purpose which including bread making, chapatti making, and blending with weak quality flour.

5.2.3. High molecular weight glutenin subunit composition in wheat

A clearer understanding of the genetic control of flour quality for bread and biscuit making would increase the probability of identifying superior genotypes and make possible subsequent development of improved cultivars. Proteins are recognized as the most important components governing bread making quality and both quantity and quality of proteins play a major role in variation in bread making quality.

Storage proteins from bread wheat (Triticum aestivum) endosperm is being a major focus in bread making quality characterization owing to their major role in determining the nutritional value and the technological quality of wheat flour. The storage proteins in wheat are usually classified into two main fractions Gliadins and Glutenins according to their state of aggregation in dissociating media. Gliadins are small, 35-70 KD and they do not have disulphide bonded subunit structures. The Glutenins on the other hand are large, heterogeneous molecules built up of different subunits connected by disulfide bonds. The term ‘Glutenin’ is now reserved for the native oligomeric molecules. The term ‘subunit’ refers to the single chain polypeptides obtained after reduction of the disulphide bonds of glutenin. Technique such as SDS-PAGE divides glutenin subunits into two relatively distinct groups. The High Molecular Weight (HMW) and the Low Molecular Weight (LMW) subunits. (Lawrence and Shepherd, 1981; Payne et al., 1982; Shewry et al., 1992). It is well known that HMW Glutenin subunits play an important role in determining wheat technological quality.
Gluten consists of two proteins, gliadin and glutenin. Gliadin (the prolamine of wheat) consists of numerous different polypeptides. Glutenin (the gluten of wheat) only partially dissolves in dilute acids and has a very large aggregate molecular weight. The two protein types impart different properties to a dough; gliadin is viscous and gives extensibility, allowing the dough to rise during fermentation, whereas glutenin gives elasticity, preventing the dough from being over-extended and collapsing either during fermentation or during baking.

High Molecular Weight (HMW) subunits of Glutenin are coded by genes at three complex loci Glu-A1, Glu-B1 and Glu-D1 located on the long arms of chromosomes 1A, 1B, and 1D respectively (Payne et al., 1982). There is strong evidence indicating that the presence of certain HMW Glutenin subunits in common wheat can be related to bread and biscuit making quality (Payne et al., 1979; Payne, 1987). Based on this, Payne et al. (1987) proposed a system of quality scores assigned to individual HMW Glutenin subunits or subunit pairs. Of these three complex loci, the allelic variations at the Glu-D1 locus are particularly important for good or poor bread making quality. The allele Glu-D1a, coding for subunits 2+12, has a Glu-1 score of 2 (poor), where as Glu-D1d coding for subunits 5+10 has a Glu-1 score of 4 (good). The allele coding the subunit combination 5+10 (at locus Glu-D1) had a better effect on bread making quality than the 2+12 allele (Payne et al., 1987; Ng and Bushuk, 1989; Khan et al., 1989; Dong et al., 1991; Hamer et al., 1992). The allele coding the subunit combinations 7 and 20 (at locus Glu-B1) had a better effect on biscuit making quality. (Carrillo et al., 1990). These alleles are usually analysed by SDS-PAGE. Discrepancies in relative mobility of some subunits make their identification ambiguous (Payne and Lawrence, 1983; D'Ovidio et al., 1994).
In the present investigation, fifty Indian hexaploid wheat varieties were analysed for their allelic variations for High Molecular Weight Glutenin subunits (HMW-GS) by Sodium Dodecyl Sulphate Poly Acrylamide gel electrophoresis (SDS-PAGE). A total of 11 alleles were identified in the analysed cultivars. At the Glu-A1 locus, three alleles a, b and c were present which encode for HMW Glutenin subunit 1, 2*, N (null), respectively. The presence of only three alleles was in agreement with the results obtained by Payne and Lawrence (1983). In the present study, the HMW Glutenin subunit 2* was present in a total of 19 varieties (38%). A total of 61% of Portuguese wheat were shown to possess this subunit (Rodriguez-Quijano et al., 1998). However, in contrast when Payne and Lawrence, (1983) analysed the world collection of hexaploid wheats, only 28% of wheats were recorded the HMW Glutenin subunit 2*. Rodriguez-Quijano et al. (1990) reported that 32% of Spanish landraces were found to possess this subunit. In the present study 19 varieties (38%) had the HMW Glutenin subunit 1 ('a' allele) and 12 varieties (24%) had the HMW Glutenin subunit N ('c' allele). Payne and Lawrence, (1983) reported that Chinese varieties (5.2%) possessing the Glu-A1 allele subunit 1, appeared less frequently than in lines of the Japanese (12.2%).

