MECHANISM OF SLOW LEAF RUSTERS, MOLECULAR CHARACTERIZATION IN BREAD WHEAT AND VARIABILITY IN *Puccinia triticina* ERIKS.

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CONTENTS

LIST OF TABLES

	Puccinia tritcina
20	Seedling reaction of *Lr* isogenic lines against thirteen pathotypes of *Puccinia triticina*
21	List of genotypes positive for slow rusting resistance genes at molecular level and their AUDPC values
22	Percent germination of uredospores at different intervals on selected bread wheat genotypes with two *P.triticina* races
23	Percent appresoria formation at different intervals on selected bread wheat genotypes with two *P.triticina* races
24	Effect of leaf rust on yield and quality traits of bread wheat genotypes during *rabi* 2012-13
25	Genotypic correlations of yield and quality traits of bread wheat genotypes during *rabi* 2012-13
26	Effect of leaf rust on starch damage, wet gluten content, dry gluten content and gluten index of selected bread wheat genotypes
27	Effect of leaf rust on micronutrients of slow rusters in comparison with resistant and susceptible selected bread wheat genotypes during *rabi* 2011-12
28	Genotypic correlations between micronutrients and disease observations
29	Effect of chemical treatments on leaf rust severity, thousand grain weight and yield during *rabi* 2012-13

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

INTRODUCTION

Bread wheat an important cereal crop in global agricultural economy is cultivated in a range of mega environments of the world. It is the most widely grown and consumed food crop of the world cultivated on a larger area and produce more tonnage of food than any other cereal.

In Indian subcontinent wheat has been under cultivation from pre-historic times which dates back to indus vally civilization. It has been established that the carbonized grain belongs to a wheat species *Triticum spherococcum* Mihi. popularly known as Indian dwarf wheat, which is almost vanished now. Presently, three wheat species namely *T. aestivum* L. em. Thell (Bread Wheat) *T. durum* Desf (kothia, macroni wheat) and *T. dicoccum* (Schrank) Schubler (Khapli, Sadaka or Emmer wheat) are commercially grown in different parts of the country.

The world acreage under wheat crop is 240 million ha with production of 600 m. tonnes, with an average yield of 2717 kg/ha. In India it is grown in an area about 29.90 m. ha with production of 93.90 m. tonnes with an average productivity of 3140 kg per hectare (Anon, 2012). It is one of the important *rabi* cereals in Karnataka wherein all three cultivated species *viz., T. aestivum, T. durum* and *T. dicoccum* are grown in an area of 2.30 lakh ha with a production of 1.94 lakh tonnes with low productivity of 843 kg/ha as compared to the national average 3140 kg/ha (Anon, 2012), which is attributed to the fact that more area (60%) is grown under rainfed (Hanchinal, *et al.,* 2011) as well as biotic stresses such as rusts and abiotic stresses such as heat and drought causes heavy losses in yield.

Diseases are the major threats to wheat production in the country and they are taking heavy toll of the crop. According to Pal (1966), among all the factors affecting yield, none is so important as the diseases.

Wheat like any other crop, suffers from many diseases caused by fungi, bacteria, viruses and nematodes. However, the diseases caused by fungi are responsible for taking heavy toll of the wheat crop in the country. Among the major diseases of wheat are the three rusts *viz*., leaf (brown) rust (*Puccinia triticina* Eriks.), stem (black) rust (*Puccinia graminis* Pers.:Pers. = *P. graminis* Pers.:Pers. f. sp. *tritici* Eriks. & E. Henn.) and stripe (yellow) rust (*P. striiformis* Westend.). The other major diseases are spot blotch, flag smut, Karnal bunt, hill bunt and loose smut.

Among the rusts, the leaf rust (brown rust or orange rust) caused by *P. triticina* is known to occur in all the wheat growing areas of the world, and its importance has been studied since many decades (Bhardwaj *et al*., 2011). Saari and Prescott (1985) considered leaf rust to be the most serious of the rusts and universal in occurrence. The “Sonalika epidemic” of the leaf rust which flounced over the entire Uttar Pradesh and part of Bihar in 1980 has caused loss of one million tonnes (Joshi *et al.,* 1984). Wheat rusts have played significant role in the agriculture of early civilization and today, there is a well documented account of their ability to cause epidemics throughout the world. In epidemic years it can cause economic losses (Nayar *et al*., 2002).

Karnataka plays an important role in wheat rust epidemiology. It serves as donor of stem and leaf rust inocula to different parts of the country (Kulkarni, 1984). Studies indicated that stem and leaf rust occurs on off-season wheat sown in the district of Chikmagalur of Karnataka (Kulkarni, 1986; Navi, 1986 and Jalinder *et al.,* 1989). Leaf rust virulence survey since many decades reveals that, the population is highly variable leading to the evolution of new pathotypes through mutation and rarely through somatic hybridization (Bhardwaj *et al*., 2005).

It is necessary to conduct a systematic survey and surveillance of the disease so that, it's distribution and extent of its spread can be understood and epidemic areas or hot spots may be identified, which would help for screening of genotypes for resistance under natural conditions. Further, such studies would help in creating genetic barriers or deployment of resistance genes across the "Puccinia path" or designing suitable "integrated management practices". It also gives information about existence of physiologic races in particular agro-climatic zones. Existence of variability in rust pathogens makes it necessary to have an effective system of virulence analysis.

The dearth of molecular information related to the inability to culture *P. triticina in vitro* and the relatively large genome size estimated to be 100–124 Mbp (Eilam *et al*., 1994). The recent development of Simple Sequence Repeats (SSR) molecular markers for *P. triticina* (Szabo and Kolmer, 2007) has made it possible to determine multilocus dikaryotic genotypes for population studies of this pathogen. Previously, random amplified polymorphic DNA (RAPDs) and amplified fragment length polymorphism (AFLPs), which are dominant markers, have been used to characterize

populations of *P. triticina* in North America (Kolmer, 2001) and Europe (Park *et al.,* 2000). The information on molecular diversity of *P. triticina* is very less in India and hence, SSR molecular markers are being used to identify the molecular diversity of *P. triticina* populations collected from wheat growing areas of Karnataka.

Economic losses estimates due to rusts have been published from time to time. Mehta (1941) reported the losses upto Rs. 60 Millions annually and Prasada (1965) estimated the losses of Rs. 392 Millions in India. The extent of yield losses caused by leaf rust depends on the nature and number of leaf rust resistance genes in the cultivars and the composition of virulence in the respective geographical region (McIntosh *et al.,* 1995).

The most environmentally sound, low cost method of controlling leaf rust is to breed and grow resistant wheat varieties. To date, 71 leaf rust resistance genes in wheat have been mapped to chromosome location and given gene designations (McIntosh *et al*., 2010). In addition, a number of temporarily designated resistance genes and quantitative loci (QTLs) are able to provide total or partial protection against various rust pathotypes (McIntosh *et al*., 2008). The effectiveness of resistance genes depends on the composition of the pathogen population. As this changes dynamically, new pathotypes virulent to the given resistance gene multiply from time to time, so the resistance of a variety is not a constant trait. Any variety carrying a single resistance gene may become susceptible within a short time.

The search and use of durable resistance (minor gene resistance, slow rusting resistance, non-specific resistance, and adult plant resistance are terms that have been used interchangeably in the literature. For the convenience of the reader, the term slow rusting resistance throughout this thesis has been used) in the germplasm, landraces and cultivated varieties is an important management strategy to get rid of most widely influential and shifty enemies like leaf rust pathogen. The mounting or growing of slow leaf rusters is an outstanding tactic to reduce the disease incidence as the slow leaf rusters are characterized by slow progress of the disease, which endow with very less terminal disease severity and also reduces the extent of losses due to leaf rust. It is imperative to identify the slow leaf rusting genotypes based on various parameters of slow rusting mechanism along with molecular level confirmation of reported slow rusting genes.

The quality of wheat grains deteriorate by several factors and diseases contribute more in deteriorating the quality of wheat grains. The quality and productivity of wheat is often reduced because of leaf rust caused by *P. triticina*. The effect of leaf rust on quality parameters such as protein content, sedimentation value, mineral nutrients, damaged starch, glutin analysis and yield directs the producers to manage the disease and to influences fetch higher market price of the produce.

In the concept of slow rusting we may have to allow the pathogen for their slow growth and development, in such situation it is highly necessary to estimate loss of mineral nutrients from wheat grain. Currently, mineral malnutrition is considered to be among the most serious global challenges to humankind and is avoidable (Copenhagen Consensus 2004; http://www.copenhagenconsensus.com). The main emphasis is to identify varieties with slow rusting characteristics as well as acceptable end use quality without compromising crop productivity.

In view of these facts, present investigation was carried out to study the “Mechanism of Slow Leaf Rusting in Bread Wheat and Variability in *Puccinia triticina* Eriks.” during *rabi* 2011-2012 and 2012-13 at All India Co-ordinated Wheat Improvement Project, Main Agricultural Research Station (MARS), University of Agricultural Sciences (UAS), Dharwad (Karnataka) with the following objectives:

1. Survey, surveillance and race identification of wheat leaf rust in wheat growing region of Karnataka.
2. Study of genetic diversity in *Puccinia triticina* population of Karnataka through molecular techniques.
3. Identification of wheat slow leaf rusters.
4. Evaluation of identified slow leaf rusters for quality traits.
5. Management of leaf rust through chemicals.

REVIEW OF LITERATURE

Wheat crop in India suffers from several diseases which cause substantial losses in yield as well as grain quality. Out of various biotic stresses known to affect this crop, the rusts have been regarded as the most dreaded pathogens. Among all the three rusts *viz*., the leaf rust (*P. triticina)*, stem rust (*P. graminis* f. sp. *tritici*) and stripe rust (*P. striiformis*), the leaf rust is prevalent throughout in India whereas stem rust prevalent in southern India while stripe rust in northern India. The earlier work on leaf rust of wheat is reviewed in brief under the following subheads.

2.1 Survey, surveillance and race identification of leaf rust in wheat growing region of Karnataka

Since the discovery of the pathogenic variability within the fungus by Eriksson (1894), race surveys have been carried out in the major wheat growing areas of the world. It provides information on new rust genotypes, track changes in the frequencies of races and of virulence on specific resistance genes (Knott, 1989).

Mehta (1941) reported six races *viz*., 15, 21, 24, 40, 42 and 75 from the 586 collection of samples of black rust; six races of brown rust (10, 20, 63, 106, 107 and 108) from 408 collections.

Among plant pathogens, *P. triticina* has a relatively long history of population studies, with nationwide race surveys for leaf rust beginning in US during 1926 (Johnston *et al*., 1968), in Canada during 1931 (Johnson, 1956) and in Australia during 1920 (Waterhouse, 1952).

The wheat cultivars Malakof (*Lr1*), Webster (*Lr2*a), Carina (*Lr2b, LrB*), Loros (*Lr2c*), Brevit (*Lr2c, LrB*), Hussar (*Lr11*), Democrat (*Lr3*) and Mediterranean (*Lr3*) were designated as the International Standard set of leaf rust differentials and used in the early race identification studies. By 1932, 26 races had been identified in US (Johnston and Mains, 1932). The International Standard differentials were also used for the initial leaf rust race studies in Europe (Chester, 1946). In Australia, these differentials did not adequately identify leaf rust races (Waterhouse, 1952).

The development of gene-for-gene theory by Flor (1942) and discovery of isogenic lines for rust resistance (*Sr, Lr, Yr*) the systems of virulence analysis were changed in many countries. In India, Nagarajan *et al.* (1983) proposed brown rust of wheat (*P. recondita* f. sp. *tritici*) virulence monitoring system, where, selected wheat lines were categorized into three sets of differentials such as O, A and B. In India, the procedure is tailored on the pattern of binary notation system proposed by Habgood (1970) wherein virulence is quantified.

Browder and Eversmeyer (1977) calculated frequency of virulence on each gene and each pair of gene and define two coefficients as pathogenicity association coefficient and virulence association coefficient.

Kulkarni (1978 and 1979a) reported common leaf rust race flora as 12, 77, 77A, 77B, 162 and 162A at different locations in Karnataka. Pathogenicity survey after 1980 revealed the dominance of 77 and 104 race group (Nayar *et al.,* 1985). Farmers grow wheat crop during the off season in the plains of Chikmagalur and Chithradurga districts of Karnataka were heavy incidence of stem rust and less incidence of leaf rust have been reported (Kulkarni, 1984 and Hegde, 1991).

Nagarajan and Joshi (1985) concluded that once the leaf rust of wheat appears, subsequent development is dependent on prevailing local weather conditions *viz.,* temperature and relative humidity. The component of parasitic population may be genetically competent however their reproductive potential will also be determined by temperature, humidity and other factors (Bahadur, 1986).

Joshi (1986) enumerated the sequence of virulence population shifts in the pathogen into four categories, as effect of wheat without resistance (1931-45), with vertical resistance (1946-55), with combined resistance (1956-65) and dwarf wheat with complex resistance (1966-80). Races of leaf rust pathogen, 12 and 77 remained prevalent throughout the period of 1965 to 1980, while 162 in 1965-75, 162A in 1966-70 and 104 in 1971-80, dominated the population at national level (Joshi, 1986). Nargund (1989) found that, the leaf rust races *viz.,* 77(45R31), 77A (109R31) and 77A-1(109R23) were the most predominant in Karnataka during rabi 1987-88.

Bahadur *et al.* (1994) proposed revised strategy for gene deployment following effectiveness of different vertical resistance genes and distribution of avirulence / virulence combinations.

Nagarajan and Muralidharan (1995) explained appearance of pathotypes towards higher virulence with matching complex resistance in the host. Pathotypes 77-1 to 77-5 appeared in nature with gaining additional virulence towards *Lr* 23 and *Lr* 26. Singly or in combination over previous 77A pathotype. The cultivars Gaza (*Lr23*), Thew (*Lr20*) and other cultivars were used to supplement the International Standard differentials in Australian studies (Park, 1996).

Regional populations of *P. triticina* races can arise within a continent due to use of wheat cultivars with different leaf rust resistance gene composition in different regions. Winter wheat cultivars with *Lr3* and *Lr26* have been grown in central Europe, where races with virulence to *Lr3*, *Lr3bg*, *Lr3ka* and *Lr26* were generally avirulent to these resistance genes (Park and Felsenstein, 1995).

Hasabnis (1998) surveyed different districts in Karnataka and Maharastra states during 1996-97 and 1997-98 for virulence monitoring. He reported that, pathotypes from group 77 were widely distributed in surveyed areas. Kiyar (2002) reported that race group 77, 104 and 12 with frequency of 40 per cent, 30 per cent and 30 per cent respectively were found in leaf rust samples collected from farmers fields of Dharwad and Belgaum districts of Karnataka.

There was also evidence of long-distance movement of *P. triticina* in Europe, as only four races accounted for 64% of tested isolates. In 1998, 105 races were identified in France, Hungary, Italy, Bulgaria and Poland (Mesterhazy *et al*., 2000), with very few races in common between the countries. Isolates of *P. triticina* were collected from wheat leaf collections by co-operators throuout the United States and from surveys of wheat fields and nurseries in the Great Plains, Ohio valley and Gulf coast states in 1996 to 1998. They were found that, 31 phenotypes among 277 single uredial isolates in 1996, 56 phenotypes among 989 isolates in 1997 and 43 phenotypes among 989 isolates in 1998 (Long *et al*., 2000).

Singh *et al*. (2004b) reported that a new race, designated as BBG/BN, was detected that caused the most widely grown cultivar, Altar C84, which had remained resistant for 16 years, to become susceptible. Other recommended cultivars also became either moderately susceptible or susceptible. Similarly, in India Bhardwaj *et al*. (2005) reported a new pathotype of *P. triticina* virulent on *Lr19* and observed that virulence on *Lr19* (Agatha T4 line) was in approximately 2 per cent of leaf rust samples. These samples were picked from *Lr19* (NIL), cvs. Ajit, Lal Bahadur, Local Red, Lok1 and Nirbhay from Karnataka and Gujarat states. All *Lr19* virulent isolates were identical and this pathotype was detected in 6.3 per cent of samples from central and peninsular India.

Kalappanavar and Hegde (2004) reported that during 1994-95, eight leaf rust races were recorded, among them races 5R37 (12-1) and 21R55 (104-2) were most predominant with less incidence of race 121R63-1 (77-5). In 1995-96 races 21R55 (104-2) and 1R5 (12-2) were occurred in severe form. During 1996-97 and 1997-98 race 121R63-1 (77-5) was most predominant and detected from maximum samples collected from the farmers' field. Only in 1997-98, the leaf rust epidemic was noticed on DWR-162, which was cultivated on maximum area in Karnataka. The race detected was 121R63-1 and it has combined virulence on *Lr23* + *Lr26* genes present in the wheat variety DWR-162. During the years 1998-99 and 1999-2000 too only two leaf rust races recorded. Both the races namely, 121R63-1and 21R55 were becoming predominant.

Leaf rust virulence survey since many decades reveals that, the population is highly variable leading to the evolution of new pathotypes through mutation and rarely through somatic hybridization (Bhardwaj *et al*., 2005).

Kalappanavar *et al.* (2006) conducted survey in Karnataka from 1996-97 to 2005-06. They observed that, races 17R63, 29R23, 21R63 and 121R55-1 were prevalent during 2001-02 and 2002-03 respectively. Race 121R55-1 was new pathotype detected in few samples during 2002-03. In the year 2003-04 and 2004-05 the race 121R63-1 was most predominant. In the year 2004-05 a new pathotype (253R31) was reported from few samples. The other leaf rust races reported during the year were 21R55, 109R63 and 5R37. During 2005-06, seven leaf rust races were recorded. However, race 253R31 was most predominant followed by 121R63-1. Race 253R31 has the virulence on *Lr19*. A new pathotype 5R45 (12-6) was identified on one sample. In 2006-07, few leaf rust races picked up from farmer's fields. However, 21R63 (104-3), 121R63-1 (77-5) and 21R55 (104-2) were most predominant in the field. In 2007-08 along with predominant races of 2006-07, other races like 29R45 (12-5), 69R13 (12-4) and 5R13 (12A) were also most predominant. Races like 93R7 (162) and 93R47 (162-1) were less prevalent during 2007-08 but became most predominant during 2008-09. These races are most virulent on durum. Two new races like (12-7) and (12-9) were identified and were virulent on *Lr 28* (Kalappanavar *et al.,* 2007).

Wheat leaf rust pathogen *P. triticina* populations world-wide are highly diverse for virulence phenotypes or races. Seventy different leaf rust races have been identified on an annual basis in the US on 20 differential lines (Kolmer *et al*., 2007). In France, 30–50 races are identified annually (Goyeau *et al*., 2006). In Australia, 10–15 races are detected on an annual basis (Park, 1996). In the US, virulent leaf rust races increase very quickly in response to widespread use of wheat cultivars with race-specific resistance genes. As the leaf rust population is very large, it would be expected that random mutations occur in sufficient numbers, which would lead to the development of virulent races. In Australia, race-specific resistance genes have retained effective resistance for longer periods. This is probably due to the presence of fewer susceptible cultivars, which greatly reduces the population size of *P. triticina* and thus reduces the likelihood of virulent mutations being selected.

Terefe *et al.* (2009) conducted a survey during 2007 to determine the occurrence and pathogenicity of *P. triticina* Eriks. in South Africa and detected that five pathotypes out of 80 leaf rust samples collected. The most frequently detected pathotype was 3SA133 (76.8%) which was found in samples from all the localities followed by pathotype 3SA126 (11.0%). Other pathotypes detected were 3SA140 (7.3%), 3SA132 (3.7%) and 3SA137 (1.2%).

In India, Bhardwaj *et al*. (2011) reported two new pathotypes of *P. triticina* designated as 125R28 and 93R37 are described and stated that the brown rust pathogen is fast evolving with 23 pathotypes identified during the last 21 years. With these pathotypes there would be 10 pathotypes in 12 group and 13 in 77 group. Among these 9 pathotypes, 4 of 12 and 5 of 77 groups of pathotypes have combined virulence for both *Lr*23 and *Lr*26.

Kalappanavar (2010) analyzed and presented that pathotype 77-5 (121R63-1) was the most wide spread and frequent in Chhattisgarh, Gujarat, Karnataka, Madhya Pradesh, Maharashtra, Rajasthan and Tamil Nadu accounting for 41.5 % of the samples. From 2005-06 to 2008-09 proportion of pathotype 77-8 (253R31) virulent on *Lr* 19 has increased in Karnataka and Maharashtra. However, 77-5 (121R63-1) was most frequent in most of the states. Pathotype 77-5 (121R63-1) was found wide spread in Wellington followed by 77-6 (121R55-1), 77A (109R23), 77-1 (109R63). Pathotype 77-8 (253R31) virulent on *Lr* 19 was traced in samples from Karnataka, Maharashtra and Madhya Pradesh during 2006-07 also. Two new pathotypes 77-9 and 77-10 (virulent on *Lr*28) were identified during the year (2007-08) from Wellington, Karnataka and Maharashtra.

In India, realizing the importance of crop health monitoring, regular wheat disease surveys were started during 1967. These surveys were carried out through mobile units and trap nurseries which generated considerable information on the appearance of rusts in different parts of the country. The survey and surveillance programme was strengthened during 1995 through an AP-cess fund project on survey and surveillance for pests and diseases with DWR as the nodal centre and four other zonal *viz*., Ludhiana (NWPZ), Kanpur (NEPZ), Powarkheda (CZ) and Pune (PZ). Extensive surveys were conducted and pest profile was prepared. During 1995, a Wheat Crop health Newsletter was started from DWR, Karnal on monthly basis during the crop season (Anon., 2011).

Park *et al*. (2011) stated that surveillance of wheat rust pathogens, including assessments of rust incidence and virulence characterization via either trap plots or race (pathotype) surveys has provided information fundamental in formulating and adopting appropriate national and international policies, investments and strategies in plant protection, plant breeding, seed systems and in rust pathogen research. Recent survey and surveillance programme of Directorate of Wheat Research (DWR), Karnal has shown that there is a decrease in incidence of stem rust across the country whereas, increased incidence of leaf and yellow rust (Aggarwal *et al*., 2011).

Kumar *et al*. (2012) reported three pathotypes *viz*., 77-5, 77-7 and 77-8 of brown rust (*Puccinia triticina*) and two pathotypes *viz.,* 40A and 40-1 of black rust (*P. graminis* f. sp. *tritici*) of wheat prevailed in Nilgiri hills. Pathotype 77-5 and 77-8 prevailed in almost equal proportions followed by race 77-7 in brown rust. Pathotype 78S84 of yellow rust (*P. striiformis*) also existed in Nilgiris. Nallathambi *et al.* (2012) conducted systematic surveys in Thottapetta, Arakadu, Mynala, Kenthorai, Thummanahatty and Upathali area of Nilgiri hills in Tamil Nadu during June and July, 2012 and found different species of *Barberry* with rust pustules. Among 22 rust infected samples collected from *Barberry*, they could able to isolate infective nature of only five isolates on *T. dicoccum* and *T. aestivum* and also they were observed both brown and black rust pathogens (uredinospores) under microscope then purified further by using susceptible lines of wheat.

More than 426 samples of brown rust of wheat were analysed from 14 states of India, Bhutan, Nepal and Bangladesh. Among the 23 pathotypes identified in the year 2012, pathotype 121R63-1 (77-5) was most widely distributed and was observed in all the areas followed by pathotypes 21R55

(104-2), 21R63 (104-3). These pathotypes constituted more than 80 percent population of *P. triticina* in this part of Asia. Pathotype which was recorded in few samples was 121R60-1 (77-9) which occurred only in three states of India and Bangladesh. Pathotype 121R55-1(77-6) was also identified in 15 samples from four states of India. Other pathotypes were observed in few samples only. In Nepal and Bhutan pathotype 121R63-1(77-5) which was predominant in India. In Nepal, 5 pathotypes were identified in 21 samples. Likewise, in Bangladesh, 8 pathotypes were identified in 18 samples, of which 21R63 (104-3) was most common. It is evident from these results that India, Nepal, Bangladesh and Bhutan fall in one epidemiological zone (Anon., 2012a).

Analysis of 213 samples of rusts of wheat and barley indicated that there was no occurrence of new pathotypes upto January 2013. Pathotype 40A followed by 40-1 of black rust, 46S119 followed by 78S84 of yellow rust and 77-5 followed by 104-2 of brown rust were predominant in wheat growing areas of India and neighboring countries (Anon., 2013).

2.2 Study of genetic diversity in *Puccinia triticina* population of Karnataka through molecular techniques

Leaf rust of wheat is the most widely distributed and normally causes more losses than any other rust of wheat in India. It is observed in all the wheat growing areas. Because of its widespread occurrence in India (Bhardwaj *et al*., 2006), it is probably the most variable pathogen in India (Bhardwaj *et al*., 2011).

The global leaf rust population varies in virulence and this variation may result from one or more factors like weather and host cultivars carrying specific resistant genes (Roelfs, 1988). Thirty-seven distinct virulence phenotypes were identified using the Thatcher lines and 69 molecular phenotypes were identified with 164 AFLP markers. The presence of distinct groups of isolates based on virulence and AFLP variation provides evidence that a number of different *P. triticina* phenotypes have been introduced to North America (Kolmer, 2001).

The dearth of molecular information related to the inability to culture *P. triticina in vitro* and the relatively large genome size estimated to be 100–124 Mbp (Eilam *et al*., 1994). As *P. triticina* relies upon clonal reproduction nearly everywhere in the world, an effective linkage between virulence and molecular markers is maintained. However, in an experimental greenhouse population of *P. triticina* derived from aeciospores, disequilibrium between individual virulence genes and RAPD markers was often eliminated or reduced (Liu and Kolmer, 1998).