At the Glu-B1 locus the alleles a, b, c, d and e encoded HMW Glutenin subunits 7, 7+8, 7+9, 17+18 and 20 respectively. Similar results were reported earlier by Vallega and Waines (1987) and Galili et al. (1988). In the present study, 6 varieties (12%) had the HMW Glutenin subunit 7 (Glu-B1a allele), 8 varieties (16%) had the HMW Glutenin subunits 7+8 (Glu-B1b allele), 12 varieties (24%) had the HMW Glutenin subunits 7+9 (Glu-B1c allele), 22 varieties (44%) had the HMW Glutenin subunit 17+18 (Glu-B1d allele) and only two varieties(4%) had the HMW Glutenin subunit 7 and 20 (Glu-B1a and Glu-B1e alleles) Results of Ng et al. (1988) and Rabinovich
et al. (1998) also revealed presence of HMW Glutenin subunit 7 in large number of wheat cultivars studied by them.

At the Glu-D1 locus, three alleles viz., a, b and d were identified which encoded HMW Glutenin subunits 2+12, 3+12 and 5+10 respectively. Among all the allelic variants found at the Glu-D1 locus, the allele Glu-D1d (subunits 5+10) are related to good bread making quality (Burnouf and Bouriquet, 1980; Moonen et al., 1982; Branlard and Dardevet, 1985). Payne (1986) also reported that the wheat possessing subunits 5+10 were found to be associated with good bread making quality.

In the present study, the Glu-D1a (2+12) allele is associated with poor bread making quality is present in 23 varieties (46%). The subunit 3+12 (Glu-D1b allele) were present only in two varieties (4%) and the subunits 5+10 (Glu-D1d allele) were present in 25 varieties (50%). The Importance of 5+10 in bread making quality is emphasized by the fact that several authors are of the opinion that the HMW Glutenin on chromosomes 1D have a greater influence on flour quality than the alleles on chromosomes 1A and 1B (Payne et al., 1981, 1984, 1988; Burnouf and Bouriquet, 1983).

The earlier studies on HMW Glutenin subunits of spring wheats indicate that among the 122 wheats from the U.S. and Mexico, subunits 2* (64) and 1 (28%) predominate at the Glu-A1 locus, 7+9 (44%); 17+18 (23%) and 7+8 (18%) predominate at Glu-B1; and 5+10 (7.3%) is the main subunit at Glu-D1. Among 104 wheats from 12 Western and Eastern European countries, subunits 1 (47%) and 2* (33%) predominate at locus Glu-A1; 7+9 (47%) and 7+8 (21%) predominate at Glu-B1; and 5+10 (69%) at Glu-D1.

However, among wheats from seven countries (mainly Sweden and Germany), subunit 14+15 (14%) occurs at locus Glu-B1, which is absent in the North American cultivars (Rabinovich et al., 2000). Wheat cultivars from
Osijek country, subunits were found with respective frequencies 1 (47%) and N (53%) at Glu-A1. 7+8 (40%) and 7+9 (60%) at Glu-B1; 2+12 (80%), 5+10 (20%) at Glu-D1 (Jurkovic et al., 2000). Among 15 Norwegian wheat cultivars have high-quality subunits 1 (7%) and 2* (93%) at Glu-A1; 7+8 (50%), 13+16 (7%) and 7+9 (36%) at Glu-B1 and 5+10 (50%) at Glu-D1 (Uhlen, 1990; Jackson et al., 1996).

The most frequent HMW Glutenin subunits were 2* at Glu-A1 locus (85%), 7 at Glu-B1 locus (40%) and 2+12 at Glu-D1 locus (60%). The most frequent protein combinations are 2*, 7+9, 2+12; 2*, 7+8, 2+12 and 2*, 7, 5+10. Similar results were reported recently by a number of workers (Vida et al., 1998; Ivano et al., 1998; Rabinovich et al., 2000; Reddy and Gothandam, 2002).

In the present study, the Glu-1 quality score ranged from 4-10. Among fifty wheat varieties, 11 varieties (Glu-1score 10) had minimum baking quality with over strong gluten strength and SDS-sedimentation value were recommended for blending with weak quality wheat flour (weak gluten strength); 33 wheat varieties (Glu-1score 7-8) had high baking quality with higher gluten strength and SDS-sedimentation value were recommended for bread and chapatti making. Remaining 6 varieties (Glu-1score 4-5) had poor baking quality with lower gluten strength and SDS-sedimentation values were recommended for biscuit making. Similar results were reported by several workers (Tohver et al., 2001; Ivanov et al., 1998; Tsoun et al., 1998; Huang and Khan, 1995; Payne et al., 1987).

The earlier studies also demonstrated that allelic variation in LMW - GS is correlated to the difference found in gluten viscoelasticity either in bread or durum wheats (Pogna et al., 1990; Gupta et al., 1991, 1994; Gupta and MacRitchie, 1994). In many countries the importance of these proteins in bread making quality were also demonstrated (Metakovsky et al., 1990;
Gupta et al., 1989). Varieties with similar HMW-GS when grown in Argentina showed variable bread making quality suggesting that investigations into the composition of Gliadins and LMW glutenin subunits are important (Feingold et al., 1993).

The prominent role of Glutenin subunits in determining dough strength lies almost entirely in their capacity to create a large variability in the size of the polymers (hence overall protein) by forming intermolecular disulphide linkages. The loss of cohesive elastic properties of the dough of polymeric protein (native glutenin) when the disulphide linkages are cleaved even partially clearly supports the positive roles of disulphide-linked polymers, particularly of the large polymers, in governing dough strength. Monomeric proteins, on the other hand, do not form intermolecular disulphide linkages, thus do not have positive effects on strength properties of wheat flour (Singh et al., 1991; Gupta et al., 1993).

Despite these advances in our understanding of the relationships between flour properties and polymeric protein (Glutenin) at the subunit (allelic) or polymer (functional) level, the basis of differences in effects of various glutenin alleles on dough properties (i.e. the role of individual glutenin subunits in the formation of polymeric protein and resulting effects of functional properties) has not be investigated in detail. For e.g. it is not known how HMW Glutenin subunits 5+10, 17+18 make a greater contribution to dough strength than subunits 2+12 and 20, respectively.

The results obtained in the present research clearly point to the presence of HMW glutenin subunits like 7+8 on chromosome 1B, 2+12 and 5+10 on chromosome 1D are highly influence the water absorption capacity, dough development time and stability time of the dough. This result also corroborate with those of Tohver et al. (2001).
Test weight, which has been considered as an indicator of potential flour yield, showed significant correlation with flour yield. Although test weight is a rough index of flour yield in several studies, Labuschagne and Deventer (1995) used as the stepwise linear regression technique for correlate the different quality parameters like test weight, protein content, Farinographic water absorption and vitreous kernels to HMW glutenin subunit composition of soft wheat samples. They observed HMW- GS 17+18 made a relatively small contribution to baking quality, with the exception of biscuit making quality. In contrast, in the present study, HMW-GS 17+18 make a greater contribution to baking quality. Milovanovic et al. (1998) studied the influence of 1BL-1RS translocation on technological quality of winter wheat, they observed sedimentation value is the best index of protein and gluten nature and it is highly positive correlation with the contents of protein and wet gluten. Considering the effect of the different Glu-1 score and SDS sedimentation value, in the present study the Glu-1 score and SDS sedimentation value are positively correlated and this results shows that both the values are important to predict the bread making quality.