Molecular markers such as RAPDs and AFLPs have been used to characterize variation in *P. triticina* populations. Different groups of *P. triticina* isolates in Canada and from international collections could be distinguished with RAPD and AFLP markers that also correlated with grouping based on avirulence/virulence to single gene differential lines (Kolmer, 2001; Kolmer and Liu, 2000). In Europe, multiple isolates of the same race from different countries had identical RAPD banding patterns (Park *et al*., 2000).

Introductions from distant sources of new races with virulence to resistance genes in commonly grown wheat cultivars can occur. Races with virulence to *Lr17*, *Lr3bg* and *LrB* became common in the southern Great Plains of the US in the mid 1990s. Genetic analysis with AFLP markers indicated that these races were most likely introduced to the Great Plains region from either Mexico or the Pacific Northwest and were not derived by mutation from the previously existing populations (Kolmer, 2001). In Australia, a new race that was distinct for virulence to genes *Lr16*, *Lr27* and *Lr31* was detected for the first time in 1984 and was probably introduced from another continent (Park *et al*., 1995).

Recently, locus-specific microsatellite or SSR markers have been developed for *P. triticina* (Duan *et al*., 2003; Szabo and Kolmer, 2007). SSR markers are co-dominant and can distinguish between heterozygote and homozygote genotypes, in contrast to RAPD and AFLP markers that are dominant. *P. triticina* populations studied with SSR markers have several attributes in common, including higher levels of heterozygosity than expected compared with populations in Hardy–Weinberg equilibrium and high levels of linkage disequilibrium and they are genetically differentiated by continental region (Kolmer and Ordonez, 2007) or due to selective effects of resistance genes in wheat cultivars (Goyeau *et al*., 2007). All populations of *P. triticina* that have been examined with SSR markers (Goyeau *et al*., 2007; Kolmer and Ordonez, 2007) have genetic characteristics typical of clonal diploid or dikaryotic populations in which high levels of heterozygosity are maintained by sequential mutation in the absence of recombination (Balloux *et al*., 2003; Halkett *et al*., 2005).

Somatic recombination has been reported in *P. triticina* (Park *et al*., 1999), although the rates of such variation remain unknown.

Keiper *et al*. (2003) assessed the high multiplex DNA fingerprinting techniques, amplified fragment length polymorphisms (AFLP), selectively amplified microsatellites (SAM) and sequence-specific amplification polymorphisms (S-SAP) for their potential in investigations of the genetic relationships among isolates of wheat rust pathogens, *P. graminis* f. sp. *tritici*, *P. triticina* and *P. striiformis* f. sp. *tritici*, the oat stem rust pathogen *P. graminis* f. sp. *avenae* and a putative new *P. striiformis*. Marker information content, as indicated by the number of species-specific fragments, polymorphic fragments among pathotypes, percentage of polymorphic loci and the marker index were highest for the SAM assay, followed by the AFLP and S-SAP assays. Within pathogen groups, the marker types differed in the amount of variation detected among isolates. Of the three marker types, SAM was the most informative and have the potential for the development of locus-specific microsatellites.

Moghaddam *et al*. (2004b) first developed protocol for extracting DNA adopting a chloroform: phenol free method involving only 30-50mg quantity of urediospore and also suggested that DNA could effectively be used for polymorphism analysis among the leaf rust pathotypes. They first time attempted with ten random primers and distinguished all the 12 leaf rust pathotypes clearly.

Kosman *et al.* (2004) observed that *Lr26* virulent rust pathotypes are as geneticaly dissimilar as the rest of the population. The cluster analysis showed that the rust population in Israel includes atleast two subpopulations. Both of which contain *Lr26* virulent and *Lr26* avirulent isolates. The results indicated that the leaf rust population in central Ethiopia is genetically distinct and this might be related to the predominant cultivation of durum wheat cultivars in this area (Mebrate *et al*., 2006).

The high degree of virulence and simple sequence repeat (SSR) genotypic similarity between *P. triticina* isolates from durum wheat in Europe, South America, Mexico and California suggested that *P. triticina* populations on durum wheat in these regions originated from a single original founder population (Ordonez and Kolmer, 2007).

Long-term monitoring of pathogenic variability in wheat rust pathogens including *P. graminis* f. sp. *tritici* across the Australian region has shown clearly rapid and unimpeded rust migration within this region and provided arguably the best evidence supporting periodic long-distance intercontinental spread of wheat rust pathogens (Watson and de Sousa, 1982; Wellings, 2007).

Szabo (2007) developed twenty-four dinucleotide simple sequence repeat markers for the phytopathogenic fungus, *P. graminis* f. sp. *tritici*. The identified loci were polymorphic, with allelic diversity ranging from 2 to 11 alleles. Observed and expected levels of heterozygosity ranged from 0.000 to 0.960 and from 0.113 to 0.846, respectively. Fourteen of the loci deviated significantly from Hardy–Weinberg equilibrium. Null alleles were observed for 10 of the 24 loci with a frequency of 4–16%. A preliminary screen of other *Puccinia* cereal rust fungi (*P. coronata, P. striiformis* and *P. triticina*) indicated that these primer pairs are specific to *P. graminis* f. sp. *tritici*.

The high degree of similarity for SSR genotype of isolates from both South America and North America suggested a common European origin of *P. triticina* that was introduced to both continents. The emergence of the same *P. triticina* virulence phenotypes with highly related SSR genotypes in the United States in 1996 and in Uruguay in 1999 indicated the likely intercontinental migration of these genotypes from Mexico to both South America and North America (Ordonez *et al*., 2010).

Ordonez and Kolmer (2009) collected in total, 148 isolates of *P. triticina* from 1980s to 2005 from wheat-growing regions of the United States and Canada and tested for virulence on 20 lines of wheat with single genes for leaf rust resistance and for molecular genotype with 23 simple sequence repeat (SSR) markers. In total, 91 virulence phenotypes and 65 SSR genotypes were found. After removal of isolates with identical virulence and SSR genotypes, 125 isolates were included for further analysis.

Mantovani *et al*. (2010) characterized twenty-four isolates of *P. triticina* from Italy for virulence to seedlings of 22 common wheat Thatcher isolines, each with a different leaf rust resistance gene and for molecular genotypes at 15 simple sequence repeat (SSR) loci. The isolates were compared to a set of 13 previously characterized *P. triticina* isolates from either durum or common wheat. Clustering based on virulence phenotypes and SSR genotypes grouped the Italian *P. triticina* isolates into three groups.

Wang *et al*. (2010) developed gene-associated simple sequence repeat (SSR) markers for *P. triticina* through the data mining of existing EST libraries and they analysed of 7134 expressed sequence tags (ESTs) from cDNA libraries of *P. triticina* detected 204 EST-SSRs with a minimum of 12 repeating nucleotides. These EST-SSRs were evaluated on 35 *P. triticina* isolates collected in Canada and 21 EST-SSRs were polymorphic and informative in determining intraspecific genetic diversity. A comparison of virulence and EST-SSR genotypes showed a strong correlation between virulence to *Lr2a*, *Lr2c* and *Lr17a* and EST-SSRs genotypes. The differentiation of the *P. triticina* population based on EST-SSR genotypes was comparable to that obtained with genomic SSRs, despite differences between two types of SSR markers. They suggested that the data mining of EST databases is a feasible way to generate informative molecular markers for genetic studies of *P. triticina*.

To support gene discovery and gene model verification in the genome of the wheat leaf rust fungus, *P. triticina*, Xu *et al*. (2011) were generated Expressed Sequence Tags (ESTs) by sampling several life cycle stages.

Kolmer *et al*. (2011) collected in total, 118 isolates of *P. triticina* from common wheat and durum wheat in Egypt, Israel, Turkey, Ethiopia and Kenya were tested for virulence on 20 lines of wheat with single genes for leaf rust resistance and for molecular genotypes with 23 simple sequence repeat (SSR) markers. Clustering of SSR genotypes based on two-dimensional principal coordinates and virulence to wheat differential lines grouped the isolates into four Middle East (ME) groups. All pairs of ME groups were significantly differentiated for SSR genotype.

Singh *et al*. (2011) reported the abundance and inherent potential for extensive allelic variations in simple sequence repeats (SSRs) or microsatellites resulted in valuable source for genetic markers in eukaryotes. They analyzed and compared the abundance and organisation of SSR in the genome of two important fungal pathogens of wheat leaf rust (*P. triticina*) and stem rust (*P. graminis* f. sp. *tritici*). *P. triticina* genome with two fold genome size as compared to *P. graminis* f. sp *tritici* has lower relative abundance and SSR density. The distribution pattern of different SSR motifs provides the evidence of greater accumulation of dinucleotide followed by trinucleotide repeats. More than two-hundred different types of repeat motifs were observed in the genomes. The longest SSR motifs varied in both genomes and some of the repeat motifs are found in higher frequency. The information about survey of relative abundance, relative density, length and frequency of different repeat motifs in *Puccinia* sp. are useful for developing SSR markers that could find several applications in analysis of fungal genome such as genetic diversity, population genetics, race identification and acquisition of new virulence (Wang *et al*., 2010 and Singh *et al*., 2011).

Kolmer (2013) reviewed that cereal rust fungi are highly variable for virulence and molecular polymorphism. Leaf rust is the most common rust of wheat on a worldwide basis. Many different races of *P. triticina* that vary for virulence to leaf rust resistance genes in wheat differential lines are found annually in the US. Molecular markers have been used to characterize rust populations in the US and worldwide.

2.3 Identification of slow leaf rusters

Availability and utilization of only one known leaf rust resistance gene (*Lr34*) in India conferring durable resistance is a major limitation in wheat breeding, therefore; more genes conferring such resistance need to be searched from various germplasm collections. Characterization of novel sources of durable resistance and accelerated breeding in conjunction with elucidating the basis of resistance would provide at least, a sustainable resistance management strategy. Hence, the present study was undertaken to identify new sources of slow leaf rust resistance in Indian bread wheat genotypes. Related earlier findings were reviewed as follows,

The term slow leaf rusting has been first used by Caldwell *et al*. (1970) for brown rust resistance of wheat. Slow rusting resistance is characterized by a reduced rate of epidemic development, despite a compatible host-pathogen interaction (Caldwell, 1968; Parlevliet and Van Ommeren, 1988; Parlevliet, 1975; Rubiales and Niks, 1995). Therefore, a cultivar that only has slow rusting resistance to leaf rust will display susceptible infection-type responses throughout the entire life cycle of the plant (Rubiales and Niks, 1995). Slow rusting resistance can be measured in the field by recording disease severity at weekly intervals and then calculating the area under the disease progress curve (AUDPC) (Wilcoxson *et al*., 1975). This type of resistance is characterized by the combined effect of a longer latent period, smaller uredinium size, lower receptivity (i.e., lower infection

frequency) and reduced spore production are known as slow rusting components (Ohm and Shaner, 1976; Nayar *et al*., 2003).

Dyck and Samborski (1974) reported that cultivar background can affect the expression of resistance genes. Gene *Lr2b* in a Prelude background was partially dominant in crosses with Thatcher and completely dominant in crosses with Red Bobs. The *Lr2c* allele in Prelude was recessive in crosses with Thatcher and dominant in crosses with Prelude and Red Bobs. The *Lr2* alleles expressed most resistance in the Thatcher background and least resistance in Red Bobs. Similar differences were noted for *Lr3* in Thatcher and Red Bobs. Pretorius *et al.* (1990) noted background effect on the expression of *Lr22a*.

Buchenau (1975) reported relationship between yield loss and AUDPC of stem and leaf rust of wheat. The whole culm of area under disease progress curve was highly correlated with the yield in every test where treatment affected yield.

Mackenzie (1976) noticed reduced spread and slow rate of increase in stem rust of Bonaza 55 compared to the other wheat varieties, which is the characteristics feature of slow rusting and proposed the application of these relationships to the identification of slow rusting mechanism. The leaf rust infection started from third march in WL-711, C-306, CPAN-1676, Lal Bahadur and DWL-5023 and rust intensity reached to 60, 40, 80 and 20 respectively. Whereas, cultivars NI-5439 and HD-2255 remained resistant. (Thombare, 1981).

Sharma and Gupta (1986) reported that wheat cultivars VL-422 and Sonalika were found to be slow rusters against two Indian cultures of *Puccinia recondita*, IL 005 and IL 007, at the adult plant stage, showing lower average disease score and maximum rust severity compared to cultivar CPAN 1425. This slow rusting could not be detected in seedlings of these cultivars.

The use of cultivars with single-gene resistance permits the selection of mutations at a single locus to render the resistance effective in a relatively short time. However, due to selection pressure and evolution, new virulent races of the fungus appear which increase the need to develop durable resistance. Hence, the use of combinations of genes, irrespective of whether they are major or minor, has been suggested as the best method for genetic control of leaf rust. The South American cultivar Frontana is considered to be one of the best sources of durable resistance to leaf rust (Roelfs 1988).