Correlation between Glu-1 score and the quality parameters of Osijek wheat cultivars were studied by Jurkovic et al. (2000). Pi- Fen Lin et al. (2003) reported that correlation coefficient between Glu-1 quality score and their corresponding farinographic and extensographic properties. In the above studies a significant positive correlation between Glu-1 score and resistance to extension was noticed. A negative correlation was also found between Glu-1 score and protein content, mixing tolerance index and extensibility. Similar observations were also made in the present study. The results of the present study also revealed that, a positive correlation between Glu-1 quality score and farinographic properties (stability, DDT) and a negative correlation between Glu-1 quality score and extensibility, which implies higher Glu-1 quality score had low extensibility with high strength of
dough and lower Glu-1 quality score had higher extensibility with low strength of dough.

5.2.4. Grouping of wheat varieties based on physical, chemical, rheological and glutenin quality

Based on various important physical properties of wheat grains viz. test weight, 1000-grain weight and grain appearance score; chemical properties of wheat flour viz. protein, fat, ash, wet gluten and SDS sedimentation value; rheological properties of wheat dough viz. water absorption, stability time, quality number, mixing tolerance index, extensibility, area; and glutenin quality in seed proteins are taken together, the studied 50 hexaploid wheat varieties were grouped into 5 different quality groups. The wheat varieties in each group significantly differ with other wheat varieties in several quality parameters. Group one consisting of a total of 13 wheat genotypes, followed by 5 varieties in group two, 22 varieties in group three, 4 varieties in group four and 6 varieties in group five. Physical quality of the grains and chemical composition of the flour, rheological properties of the dough strength and Glu-1 quality score was high in group one; the lower content of ash, fat and mixing tolerance index was prominent in this group. The results indicate that the wheat flour is 'over strong' type in nature. Therefore the varieties in this group were more suitable for blending purpose; that is the flour of these wheats should be mixed with 'weak quality' flour so that the flour quality of these wheat genotypes can be modified and by changing the blending ratio of 'over strong' wheat flour and 'weak flour', any type of end product therefore can be obtained. Blending enhances both milling and baking properties of wheat (Sharma et al., 1988). Wheat varieties in group five characterized with poor quality in grain structure and weight, high content of fat and ash in the flour, high extensibility, mixing tolerance index and low Glu-1 score, makes these
varieties are suitable only for biscuit making. Wheat varieties in group three possessed grains with medium physical quality and medium contents of various other chemical constituents (protein, fat, ash, wet gluten and SDSS). The rheological properties of these wheat doughs were also mid-strength in nature. The Glu-1 score of these wheats ranged from 7-8. All these properties suggest that these wheat varieties were highly suitable for bread and chapatti making. Wheat varieties in group two possessed various quality characters (physical, chemical, rheological, glutenin) which are intermediate to that of group one and three. Therefore, these varieties were more suitable for bread making. Varieties in fourth group possessed characters which are intermediate to that of group three and five. These varieties therefore suitable for chapatti as well as biscuit making purpose.