Singh and Rajaram, (1992) Stated that slow rusting is characterized by slow disease development in the field despite a high infection type and involves a longer latent period, a low infection frequency, smaller uredial size and reduced duration of sporulation and less adult-plant resistance (APR) of the slow rusting type is considered more durable than seedling resistance. The Rockefeller-Mexican Program first used the variety in the 1950s. Later derivatives, such as Penjamo 62, Torim 73, Kalyan/Bluebird, etc., showed slow rusting characteristics possibly derived from Frontana. Genetic analysis of Frontana and various CIMMYT wheat possessing excellent partial resistance to leaf rust worldwide has indicated that such adult plant resistance is based on the additive interaction of *Lr34* and two or three additional slow rusting genes. Relative tolerance of wheat varieties *viz*., Arjun, RAJ-1555, HD-2285 and HD-2329 to leaf rust was studied, low values of AUDPC and 'r' values as compared to Kalyanasona, Lal Bahadur and WL-711 (Meenakumari *et al*., 1994).

Khan *et al.,* (1997) reported that out of 10 wheat varieties evaluated for slow rusting, the varieties Chenab-70, WL-711 and Pak-81 were fast rusting cultivars suffering from 11.22, 19.73 and 13.88 per cent grain yield losses, respectively. The cultivars LU-26, V-87094 and V-8829 were moderately slow rusters with 8.10, 9.28 and 5.19 per cent yield losses, respectively and Paven, FSD-85 and INQ-91 were slow rusters. Although the cultivar SH-88 was fast rusting, it responded as rust tolerant with respect to yield loss.

In the sustainable agriculture, which is economical both for the farmer and nature, durable disease resistance is an essential tool against pathogens attack beside cultural practices, like crop rotation, seed treatment etc. Moreover, with the biotrophic fungi like rusts and powdery mildew, the only solution is the durable disease resistance (Nagarajan *et al*., 1998).

Singh *et al*. (1999) evaluated 102 wheat cultivars from China and a set of testers, carrying named *Lr* genes for resistance at seedling stage against leaf rust races. Variation in seedling infection types of cultivars was compared with that of the testers and genes conferring low infection types were postulated. Over all, nine named genes *viz*., *Lrl* (in 13 cultivars), *Lr3*(*12*), *Lr3bg*(*2*), *Lr10*(*1*), *Lr13*(*4*), *Lr16*(*49*), *Lr23* (*9*) and *Lr26*(*81*) were identified.

Mahoto *et al*. (2001) analyzed 43 bread wheat varieties from Nepal against fifteen pathotypes of *P. recondita* f. sp. *tritici* prevalent to South East Asia. Probable leaf rust resistance genes in these lines were postulated. Genes *viz*., *Lrl*, *Lr3*, *Lr9*, *Lr10*, *Lr13*, *Lr23* and *Lr26* were identified either singly or in combination in the bread wheat lines. Since Central Nepal also serves as the source for the recurrence of leaf rust for the Indo Gangetic plain, use of wheat varieties with diverse resistance would reduce the leaf rust severity and minimize crop losses.

Bhardwaj *et al*. (2005) stated that an integrated strategy using a combination of diverse resistance genes, deployment of cultivars by using pathotype distribution data, slow rusting and adult plant resistance to curtail selection of new pathotypes and prevent rust epiphytotics.

Aslam, *et al.* (2009) reported that out of eighty four test entries/varieties screened against leaf rust, 5 exhibited resistant, 21 moderately susceptible, 20 susceptible, 28 moderately resistant and 10 were highly susceptible.

Bhardwaj *et al*. (2011) expressed their views that the presently followed strategy to manage wheat rusts through pathotype monitoring, varietal deployment, diversity of resistance and combination of seedling stage, slow rusting, adult plant and minor gene resistance would definitely keep the wheat rusts under check.

Kolmer (2013) opined that cultivars that only have race-specific leaf rust resistance genes that are effective in seedling plants lose their effective resistance and become susceptible within a few years of release. Cultivars with combinations of race non-specific resistance genes have remained resistant over a period of years even though races of the leaf rust population have changed constantly.

2.3.1 Average Co-efficient of Infection (ACI), Rate of Infection (r) and Area Under Disease Progress Curve (AUDPC)

According to Van der Plank (1963) slow rusting is a partial resistance partitioned in variable proportion between horizontal and vertical resistance. He related AUDPC to crop loss and established a linear relationship between parameters in an empirical way.

Buchenau (1975) reported relationship between yield loss and AUDPC of stem and leaf rust of wheat. The whole culm of area under disease progress curve was highly correlated with the yield. Evaluation of wheat cultivars for ability to retard development of stem rust. AUDPC was a convenient and reliable approach for data summation but rate of disease development was not (Wilcoxson *et al.,* 1975).

Gupta and Singh (1982) reported that wheat varieties UP310, Janak and WH 147 showed slow rusting behavior as they allowed lowest rate of infection of brown rust as compared to Lal Bahadur and Kalyansona. Sabharwal (1986) studied the leaf rust development and AUDPC on four wheat cultivars under field conditions. Maximum AUDPC value was found in Lal Bahadur followed by WH 711 and least in Arjun.

Nargund (1989) identified DWR 39, HD 2189, HI 977, Keerti, Sonalika, WH 147 and WH 416 of *T. aestivum* and Bijaga Yellow, Kiran, MACS 1967 and Raj 1555 of *T. durum* as slow leaf rusters on the basis of values of AUDPC.

Singh and Rajaram (1991) evaluated 50 Mexican wheat varieties against leaf rust at two locations. Values of AUDPC for 25 varieties indicated variable levels of resistance. Prabhu *et al.,* 1993, conducted a field experiment and reported that among the wheat varieties S-57, S-69 and HB-208 expressed stable slow rusting resistance, as measured by AUDPC.

Dalal and Singh (1994) evaluated genetics of slow leaf rusting in wheat. The genetics of slow leaf rusting was studied by determining the AUDPC in the parental F1, F2 and backcross generations from 3 crosses. Partial dominance indicated by all crosses and genes reducing the spread of the disease were dominant over alleles causing spread of the disease.

Luthra *et al*. (1996) studied genetics of slow leaf rusting cultivars of wheat based on three and six parameter models for pustule size, latent period, coefficient of infection, ‘r’ and AUDPC.

Kloppers and Pretorius (1997) studied the effects of the leaf rust resistance genes *Lr13*, *Lr34* and *Lr37* in combination on the components of resistance to leaf rust. The results showed that the level of resistance of the lines was determined by the gene combination, environment and pathogen race. They concluded that resistance was stronger when below mentioned all three genes were

combined. In single gene analysis under field conditions, *Lr13* was totally susceptible, while *Lr34* showed very little resistance (80S). In the *Lr13* and *Lr34* combination, different lines showed severity ratings varying between 10MR-50MS. *Lr37* showed complete resistance in single gene analysis as well as in combination with *Lr13* and *Lr34*. They suggested that careful selection should be practiced when these genes are involved in segregating populations.

Kalappanavar and Yashoda Hegde (2001) screened 100 genotypes of wheat at MARS, Dharwad, (Karnataka) and reported that genotypes like HD-365, WH-542 and HW-2045 were having less than 5.0 ACI values to both stem and leaf rusts over years. Other nine genotypes were showing moderately resistant to rusts. Results indicated that HD-365, WH-542 and HW-2045 can be used as rust resistant source in resistance breeding programme.

Hasabnis and Srikant Kulkarni (2002) evaluated 17 wheat genotypes for slow leaf rusting characteristics during rabi 1997-98 at Main Research Station, Dharwad. They reported that the genotype HD 2189 had lowest (96.35) value of AUDPC as compared to 2173.05 of Agra local.

Hasabnis *et al.* (2002) reported that in susceptible varieties of wheat for leaf rust, flecks of infection were observed within a week. The initial average coefficient of infection in the cultivars tested ranged from 0.8 to 20. Wheat cultivars CPAN 4011, K 9324, UP 2358, CPAN 4059, YCBW 13 and K 9305 remained in the logarithmic phase throughout the course of leaf rust epidemic; 'r' and AUDPC in these cultivars were low.

Hasabnis *et al.* (2003) evaluated 55 wheat cultivars to leaf rust during 1999-2000. They showed that 34 wheat cultivars expressed hypersensitive type of resistance against the pathogen. Twenty-one cultivars expressed susceptible to moderately susceptible type of rust reactions. Wheat cultivars HD 2285 and Sonalika expressed terminal Average Co-efficient of Infection of 80.00 and 70.71, respectively. The Area Under Disease Progress Curve (AUDPC) was 1186.20, 821.00 and 712.60 in cultivars Sonalika, UP 2425 and HD 2285, respectively. The Average rate of infection (units/day) was highest in cultivars HUW 234 (0.34) and UP 2425 (0.35). Ten wheat cultivars, namely B. Yellow, C 306, GW 173, HD 2189, HD 2501, HD 2687, K 8962, PBW 396, Sujata and WH 542, had AUDPC values ranging from 10.00 to 77.50 and rate of infection from 0.00 to 0.12 units / day. Considering lower terminal disease score, smaller values of AUDPC and slow rate of infection, these 10 wheat cultivars recommended for future wheat improvement programme as good donors of desirable and durable leaf rust resistance.

Todorova and Andonova (2004) evaluated ten Bulgarian winter wheat cultivars (Zora, Kristal, Enola, Todora, Laska, Elitza, Albena, Milena, Pryaspa and Preslav) at adult plant stage under field conditions during 2002-03 for incomplete resistance to *P. recondita* f.sp. *tritici* using AUDPC as the criteria. The lowest AUDPC was found with Pryaspa, Zora, Preslav and Elitza. Laska, Albena and Milena showed relatively the same level of AUDPC, whereas Todora, Enola and Kristal expressed the highest AUDPC. Some of the cultivars such as Pryaspa, Preslav and Todora, have been studied for many years and did not lose their resistance irrespective of changes in the pathogen population structure in the country. Patil *et al.* (2005) studied the rusting behavior of some wheat cultivars against leaf rust under artificial conditions. They reported that the genotypes HD-2501, GW-173 and HD-2189 revealed low AUDPC values i.e. 2.4, 8.8 and 8.9, respectively, which also carry *Lr*34.

Hasabnis and Srikant Kulkarni (2004) reported that in intraspecific cross HD 4502/Amrut, the estimated additive gene effects and dominance gene effects were highly significant. The magnitude of additive X additive gene effects was higher and positive whereas dominance X dominance was negative. In interspecific crosses *viz.*, DDK 1001/Amrut and NP 200/Amrut, the estimates of additive and dominance effects were highly significant in the cross NP 200/Local Red. Only additive gene effects were highly significant for average coefficient of infection (ACI).

Oelke and Kolmer (2005) identified the leaf rust resistance genes present in the wheat cultivars Alsen and Norm. Alsen was released in 2000 by the North Dakota Agricultural Experimental Station for resistance to Fusarium head blight as well as good stem and leaf rust resistance. Norm has maintained high levels of leaf rust resistance and high yields since its release in 1992.

Patidar *et al.* (2007) characterized that genotypes HD-2189, HW-2021 showed the typical characteristics of slow rusters. These genotypes recorded low ACI, low AUDPC, considerable more latent period, medium pustule size, pustule density and less rate of infection with good quantity of yield per hectare. However, genotypes DWR-195, DWR-162 and MACS-2496 recorded maximum ACI and relevant adverse values compared to slow leaf rusting genotypes and identified as fast rusters.

Sareen *et al*. (2012) investigated slow rusting resistance at pathological and molecular level. Fifteen wheat genotypes with the aim to characterize pyramid resistance genes, including slow rusting genes like *Lr46* and *Lr50* were evaluated for disease severity percent, latent period and incubation period under field conditions. AUDPC, that is, <250.0 in the resistant genotypes, while it was beyond 1000.0 in 'Agra Local'. The shorter mean latent (7.67) and incubation period (6.0) was observed in susceptible genotypes, that is, 'Agra Local' as compared to all the resistant genotypes, that is, LP (10 to 12) and IP (9 to 10); while testing against all the three different pathotypes (21R55 (104-2), 121R63-1 (77-5) and 29R45 (12-5)). Genotypes which showed slow rusting, had longer latent period and incubation period as well as reduced percent disease severity and confirmed the presence of four to five resistance genes including slow rusting genes, that is, *Lr46* and *Lr50.* This indicates that these genotypes have potential durable resistance and can be used as parental lines in the development of more durable rust resistance.

2.3.2 Latent period

The latent period is the number of days from inoculation to the appearance of 50 per cent of the uredinia on host (Das *et al*., 1993).

Kapoor (1979) opined that the latent period of the stem rust of wheat was found to be an important component both in seedling and adult stages. The latent period was always found to be longer for all the cultivars in adult stage than in seedling stage.

Johnson (1980) studied the effect of temperatures on the latent period of slow and fast rusting wheat genotypes. He concluded that the longer latent period of slow rusting cultivars may be effective in reducing the rate of leaf rust development during winter in Texas.

Kulkarni *et al.* (1982) determined the relative importance of various components affecting the progress of leaf rust in wheat in the field by calculating the effect of equivalent changes in the individual components. Four components *viz*., latent period, infectivity, sporulation and weather were found to be important. Further, it was found that these four components were equally important and that they collectively determined the rate of leaf rust in an additive manner.

Lehman and Shaner (1996) studied the genetic variation in latent period among isolates of *P. recondita* f.sp. *tritici* on partially resistant wheat cultivars. They found that the latent period among isolates differed by 24 to 27 per cent on individual partially resistant cultivars. In simulated epidemics, isolates with short latent periods caused 2 to 2.5 times more disease and overcame 13 to 35 per cent of the resistance of 4 partially resistant cultivars.

Basandrai *et al.* (1998) crossed the susceptible single gene lines carrying the genes *Lr1*, *Lr3Bg*, *Lr10* and cultivar WL 711 carrying the defeated leaf rust resistance gene *Lr13* and the F1 seedlings were studied for latency period and uredinial size against race 77A. They found that F1 seedlings from the crosses *Tc*+*Lr3Bg* x WL711 (*Lr13*), Tc+*Lr10* X WL711 (*Lr13*) and Prelude+*Lr10* x WL 711 (*Lr13*) developed smaller uredinia than those seen on the parental lines. The F1 seedlings from the cross *Tc*+*Lr3Bg* x WL711 (*Lr13*) showed longer latency period than that observed on both parents.

Wheat cultivars HD 2687, HD 2501, HW 2004, UP 2425 and RAJ 3765 were genetically analyzed with three pathotypes 0R9 (106), 109R31-1 (77-2) and 17R23 (104) of leaf rust pathogen. Segregation of F2 seedlings suggested three dominant independent genes for resistance in HD 2687, one dominant gene for resistance each in HD 2501 and HW 2004 and two dominant independent genes each in UP 2425 and RAJ 3765 against Pathotype 0R9 (106). Analysis of reciprocal crosses, BC1 and BC2 of above cultivars confirmed the above findings. The gene *Lr23* in HD 2687; *Lr23* and *Lr26* in UP 2425; *Lr23* and *Lr34* in HD 2501; *Lr24* in HW 2004 and *Lr13* in RAJ 3765 were validated based on pedigree and ITs of cultivars (Moghaddam *et al.,* 2004a).

Marina and Putnik-Deliac (2009) studied ten wheat genotypes were tested for resistance characteristics to *P. triticina*. Infection intensity in the field was evaluated at different growth stages and time of spike appearance and leaf senescence were recorded. At seedling stage, under the controlled conditions of greenhouse, latency period, infection frequency and reaction type were determined. Resistance characteristics at different wheat growth stages were strongly correlated. Correlation coefficient between LP x RT x IF and AUDPC values, was 0.828. The highest coefficients of correlation between particular resistance characteristics and maximal intensity in the field were determined with the last evaluation in the field (0.665, 0.476 and 0.834). Time of spike appearance was very variable for different genotypes, whereas leaf senescence was recorded concomitantly for

near all genotypes. The exception was Rusalka, as the most resistant in the field. All genotypes included in this three-year long experiment expressed stability with respect to infection intensity at different growth stages.

2.3.3 Pustule Size and Density

Shaner *et al.* (1978) selected two slow leaf rusting wheat cultivars Suwan 85 and P 6028 and two susceptible cultivars Monon and Suwon 92 and inoculated the flag leaves uniformly with uredospores of *P. recondita* f. sp. *tritici* to measure the components of slow rusting. Uredium size was universally related to density of uredia, but uredia were consistently larger on Monon and Suwan 92 than on Suwa 85 and P-6028, the production of more uredospores per day per uredium on Monon and Suwan 92 compared to Suwan 85 was because of larger uredia on these two cultivars, low production of uredospores on P 6028 was due to less production per cm^2 of uredium and smaller uredia.

Shaner and Finney (1980) developed new sources of slow leaf rusting resistance in wheat. Three cultivars of wheat were selected from International Winter Wheat Rust Nursery since they had a low severity of *P. recondita* f.sp. *tritici* coupled with a compatible infection type. In hills, leaf rust developed more slowly on cultivar that showed longer latent periods and smaller and fewer uredia in glass house conditions.

Kapoor and Joshi (1981) studied the slow leaf rusting in wheat. Latent period, flecks and pustules number per square cm of leaf area were compared in glasshouse during 1977-78 and 1978-79 at seedling stage on six wheat cultivars. All six wheat cultivars showed susceptible reaction to race 122 of *P. graminis* f.sp. *tritici.* Cultivar “Sonalika” produced comparatively fewer flecks and pustules per cm of leaf area than “Agra Local”. The latent period for cultivar “Sonalika” was longer by 1-2 days than for ”Kharchia” and “Agra Local”.

Sokhi and Singh (1984) studied the slow rusting cultivars and reported that the latent period was longer whereas, number and size of uredia and uredospores production were less in most of the cultivars that they rusted slowly.

Prabhu *et al.,* (1993) conducted a greenhouse experiment under controlled conditions on wheat varieties and inoculated uredospores, showed that long latent period and small pustules were the components functioning in the slow rusting varieties S-57, S-69 and HB-208.

Ahamed and Singh (2003) screened wheat variety Kundan, along with the fast ruster variety Agra Local for seedling reaction and adult plant response for two years. They reported that seedlings of Kundan were susceptible while adult plants showed lower susceptible response than Agra Local in the field and lower pustule number, uredial size and longer latency period under glasshouse conditions for both the years, which indicates potentially consistent performance of the components over years and that these measures can be used as direct parameters for identifying slow rusting genotypes or varieties.

Singh and Huerta Espino (2003) studied slow rusting components in bread wheat and found that temperature and growth stage had little effect on uredinium size. Pretorius *et al*. (1994) did not find an effect of inoculum concentration on uredinium size. In addition, Das *et al*. (1993) showed that the narrow sense heritability of uredinium size was higher than that for latent period and receptivity, meaning that breeding for slow rusting resistance in bread wheat would be more efficient if selection is based on this component rather than on the other two. Use of digitized imaging can be used to further expedite the measurement of uredinium size.

Leyva-Mir *et al*. (2008) determined the components of slow-rusting resistance to rust (*P. triticina*) in wheat, evaluated seedlings resistance of 20 flour wheat (*T. aestivum*) genotypes in a growing chamber using the *P. triticina* physiological race MCJ/SP. Dormant period, size and number of pustules were measured. The genotypes Kakatsi, Tarachi F2000, Kuruku, Jupateco+*Lr34-Sr2*, Oaxaca local flour race and Rayon F89 exhibited a longer dormant period. The Kuruku and Kakatsi genotypes had smaller and fewer pustules per square cm. It is concluded that a genotype with a high level of this type of resistance can be identified by measuring any of the SRR components during the seedling stage.

Statler *et al.* (1977a) used durum wheat varieties *viz*., D 6618, Hercules, Rolette, Bolone, Thatcher and Wodron for slow rust development. The wheat cultivars Thatcher and D 6618 showed susceptible reaction to leaf rust of wheat and rest cultivars showed resistant reaction.

Statler *et al.* (1977b) studied hard red spring wheat cultivars namely, Justin, Fortuna and Tioga with susceptible reaction type to *P. recondita* f.sp. *tritici.* Justin, Fortuna and Tioga consistently exhibited less rust in the field and slow rates of rust development than Thatcher.

Jalinder (1983) studied slow rusting mechanism in 16 varieties of wheat. UP-301, DWR-16, HD-2189 and DWR-26 remained free from infection throughout and varieties WH-147, KIL-711, Sonalika, C-306 and HD-4502 were identified as slow rusters. whereas, Agra Local, Kalyansona, Narmada, NI-5439 and Lal Bahadur were identified as fast rusters.

Navi (1986) while working with slow rusting of leaf rust in bread and durum wheat varieties found that HD-2278 remained tolerant and C-464, DWR 39, HD-2189 and DWR-16 were identified as slow rusters. Raj 155, DWR 137, HD-4502 and DWL-5023 of *T. durum* infected later in the season indicating the operation of slow rusting mechanism. Wheat varieties *viz*., NI-5439, Lal Bahadur, Kalyansona and Sonalika of *T. aestivum* and Local Red, Agra Local, N-59, A-9-30-1 and MACS-1967 of *T. durum* were identified as fast rusters.

Borkar and Patil (1995) evaluated 17 wheat varieties at Wheat Research Station, Niphad (Maharashtra) under both natural and artificial conditions. They reported that varieties like PBN-142, HD-2501, HD-4502, HD-2380, HD-2278, Unnath-Sonalika, Unnath-Kalyansona, Unnath-NI-5439, MACS-2496 and MACS-1967 were free from leaf rust and varieties like HI-977 and AKW-381 showed low leaf rust incidence under both natural and artificial epiphytotics.

Grewal *et al.* (1998) evaluated 165 lines for resistance to stripe rust, leaf rust and loose smut. They found that 14 lines exhibited durable resistance to predominant races of stripe and leaf rust. Among others, 36 lines showed durable resistance to stripe rust and 26 lines exhibited durable resistance to leaf rust.

El-Nashar and El-Ghamry (1999) evaluated the slow rusting resistance in some Egyptian wheat cultivars to leaf rust. They reported that Sids1, Sids2 and Sids3 showed slow rusting resistance and these cultivars can be included in the breeding programme.

Kaur *et al*. (2000) evaluated the adult plant resistance of 111 wheat cultivars from all over the world against Indian leaf rust race 77 and five of its virulent variants. Out of 111 cultivars tested, 65 showed seedling susceptibility and low infection levels to leaf rust races at the adult plant stage. A non-hypersensitive type reaction to leaf rust races was observed in 65 cultivars. In 45 of these cultivars, a non-hypersensitive type reaction was linked to the adult plant resistance gene *Lr*34 due to the presence of leaf tip necrosis. The reaction pattern to different leaf rust races indicated the presence of at least six or seven adult plant resistance genes.

Kalappanvar *et al.* (2003) evaluated 100 genotypes of wheat at MARS, Dharwad (Karnataka) for three years and reported that PBW-500 and HW-2004 genotypes were almost free from both the rust, however, VL-822 and VL-829 recorded resistant reaction.

Hasabnis and Srikanth Kulkarni (2004) reported that genotype DWR 162 and MACS 2496 showed susceptible reaction to leaf rust with the severity of 50 and 40 per cent respectively. The genotypes NIAW 34 and HD 2189 showed moderately susceptible reaction to leaf rust of wheat.

Khanna *et al*. (2005) studied the inheritance of leaf rust resistance in a partially leaf rust resistant Indian cultivar, HD 2009 and a susceptible cultivar, WL711. The segregation of progenies in the F2, F3 and F5 generations for resistance to leaf rust indicated the presence of two resistance genes with an additive effect. Although the resistance pattern of HD2009 is similar to that of *Lr*34, HD2009 does not possess the leaf tip necrosis phenotype. They concluded that *Lr*34 is not one of the partial resistance genes present in HD 2009. They also ruled out the possible involvement of *Lr*46/Yr29 in leaf rust and stripe rust resistance of HD 2009, since gene *Lr*46 is not effective in India.

A high level of adult-plant resistance (APR) to leaf rust was found in the CIMMYT-developed spring wheat 'Brambling'. To determine the genetic basis of resistance in seedlings and adult plants and the magnitude of genotype × environment effects on the expression of APR. Brambling was crossed with spring wheat 'Jupateco 73S' that is highly susceptible to current predominant *P. triticina* races in Mexico and the United States. The F1, F2:3, F4:5, F4:6 and F5:7 recombinant inbred lines (RILs) were evaluated under artificial field epidemics in Mexico and St. Paul, MN. The RILs also were tested with five races of *P. triticina* in greenhouse seedling experiments. A DNA marker was used to postulate the presence of slow-rusting gene *Lr34* in the RILs. F1 data suggested strong dominant effect of the APR genes in Brambling. Expression of APR was influenced by the environment in the

RILs, even though Brambling displayed a consistent response, indicating that stability of APR can be achieved by combinations of slow-rusting resistance genes (Zhang *et al*., 2008).

2.3.4 Yield and thousand grain weight

Sayre *et al*. (1998) found that leaf rust caused losses irrespective of the level of resistance possessed by the cultivars. Smedegaard-Petersen and Tolstrup (1985), who studied powdery mildew resistance in barley, argued that the fact that highly resistant plants do not show any visible disease symptoms after inoculation does not mean that the plants are not affected.