5.2.5. Effect of additive on the rheological, baking and pasting quality

Additives are substances that are added in small quantities to improve either the processing characteristics or the quality of end products. Food additives are used to assist processing, assuring quality of end products, reducing costs, and to preserve the foods and increase the strength of doughs (Rao et al., 2003). In the present study the effect of the additive Sodium Stearoyl-2-Lactylate (SSL) with different concentrations (0.0, 0.1, 0.2, 0.3, 0.4, 0.5, and 0.6%) was evaluated on various Farinographic and Extensographic parameters of wheat dough derived from three different quality wheat groups namely high, medium and low. The wheat dough from high quality groups is strong type; dough from low quality wheat group is of weak type, while dough from medium quality wheat group is of medium-strong type. The main objective of studying the supplementation of the additive SSL to wheat dough is to improve the quality of the end products viz. bread, chapatti and biscuit. Farinographic data showed a gradual
increase in farinographic water absorption with increase in the level of supplementation of SSL. Kurakke et al. (2004) found that SSL has normal tendency to absorb water due to hydrophobic nature. In the present investigation, the water absorption of supplemented test doughs values are distinctly higher than control. Water absorption enhances the yielding of bread. Such observations were also made in wheat flour supplemented with SSL and soya protein blends (Chen et al., 1982).

Addition of any additive affects the strength of the dough consequently affects the quality of end products. The effect may be positive or negative depending upon the type and nature of the additive (Kamaliya and Patel, 1998). Dough development time and mixing tolerance index value of the supplemented dough gives an idea about the strength (strong and weak) of the dough. In the present study due to addition of SSL there was no significant deviation in dough development time and in mixing tolerance index value indicating that there was no adverse affect with respect in weakening of the dough. These results are in accordance with results of Volpe and Zabik (1981). Any reduction in the strength of the dough severely affects the quality of the dough and ultimately end products. On the other hand, in the present study there was considerably increase in stability time of the dough (only upto 0.4% of SSL), this indicates that there was an improvement in the strength of the dough due to addition of SSL. The additive might have interacted with proteins and starch and thereby enhanced the dough strengthening. Similar results were reported by Chen and Rasper (1982). Being a natural improver and conditioner of gluten, the SSL enhances the quality of Gluten in terms of strength and in extensibility. The extensibility values increased continuously with increase in the level of SSL supplementation; however beyond a particular level of extensibility, which is beyond 100 BU, the wheat dough is not desirable for bread making; however it may be useful for making biscuits. Janssen et al. (1995)
suggested that extensibility exceeding a minimum level for obtaining high loaf volume and a fine crumb structure. The area (cm$^2$) of the Extensographic curve values of supplemented dough gradually increased over the control with the addition of SSL. The results obtained from Farinographic and Extensographic studies, in general, revealed that the addition of SSL up to 0.4% improved the strength and quality of the wheat dough in all the three different quality of wheat varieties.

(A) Effect of SSL on bread-making quality

One of the major objectives in wheat breeding in India is the production of new varieties with improved yield. Not much attention was paid to the breeding wheats for improving bread-making quality. However, in recent years, in India, much interested was generated for the development of wheat varieties with improved yield and bread-making quality.

Baking test is the last and the most important test showing the suitability of wheat varieties for bread making. To make bread, flour is mixed with water, salt and yeast, worked to form dough, rested to allow fermentation and aeration of the dough to take place and oven baked. Universally accepted that gluten, the water and salt-insoluble protein complex of the flour makes a valuable contribution to dough Rheology and bread yield (Wall, 1979).

Surfactants have been found to improve the quality of bakery products and are commonly used in many bakery industries elsewhere. Kawka and Wlad (1999) suggested that surfactants in dough are reported to complex with the protein and carbohydrate fractions of flour to strengthen the extensible gluten-starch. The additions of surfactants at the appropriate levels have been reported to increase the volume, shorter proof time, improve bread texture, increase water absorption shelf-life and taste of
bread. In the present study the addition of SSL increased the loaf volume, crumb texture, bread score shelf- life and taste upto 0.4% level of supplementation for obtaining good quality bread.