Singh *et al*. (2004a) evaluated losses for selected race-specific, slow-rusting and susceptible durum genotypes under artificial inoculations. Hussain *et al.,* (2004) studied the genetics of leaf rust and agronomic traits in wheat (*T. aestivum*) gene action for plant height, number of tillers per plant, number of grains per spike, 100 grain weight, grain yield per plant, biological yield per plant, harvest index and leaf rust incidence was studied in 8X8 diallel crosses produced from susceptible and resistant wheat cultivars (SA 42, LU 26, MH 97, Nacozari F 76, Chenab 7, Crow, Parula and Inqilab 91). The greatest incidence of dominant genes was observed in 'Crow' for number of grains per spike, grain yield, biological yield, harvest index and leaf rust incidence; in 'LU 26' for plant height and grain weight; in '97' for number of tillers; in 'Parula' for grain yield; and in MH 97, Nacozari and Inqilab 91 for leaf rust incidence. The highest number of recessive genes was evident in Chenab 70 and SA 42 for grain yield, biological yield, harvest index and leaf rust incidence. The superior crosses consisted of MH 97 x Crow for number of tillers (11.97), SA 42 x Parula for grain number (60.1), LU 26 x Nacozari for grain weight (5.58 g) and MH 97 x LU 26 for grain yield (24.67 g). The following crosses with the lowest values of area under disease progress curve were resistant to leaf rust: LU 26 x Crow, LU 26 x Parula, LU 26 x Inqilab 91, SA 42 x Crow and MH 97 x Crow. Hence, Crow, Parula and Inqilab 91 could be successfully exploited as a source of leaf rust resistance for wheat breeding programmes.

Herrera-Foessel *et al*. (2006) reported that, slow-rusting durum wheat lines with low disease levels and low yield losses, as well as genotypes with low yield losses despite moderate disease levels, were identified. Such genotypes can be used for breeding durum wheat genotypes with higher levels of resistance and negligible yield losses by using strategies that previously have been shown to be successful in bread wheat.

Afzal *et al*. (2008) reported that wheat yield losses in the northern Punjab and North West Frontier Province (NWFP). There exists a direct linkage between the disease level of *P. striiformis* and weight loss of kernel in the most common wheat varieties sown in Pakistan. The kernel weight was significantly negatively correlated with the proportion of leaf area affected by stripe rust. The correlation coefficient (-0.9185) depicted highly significant effect of stripe rust on lowering 1,000 grain weight, ultimately the wheat yield.

2.3.5 Seedling reaction test and gene postulation

Nagarajan *et al*. (1984) identified the various types of genes by selecting appropriate pathotypes by matching techniques in large number of wheat genotypes. McIntosh *et al*. (1995) catalogued resistance genes to three rusts of wheat. Nayar *et al*. (1988) postulated *Lr14a* in HUW 12 and *Lr10* in WH 322 varieties. Total nine known and one unknown gene was postulated singly or in combinations from CIMMYT germplasm by Singh and Gupta (1991). Twenty commercially grown varieties of wheat under study possessed *Lr13*, *Lr23*, *Lr24*, *Lr26* and *Lr34* genes singly or in combinations. The leaf rust resistance genes *Lr26* as singly as in combinations with *Lr10*, *Lr13* and *Lr23* were detected at higher frequency from 36 wheat lines developed at Dharwad (Hasabnis, 1998).

Pretorius *et al.* (1984) showed that the resistance of the adult-plant gene *Lr13* was expressed at 25°C in seedling plants to three isolates of *P. recondita* from Mexico, China and Chile. However, the resistance was not expressed in seedlings to isolates from North America.

Nayar *et al*. (1993) showed presence of gene *Lr23* in more than 30 per cent of wheat lines. Some of the released varieties having *Lr23*+ *viz*., Bijaga yellow, DWR-39, HD 2278, HD 2380 and HD 2501. Sharma and Saini (1993) explained diversity for resistance to seven pathotypes of *P. recondita* in 88 lines of *T. aestivum* using infection type matching technique. The genes identified were *Lr1*, *Lr3* and *Lr10* in 38.6, 31.84 and 14.7 per cent of wheat lines, respectively. Bahadur *et al*. (1995) analyzed 28 improved wheat lines and identified genes as *Lr*1, *Lr*3, *Lr*10, *Lr*19, *Lr*23, *Lr24* and *Lr26* alone or in combinations. The observed combinations were *Lr1*+ *Lr23*(in 3 lines), *Lr10* + *Lr23* (*3*) and *Lr10*+*Lr26* (*3*).

Saini *et al*. (1993) concluded that, *Lr1*, *Lr10*, *Lr13*, *Lr14a*, *Lr17*, *Lr23* and *Lr26* genes were not useful singly or in combination against pathotypes *viz*., 77A, 77-1 and 77-2. But *Lr3*, *Lr16* and *Lr34* genes confirmed resistance against leaf rust. Sawhney *et al*. (1992) studied genetic diversity to leaf rust in near isogenic lines and discussed the possible presence of *Lr34* gene in Indian bread wheat varieties and its role in durable resistance.

Hasabnis (1998) reported that a variety N-59 did not carry any known or unknown *Lr* genes. The varieties MACS-2846, NI-146, with an additional unknown gene. The *Lr24* was postulated from DWR-185 and MACS-2884. Adult plant resistance gene *Lr34* was detected in HD-2189, HD-2501 and NIAW-34 in addition to other genes. HD-2380 possessed known gene combination (*Lr10+Lr23+Lr26*).

Kiyar (2002) postulated *Lr* genes from the varieties *viz*., DDK1001, DDK1019 and DDK1022 were found to carry gene combination of *Lr23+Lr26*, *Lr23+Lr26 and Lr10+Lr26* respectively with an unknown additional gene. Whereas, DWR 225 carries *Lr26* with an unknown additional gene and DWR-2006 possessed gene combination (*Lr13+Lr23*). But *Lr* genes from DWR1006, NP200 and DDK1009 could not be postulated.

Singh *et al*. (2004a) evaluated 44 cultivars and lines of wheat for resistance to leaf rust. They found that 14 wheat lines showed seedling resistance, while 30 cultivars/lines showed seedling susceptibility to race 77-5. The 14 wheat lines possessing seedling resistance against 77-5 also showed adult plant resistance against pathotypes 77-5, 77-2 and 104-2.

Mebrate *et al*. (2008) stated that gene postulation helps to undertake a quick identification of the probable leaf rust resistance genes (*Lr* genes) present in a large number of wheat cultivars at a time. To identify the race-specific *Lr*-genes present in 36 wheat cultivars from Ethiopia and Germany. Seventy-six wheat genotypes, including 40 near-isogenic lines (NILs), were tested against 31 isolates of *P. triticina* isolates collected from both countries. *Lr*-genes *Lr1*, *2c*, *3*, *3ka*, *9*, *10*, *14a*, *14b*, *13*, *16*, *18*, *21*, *23*, *27+31*, *30*, *37* and *44* were postulated to be present in the Ethiopian wheat cultivars. *Lr* genes *Lr9*, *20* and *21* were present in the German wheat cultivars. The *Lr*-genes present in some wheat cultivars could not be postulated because of non-matching virulence combinations with any of the NILs.

Li *et al*. (2010) characterized by a total of 102 Chinese winter wheat cultivars and advanced lines were inoculated with 24 pathotypes of *P. triticina* for postulation of leaf rust resistance genes effective at the seedling stage. These genotypes were also planted in the field for characterization of slow rusting responses to leaf rust in the 2006–07 and 2007–08 cropping seasons. Fourteen leaf rust resistance genes *Lr1, Lr2a, Lr3bg, Lr3ka, Lr14a, Lr16, Lr17a, Lr18, Lr20, Lr23, Lr24, Lr26, Lr34 and LrZH84* either singly or in combinations, were postulated in 65 genotypes, whereas known resistance genes were not identified in the other 37 accessions. Resistance gene *Lr26* was present in 44 accessions. Genes *Lr14a* and *Lr34* were detected in seven entries. *Lr1* and *Lr3ka* were found in six cultivars and five lines possessed *Lr16*. *Lr17a* and *Lr18* were identified in four lines. Three cultivars were postulated to possess *Lr3bg*. Genes *Lr20*, *Lr24* and *LrZH84* were each present in two cultivars. Each of the genes *Lr2a* and *Lr23* may exist in one line. Fourteen genotypes showed slow leaf rusting resistance in two cropping seasons.

Twenty four differential lines with individual known leaf rust resistance genes were tested with 17 different pathotypes of leaf rust collected from Argentina. Leaf rust infection types produced on seedling plants of the 66 local cultivars were compared with the infection types produced by the same pathotypes on *Lr* differentials to postulate which seedling leaf rust genes were present. Presence of *Lr9, Lr10, Lr19, Lr20, Lr21, Lr24, Lr25, Lr26, Lr29, Lr34, Lr35, Lr37, Lr47* and *Lr51* was also determined using molecular markers. Eleven different *Lr* genes were postulated in the material: *Lr1, Lr3a, Lr3ka, Lr9, Lr10, Lr16, Lr17, Lr19, Lr24, Lr26, Lr41*. Presence of *Lr21*, *Lr25*, *Lr29* and *Lr47* could not be determined with the seventeen pathotypes used in the study because all were avirulent to these genes. Eleven cultivars (16.7%) were resistant to all pathotypes used in the study and the remaining 55 (83.3%) showed virulent reaction against one or more local pathotypes. Cultivars with seedling resistance gene combinations including *Lr16* or single genes *Lr47* (detected with molecular marker), *Lr19* and *Lr41*, showed high levels of resistance against all pathotypes or most of them. On the opposite side, cultivars with seedling resistance genes *Lr1*, *Lr3a*, *Lr3a + Lr24*, *Lr10*, *Lr3a + Lr10*, *Lr3a + Lr10 + Lr24* showed the highest number of virulent reactions against local pathotypes. Occurrence of adult plant resistance genes *Lr34*, *Lr35* and *Lr37* in local germplasm was evaluated using specific molecular markers confirming presence of *Lr34* and *Lr37*. They suggested that combinations including seedling resistance genes like *Lr16, Lr19, Lr21*, *Lr25, Lr29, Lr41* and *Lr47* with

adult plant resistance genes like *Lr34, Sr2, Lr46* will probably provide durable and effective resistance to leaf rust in the region (Vanzetti *et al*., 2011).

Abdelbacki *et al*. (2013) observed the most frequently occurring gene in ten Egyptian wheat cultivars was *Lr35* (70%), followed by *Lr22 (60%), Lr27 (40%), Lr34 (30%), Lr19 (30%), Lr18 (10%), Lr36* (10%) and *Lr46* (10%), eight out of sixteen *Lr* genes were not present in the tested cultivars. Four genes; *Lr28*, *Lr24*, *Lr34* and *Lr19* were confirmed using molecular marker. It is concluded that there was a good variation in *Lr* genes carried by wheat cultivars commercially grown in Egypt.

2.3.6 Genetics and molecular characterization of bread wheat varieties for durable/slow leaf rust resistance

Genetic studies of leaf rust resistance in wheat have been conducted by wheat researchers world-wide. In the first of these studies, Mains *et al*. (1926) determined that the wheat cultivars Malakof and Webster each had a gene that conditioned leaf rust resistance, later designated as *Lr1* and *Lr2*, respectively (Ausemus *et al*., 1946).

Soliman *et al*. (1964) mapped *Lr* genes by identifying the chromosomes that carried leaf rust resistance genes *Lr1*, *Lr3* and *Lr11*. Dyck and Samborski (1968) demonstrated allelic variation in *Lr* genes when they determined the presence of three alleles at the *Lr2* locus.

Leaf rust resistance genes designated *Lr1* to *Lr60* have been described (McIntosh *et al*., 2007) and molecular markers are available for many of them (Vasu *et al*., 2006 and Khan *et al*., 2011). These genes have been characterized in common hexaploid wheat, tetraploid durum wheat and many diploid wild wheat species. In hexaploid wheat, leaf rust resistance genes are widely distributed across the genome, being present on nearly every one of the 42 chromosome arms. Four allelic series have also been described. Genes *Lr2a*, *Lr2b* and *Lr2c* were mapped to a locus on chromosome arm 2DS (McIntosh and Baker, 1968), *Lr3a*, *Lr3ka* and *Lr3g* are at a locus on chromosome arm 6BL (Haggag and Dyck, 1973), *Lr17a* and *Lr17b* are at a locus on chromosome arm 2AS (Dyck and Kerber, 1977) and *Lr22a* and *Lr22b* at a locus on chromosome arm 2DS (Rowland and Kerber, 1974). Genes *Lr14a* and *Lr14b* are extremely tightly linked on chromosome 7BL (Dyck and Samborski, 1970) and are considered as alleles for all practical purposes.