Boyacioglu et al. (1994) observed that SSL interacts with both proteins and starch thereby resulting in moderate dough strengthening and improvements in crumb softness and fresh-keeping quality. The production of a well-risen loaf of bread with desirable texture and taste is the result of complex interactions between all the flour components and all ingredients used during the mixing, fermentation and baking. At higher levels of SSL incorporation, the acceptability is reduced due to poor texture of the crumb and undesirable flavour of bread. SSL is also the best disperser of fats in the dough. Therefore, a baker can reduce the quality of fats being used in the products thereby reducing the cost and at the same time getting bread which is softer and full effectiveness of the fat used to the bread.

The effect of other additives in enhancing the quality of bread has been studied by several workers from time to time. For example, Bamidele et al. (1990) obtained high quality bread by blending of 10% blanched plantain and 90% wheat flour. Satisfactory quality bread was obtained by blending of composite flour consisting of 5% full fatted soy flour and wheat flour (Rastogi and Gurmuckh, 1989). Effect of incorporating fermented cassava flour improves the wheat bread quality (Omune et al., 2001). Similarly, a combination of wheat flour and the additive Xanthan gum significantly enhanced the quality of bread (Rao et al., 1993). Guar gum addition significantly improved the overall bread making quality (Rao et al., 1985). All these earlier reports and the present results seem to indicate incorporation of different bread additives with appropriate level could result in bread with more acceptable characteristics.
(B) Effect of SSL on biscuit-making quality

Blending of different additives like, dough improvers and surfactants to wheat flour is reported to change the quality of biscuits derived from it (Leelavathi and Haridas, 1993; Rao and Manohar, 1999). Due to supplementation of SSL to wheat dough improved the density, diameter, crispness and thickness of biscuits; on the other hand the spread ratio was decreased. This is evident that the extensibility curve of the supplemented dough increased over control, which is due to shortening of the glutenin. At higher concentration, the SSL lowered the elastic recovery value and spread ratio indicating their contribution to shortening effect on glutenin, and also resulted in improvement in crispness, thickness and quality of the biscuits. The biscuits made with use of Sodium Stearoyl-2-Lactylate also brought about greater improvement in colour and appearance of the biscuits, crispness, mouth feel and overall quality of the biscuits over the control by producing finer and more uniform pattern of surface cracks due to SSL supplementation. These results agree well with those reported by Leelavathi and Haridas (1993), Chen and Rasper (1982) and Tsen et al. (1971, 1973).

(C) Effect of SSL on chapatti-making quality

Chapattis made from whole flour is generally poor in quality (Austin and Ram, 1971). Incorporation of additives enhances the softness of the dough thereby improving the quality of chapattis. In the present study, puffing of the chapattis was full upto 0.4% level of supplementation, while it was partial beyond this level, this could be due to dilution of wheat gluten. Ebeler et al. (1983) observed that similar results in puffing of the chapattis with supplementation of Isabgol. Sidhu et al. (1989) observed that shortening and addition of surfactants increased the quality of chapatti. In the present study, chapatti made with use of sodium Stearoyl-2-Lactylate particularly at the level of 0.3% and 0.4% level recorded maximum sensory
scores for colour, taste, flavour, and hand feel. Earlier Ahluwalia and Amarjeet (2001) reported that the additive Isabogol recorded maximum sensory scores at 2% of supplementation. Ebeler and Walker (1983) also noticed that with the addition of sucrose ester improved various sensory qualities of chapattis.

(D) Response of different quality wheat doughs to the addition of SSL

The wheat dough used in the present study is obtained from three quality wheat groups; accordingly the dough is strong, medium-strong and weak type. Without the addition of SSL, strong and medium-strong doughs were suitable for bread and chapatti making, while weak dough is suitable only for biscuit making. Due to SSL addition to the dough, weak dough can also now be used for bread and chapatti making. Baking and overall quality of the bread and chapatti derived from strong and medium-strong dough, supplemented with SSL, is very good compare to control. Among these three wheat doughs, the week dough responded better than medium-strong dough, and the medium-strong dough responded better than strong dough.