Most leaf rust resistance genes condition effective resistance to specific races of *P. triticina*. Race-specific resistance is usually manifested by a hypersensitive response (HR) of rapid cell death that occurs at the interface between fungal haustoria and host cells in the epidermal and mesophyll layers. Different resistance genes condition the characteristics of resistance phenotypes or infection types. For instance, the resistance response of wheat lines with *Lr3* is characterized by clearly defined hypersensitive flecks while lines with *Lr2a* have only very light flecks that are difficult to see. Other race-specific resistance responses such as those conditioned by wheat lines with *Lr3ka*, *Lr3bg* and *Lr11* are manifested by small uredinia surrounded by chlorosis and lines with *Lr16* have small uredinia surrounded by necrosis. Resistance conditioned by these last genes is probably expressed at a later point in the infection and colonization process of *P. triticina*. Race-specific *Lr* genes are effective in seedling plants and remain effective in the adult plant stage. However, the resistance conditioned by some genes, such as *Lr12*, *Lr13* and *Lr22a*, is best expressed in adult plants. In wheat lines that have combinations of resistance genes, the gene with greatest resistant infection type is epistatic to genes with less resistant infection types. To date, three genes that confer race-specific resistance have been cloned: *Lr1* and *Lr10*, originally from common wheat and *Lr21*, originally from *T. tauschii* (Feuillet *et al*., 2003; Huang *et al*., 2003; Cloutier *et al*., 2007). These genes condition an HR response to isolates of *P. triticina* that carry matching avirulence genes and encode proteins with nucleotide binding site-leucine rich repeat (NBS-LRR) regions typical of most disease resistance genes in plants.

2.3.6.1 Slow Leaf Rust Resistant gene *Lr34*

Gene *Lr*34 was first described as a modifier of adult plant resistance in the cultivar Frontana (Dyck *et al*., 1966). This gene was found in a number of wheat lines around the world (Dyck and Samborski, 1982) and was later mapped to chromosome arm 7DS (Dyck, 1987). The resistance response conditioned by *Lr34* does not involve horizontal resistance, but instead is characterized by fewer and smaller uredinia with no chlorosis or necrosis on flag leaves of adult plants, often with a decreasing gradient of uredinia density from leaf base to tip. The adult plant resistance gene *Lr34* has been reported in many genotypes of Indian origin on the basis of leaf tip necrosis and AUDPC scores (Singh and Gupta, 1991; Saini *et al*., 1998 & Bahadur, 1998). *Lr34* conditions the same resistance response to all isolates of *P. triticina* that have been tested (i.e. non race-specific resistance) and is associated with non-horizontal resistance to stripe rust and powdery mildew (Spielmeyer *et al*., 2005).

Flag leaves may exhibit spontaneous leaf tip necrosis and tips are more resistant than leaf bases. Despite the importance of this gene, the mechanism of resistance is unknown (Hulbert *et al*., 2007).

In Mexico, leaf rust severity on most cultivars can be related to the number of slow rusting genes they carry. When susceptible cultivars display 100 percent leaf rust severity, cultivars with only *Lr34* display approximately 40 percent severity; cultivars with *Lr34* and one or two additional minor genes display 10 to 15 percent severity; and cultivars with *Lr34* and two or three additional genes display 1 to 5 percent severity. Leaf rust could further increase to unacceptable levels on cultivars carrying only *Lr34* or *Lr34* and one or two additional genes. However, cultivars with *Lr34* and two or three additional genes show a stable response in environments tested so far, with final leaf rust ratings lower than 10 percent. The presence of *Lr34* can be indicated by the presence of leaf tip necrosis in adult plants, which is closely linked with it (Singh, 1992a).

Kolmer (1996) reviewed the genetics of resistance to wheat leaf rust and opined that genes expressed in seedling plants have not provided long-lasting effective leaf rust resistance. Adult-plant resistance genes *Lr13* and *Lr34* singly and together have provided the most durable resistance to leaf rust in wheat throughout the world. Continued efforts to isolate, characterize and map leaf rust resistance genes is essential given the ability of the leaf rust fungus to overcome deployed resistance genes.

The gene *Lr34* is partially effective and it is highly interactive and often additive in expression (Ezzahiri and Roelfs 1985; Kaur *et al*., 2000; Samborski and Dyck, 1982; Singh and McIntosh, 1984) and is also effective against stem rust (Dyck, 1987; Kerber and Aung, 1999), powdery mildew (Soliman *et al*., 1964; Lillemo *et al*., 2008) and barley yellow dwarf virus (Singh and McIntosh, 1984; Ayala *et al*., 2002).

Rubiales and Niks (1995) compared the effect of *Lr*34 with that of *Lr*12 and *Lr*13 (all in Thatcher background) and with the partial resistance of Akabozu and BH1146. They found that seedlings of all lines displayed a compatible infection types. *Lr*34 increased latency period and decreased infection frequency, especially at low temperature. As far as it is known that *Lr34* and minor gene combinations have not been overcome in more than 50 years. A better understanding of the molecular genetics of the rust pathogen host interaction will help us to better use of these genes. Recently history was made when *Lr34* was cloned and the mode of action hypothesized (Krattinger *et al*., 2009).

The presence of *Lr34* in a cultivar increases its general resistance to various races of the leaf rust pathogen. However, this non-specific resistance characteristic of *Lr34* makes it difficult to identify by traditional methods (Urbanovich *et al*., 2006).

Suenaga *et al*. (2003) conducted a marker assisted study on 107 double haploid wheat lines derived from Japanese wheat and Israeli wheat. Four hundred SSR primers out of 600 were selected for use in genotyping the population. A QTL at 7DS showed a strong association with rust resistance. They suggested that *Xgwm295.1* is the closest known SSR marker for *Lr34* and alleles of *Xgwm295.1* can be used for detection of *Lr34* in different cultivars.

Kadkhodaei *et al*. (2012) identified the presence of the leaf rust resistance genes using STS, the gene *Lr34* was present in six cultivars (Akbari, Bam, Tajan, Khazar 1, Sistan and Niknezhad. The *csLV34* primer amplified fragments of 150 and 229 bp in positive and negative controls, respectively.

2.3.6.2 Slow Leaf Rust Resistant gene *Lr46*

Gene *Lr46* is another adult plant resistance gene originally found in the cultivar Pavon 76. *Lr46* conditions a resistance response of fewer and smaller uredinia, but with varying amounts of chlorosis in adult plants. But, this gene is not effective in India (Singh *et al*., 1998). Genes that condition non-HR resistance will probably differ both in DNA sequence and protein function compared with race-specific, NBS-LRR resistance genes. Genetic aspects of leaf rust resistance in wheat were previously reviewed by Kolmer (1996). These genes *Lr34* and *Lr46* are linked with stripe rust resistance genes *Yr18* and *Yr29* respectively, have been known to confer durable resistance in wheat (Singh, 1992; Singh *et al*., 1998 and Suenaga *et al*., 2001). Wheat cultivars with combinations of *Lr34*, *Lr46* or other non-race-specific resistance genes are highly resistant and are immediately available sources of durable resistance (Singh *et al*., 2000). Combinations of *Lr34* with seedling resistance genes have also provided high levels of effective resistance that have remained effective for a number of years (Kolmer and Oelke, 2006). The cloning of genes *Lr34* and *Lr46* and analysis of how these genes are expressed may provide a model for understanding the biological basis of non-race-

specific resistance. Singh *et al*., (2005) reported that, *Lr46/Yr19* has dual effect and confers slow-rusting resistance to leaf rust and stripe rust shown to provide durable resistance in combination.

Molecular markers such as restriction fragment length polymorphism (RFLP) and random amplified polymorphic DNA (RAPD) have been successfully used to develop markers for *Lr* resistance genes (Chelkowski and Stepien 2001; Helguera *et al*. 2000). For more efficient identification and practical use, these markers have been converted into sequence-tagged sites (STS) and sequence-characterized amplified regions (SCAR) (Autrique *et al*., 1995; Feuillet *et al*., 1995; Prins *et al*., 1996). Therefore, STS and SCAR markers have also been used successfully to detect polymorphisms in wheat resistance to rusts (Dedryver *et al*. 1996; Feuillet *et al*. 1995; Schachermayr *et al*. 1994, 1995, 1997). The STS marker for *Lr34* (*csLV34*) was mapped 0.4 cM from this gene and validated in many genotypes from different parts of the world (Lagudah *et al*., 2006). This marker is capable of differentiating between lines with/out this gene.

Martinez *et al*. (2001) studied the effects of leaf rust adult plant resistance gene *Lr46* and compared it with another adult plant resistance gene, *Lr34*. They reported that the effect of *Lr46* resembles that of *Lr34*. Tests conducted at the seedling stage indicated that *Lr34* enhances the seedling resistance to leaf rust, whereas *Lr46* did not have any significant effect on seedling resistance of the lines carrying it. The presence of *Lr46* results in a longer latency period and lower infection levels than the susceptible cultivars. Their results further indicated that *Lr46* confers a similar non-hypersensitive type of defense to leaf rust as *Lr34*, but its effect is smaller than that of *Lr34*. They emphasized that the need of further work to understand the role of *Lr46* in *Lr34*/*Lr46* combinations.

William *et al*. (2003) established the precise genomic location of gene *Lr46* using molecular approaches and to determine if there was an association of this locus with adult plant resistance to stripe rust. Bulked segregant analysis and linkage mapping using amplified fragment length polymorphisms with the 'Avocet' × 'Pavon 76' population, F3 progeny lines of a single chromosome recombinant line population from the cross 'Lalbahadur' × 'Lalbahadur (Pavon 1B)' and the International Triticeae Mapping Initiative population established the genomic location of *Lr46* at the distal end of the long arm of wheat chromosome 1B. A gene that is closely linked to *Lr46* and confers moderate levels of adult plant resistance to stripe rust is identified and designated as *Yr29.*

The physical location of *Lr46* was previously reported to be close to SSR markers *Xwmc44* (Suenaga *et al*., 2003), *Xwms259 and Xwms140* (Mateos-Hernandez *et al*., 2006). Microsatellite locus *Xbarc80* maps 10-11 cM distal to *Xgwm259* and can be used as an alternative distal marker.

Suenaga *et al*. (2003) studied the effects of *Lr34* and *Lr46* on leaf rust and stripe rust in wheat. One of the two parents, Fukuho-komugi, involved in the cross carrying *Lr34*/*Yr18* and the other parent, Oligoculm, carried *Lr46*/*Yr29*. The results of the molecular markers analysis showed that QTL-7DS explained 45.2% of the leaf rust resistance, while QTL-1BL explained 17.4% of the resistance. Twenty-four percent of the stripe rust resistance was associated with 7DS (*Lr34*/*Yr18*), whereas the effect of 1BL (*Lr46*/*Yr29*) on stripe rust resistance was not significant. They suggested that the reduced effect of the QTL at 1BL could be due to allelic differences at the *Yr29* locus, genetic background, or segregation of several resistance genes in the cross.

Navabi *et al*. (2004) studied the inheritance of adult plant resistance to stripe rust in five spring wheats. The Australian wheat cultivar Avocet-YrA was used as a susceptible parent. In all of the crosses, the F1 was intermediate in severity, suggesting that the adult plant resistance in these genotypes was incompletely dominant. From the consistent phenotype of leaf tip necrosis, they concluded that all resistant parents have at least one gene in common, *Yr18*. Some of the population lines reached a severity level of 50-60%, suggesting that these lines might have *Yr18* alone as a source of stripe rust resistance.

The genome sequences of *P. graminis* and *Melampsora larici-populina* Kleb. (poplar rust) have recently been released and will be an important resource for *P. triticina* comparative genomics. Research on the molecular basis of infection and race specificity in *P. triticina* is just beginning, so recent progress in transformation (Webb *et al*., 2006) and new techniques for functional analysis of *P. triticina* genes (Hu *et al*., 2007a) will provide new opportunities to study these significant yet poorly understood aspects of this important pathogen.

Mateos-Hernandez *et al*. (2006) developed fourteen new markers that potentially link to *Lr46*. They narrowed down the physical location of *Lr46* to a submicroscopic region between the breakpoints of deletion lines 1BL-13 and IBL-10. A substitution line of wheat cultivar Lalbahadur, carrying *Lr46* from Pavon was used. The leaf rust score differed over the years and environment.

Substitution lines carrying *Lr46* displayed leaf rust severity of 20-30%, while the susceptible check Lalbahadur showed a susceptibility of 80-100%.

Rosewarne *et al*. (2006) identified the presence of the *Lr46*/*Yr29* locus in a population developed through the Avocet-Yr-A x Attila cross using AFLP markers. They observed that the population segregated for leaf tip necrosis (LTN), a trait previously associated with *Lr34*. Single chromosome recombinant lines were used to confirm the association of LTN with *Lr46*. The results of their study concluded that LTN is also pleiotropic or closely linked to *Lr46* and suggested that a new LTN gene designation should be given to this locus. They suggested that LTN is a good phenotypic marker when *Lr46* and *Lr34* are individually used in combination with other leaf rust resistance genes. In crosses containing both of these genes, the use of molecular markers will help to identify lines carrying both genes. They found a continuous distribution of variation for stripe rust and leaf rust resistance among the lines derived from the population. Attila, a resistant parent, scored very low for both leaf and stripe rust, while Avocet-S showed high susceptibility to both rusts. Genetic analysis of the population indicated the involvement of two additive genes in resistance and the *Lr46*/*Yr29* locus was the main contributor to resistance. They also identified an epistatic interaction for stripe rust resistance between *Lr46*/*Yr29* locus and another unmapped region (Rosewarne *et al*., 2008).

Kuchel *et al*. (2007) used marker assisted selection to combine the superior dough quality of the Australian wheat cultivar Stylet with adult plant resistance from Annuello. They used SSR markers to screen lines with adult plant resistance genes *Lr34*/*Yr18* and *Lr46*/*Yr29*. They used BC1F1 lines and some fixed advanced lines for comparison of marker assisted selection efficiency. They concluded that the marker assisted selection of the donor alleles was more effective with early generation populations rather than the fixed lines. Results of their study suggested that the use of marker assisted selection at the early stages of a breeding program can increase genetic improvement in wheat for rust resistance.

Asalf *et al*. (2008) reported that CIMMYT bread wheat line Saar has a high level of partial resistance to powdery mildew (PM), leaf rust (*LR*) and stripe rust (YR). Saar is known to carry *Lr34/Yr18*. A population of 113 recombinant inbred F6 lines from a cross between Saar and the susceptible line Avocet-*YrA* was tested for *LR* and YR in Mexico and PM in Norway and China. There was a strong association among the disease scores and they were all strongly correlated with leaf tip necrosis (LTN). A bulked segregant analysis with SSR markers was conducted to identify molecular markers associated with the resistance to PM. Two major QTLs were identified, one on chromosome 7D and the other on chromosome 1B, corresponding to the adult plant rust resistance loci *Lr34/Yr18* and *Lr46/Yr29*, respectively.

Lillemo *et al*. (2008) mapped the QTLs for resistance to powdery mildew in a cross between the powdery mildew resistant bread wheat variety Saar and the susceptible variety Avocet. The major powdery mildew resistance locus in Saar was found to be located on 7DS and 1BL, chromosomes that also carry genes for leaf rust and stripe rust resistance (*Lr34*/*Yr18* and *Lr46*/*Yr29*). The results of their study suggested that the resistance effect of *Lr34*/*Yr18* locus was stronger than that of the *Lr46/Yr29*, thus proving that the resistance conditioned by these two loci against leaf rust, yellow rust and powdery mildew is not because of the genetic linkage but because of an individual gene effect. Lillemo *et al*. (2008) designated the powdery mildew partial resistance genes located on 7DS and lBL as *Pm38* and *Pm39* that also corresponds to the *Lr34*/*Yr18* and *Lr46*/*Yr29* regions, respectively.

Sharma and Saini (2011) reported that bread wheat cultivars Capelle Desprez and Pari 73 have been showing adult plant leaf rust resistance in India since 20 years. F2 and F3 generations from crosses of Capelle Desprez and Pari 73 with susceptible cultivar WL711 were tested for percent disease severity against leaf rust race 77-5 which suggested the presence of three genes in Capelle Desprez and two genes in Pari 73 to leaf rust. Allelic tests using Capelle Desprez with RL6058 indicated the presence of linked genes *Lr34*/*Yr18* however, presence of transgressive segregants in this cross indicated that the other two genes in Capelle Desprez are also involved in leaf rust resistance. The segregation for susceptible plants observed among all the crosses used for allelic tests of Pari 73 for leaf rust indicated that nonhypersensitive resistance genes in Pari 73 are different from those in RL6058, HD2009 and Capelle Desprez. Studies using 536 primers indicated that one of the three rust resistance gene(s) in cultivar Capelle Desprez is located on chromosome 1B, at a distance of 26.3cM from the primer *Xgwm 268*. Chromosome location of leaf rust resistance gene from cultivar Pari 73 could not be achieved.

In the last two decades, advances in the field of molecular markers have contributed towards identification of genes/quantitative trait loci (QTLs) for the various leaf rust resistant genes (Mcintosh

et al., 2011). Though, various molecular markers are available for plant genotyping, simple sequence repeats (SSRs) have found large scale application in mapping of genes due to several advantages such as they are highly polymorphic, highly reproducible and uniform distribution throughout the genome in comparison to other markers. Now, a number of SSR markers have shown their linkage with various leaf rust resistance genes which are currently being used in marker assisted selection (Gultyaeva *et al*., 2009).

Despite the importance of slow rusting mechanism in the control of rust epidemics, very few efforts has been made to combine the information of differential reaction with various rust isolates and molecular markers linked with slow rust resistant genes, which can play a vital role in the development of more resistant cultivars against leaf rust. Impact of slow rust resistant genes alone or in combination to achieve durable resistance/near immunity is yet to be done. Genes responsible for slow rusting can be brought together with the help of molecular markers (Sareen *et al*., 2012).

Lillemo *et al*. (2013) reported that *Lr34* was found to constitute the main locus for spot blotch resistance and explained as much as 55 % of the phenotypic variation in the mean disease data across the six environments. Based on the large effect, the spot blotch resistance at this locus has been given the gene designation *Sb1*. Two further, minor QTL were detected in the sub-population of RILs not containing *Lr34*. The first of these was located about 40 cM distal to *Lr34* on 7DS and the other corresponded to *Lr46* on 1BL. A major implication for wheat breeding is that *Lr34* and *Lr46*, which are widely used in wheat breeding to improve resistance to rust diseases and powdery mildew, also have a beneficial effect on spot blotch.

2.3.6.3 Slow Leaf Rust Resistant gene *Lr67*

Herrera-Foessel *et al*. (2011) reported that molecular mapping using microsatellites led to the identification of five markers (*Xgwm165*, *Xgwm192*, *Xcfd71*, *Xbarc98* and *Xcfd23*) on chromosome 4DL that are associated with *Lr67*/*Yr46* gene(s), with the closest markers being located at 0.4 cM. In a parallel study in Canada using a Thatcher × RL6077 F_3 population, the same leaf rust resistance gene was designated as *Lr67* and mapped to the same chromosomal region. The pleiotropic, or closely linked, gene derived from RL6077 that conferred stripe rust resistance in this study was designated as *Yr46*. The slow-rusting gene(s) *Lr67*/*Yr46* can be utilized in combination with other slow-rusting genes to develop high levels of durable APR to leaf rust and stripe rust in wheat.

2.3.6.4 Slow Leaf Rust Resistant gene *Lr68*

Herrera-Foessel *et al*. (2012) reported that the common wheat cultivar Parula possesses a high level of slow rusting, adult plant resistance (APR) to all three rust diseases of wheat. Previous mapping studies using an Avocet-*YrA*/Parula recombinant inbred line (RIL) population showed that APR to leaf rust (*Puccinia triticina*) in Parula is governed by at least three independent slow rusting resistance genes: *Lr34* on 7DS, *Lr46* on 1BL and a previously unknown gene on 7BL. The use of field rust reaction and flanking markers identified two F6 RILs, Arula1 and Arula2, from the above population that lacked *Lr34* and *Lr46* but carried the leaf rust resistance gene in 7BL, hereby designated *Lr68*. Arula1 and Arula2 were crossed with Apav, a highly susceptible line from the cross Avocet-*YrA*/Pavon 76 and 396 F4-derived F5 RILs were developed for mapping *Lr68*. The RILs were phenotyped for leaf rust resistance for over 2 years in Ciudad Obregon, Mexico, with a mixture of *P. triticina* races MBJ/SP and MCJ/SP. Close genetic linkages with several DNA markers on 7BL were established using 367 RILs; Psy1-1 and gwm146 flanked *Lr68* and were estimated at 0.5 and 0.6 cM, respectively.

2.3.7 Studies on expression of oxidative enzymes (isozymes) in selected bread wheat under the pathogenesis of leaf rust races

The term isozyme was coined by Markert and Moller (1959) and described different molecular forms of enzymes with the same substrate specificity occurring within the same organism. In wheat and leaf rust interaction, Nargund (1989) noticed appearance of new isozymes bands of peroxidase and polyphenol oxydase. Southerton and Deveralli (1990) observed increased activity of peroxidase during resistance expression in the leaves of wheat against leaf rust fungus.

Mohan and Khanna (1988) studied peroxidase and polyphenol oxidase activities in 10 near isogenic lines carrying resistance genes to *P. recondita* and two carrying susceptible genes. All isolines with *Lr* genes showed greater activity of both the enzymes as compared to susceptible. Narsimhan and Chawla (1989) observed seven isozymes forms, greater polyphenol oxidase activity was detected in nine *Lr* lines as compared to two susceptible. Wand *et al*. (1994) observed

peroxidase activities in wheat lines carrying different *Lr* genes when inoculated with two different leaf rust races.

Juan *et al.* (1989) observed that two peroxidase (PO) activity peaks appeared in wheat leaves during the infection by *P. recondita* f. sp. *tritici*, peak first appeared in 60 hours after inoculation (hai) and peak second appeared in 84 or 132 hai. The peroxidase activity changes in slow rusting cultivars had their own characteristics. The activity of slow rusting cultivar increased sharply at 36 hai and its peak second was much higher than that of susceptible ones. They were concluded that the PO and polyphenoloxidase (PPO) activity changes in the resistant, susceptible and slow rusting cultivars, after their infection by leaf rust of wheat, all had their own characteristics. The analysis of changes in PO and PPO activities can be served as criteria for assessing the resistance of wheat varieties to leaf rust. The analysis is especially useful for differentiating slow rusting cultivars from susceptible ones.

Mohammadi and Kazemi, (2002) measured guaiacol-peroxidase (POX) and polyphenol oxidase (PPO) activities spectrophotometrically in resistant (cvs. Sumai#3 and Wang shui-bai) and susceptible (cvs. Falat and Golestan) wheat heads at flowering, milk, dough and ripening stages following the inoculation with *Fusarium graminearum* at anthesis. POX specific activity in resistant and susceptible wheat cultivars showed a significant increase during the milk stage as compared with the non-inoculated control plants. POX activity reached the highest level in heads of Wang shui-bai followed by those of Falat, Sumai#3 and Golestan cultivars at milk stage. PPO specific activity in wheat heads reached a maximum level during the milk stage and subsequently declined. This activity was three times higher in the resistant cultivars than the non-inoculated control plants. In Falat and Golestan cultivars, PPO activity level was half of those in resistant cultivars. The optimal pH for PPO was 6.4. PPO-catalyzed reaction was inhibited by ascorbic acid. Activity stain in non-denaturing polyacrylamide gel revealed the presence of one basic and six acidic isozymes in wheat heads. The susceptible Falat heads pre-treated with an autoclaved mycelial wall preparation showed induced resistance against FHB and increased activities of POX and PPO.

Nagaveni (2005) reported that peroxidase activity was higher in the resistant barley genotype than in the susceptible genotype. It was noticed that at 30 and 60 DAS in susceptible varieties, the number of isozyme bands were comparatively less with low Rm value at 30 and 60 DAS compared to the resistant varieties.

The proteome of the susceptible wheat /*P. triticina* interaction was interrogated using two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) at 9 days post-inoculation (Rampitsch *et al*., 2006). Using a matrix-assisted laser desorption/ionization (MALDI) source coupled to a tandem quadrupole/time-of-flight (Qq-TOF) tandem mass spectrometer (MS/MS), 22 fungal proteins were characterized. These belonged to several protein classes such as metabolic enzymes, structural proteins and putative virulence-related proteins, the last of which includes heat-shock and 14-3-3- like proteins (Rampitsch *et al*., 2006).

Recently, an expressed sequence tag (EST) database was developed representing each of several life-cycle stages of *P. triticina* (Hu *et al*., 2007b). cDNA libraries were constructed from resting urediniospores, germinating urediniospores, an appressorial stage on compatible leaves, a haustorial stage on compatible leaves and an incompatible interaction 24 h after inoculation. Although there was significant overlap in transcripts found between resting and germinated urediniospores, little overlap was found between the other stages, suggesting distinct coordinated gene expression changes at each stage. The presumed fungal cDNAs encoded proteins involved in general metabolism, protein synthesis and transport-, stress- or virulence-related proteins (e.g. glutathione-S transferase, catalase, heat shock proteins, chitinases and cytochrome P-450 monooxygenases). One gene generated from this study, *PtMAPK*, encoded a mitogen-activated protein kinase (MAPK), which was functionally analysed by complementation in the corn smut fungus *Ustilago maydis* (Hu *et al*., 2007a). The results suggest that *PtMAPK* is involved in pathogen development, as are its orthologues in *U. maydis* (Hu *et al*., 2007a).

Haggag *et al.* (2009) noticed that leaf rust of wheat incited by *P. recondita* f. sp. *tritici* is one of the most important wheat disease in Egypt. Methyl jasmonate (MJ) is a potential plant elicitor which induces a wide range of chemical and anatomical defense reactions in conifers and might be used to increase systemic resistance against biotic damage. In the greenhouse, different concentrations of MJ (10, 20 and 30 mM) were applied as seed soaking plus foliar spray or only as foliar spray to control leaf rust and induction of secondary compound production in leaves of wheat plants. Foliar spray was applied after 30 and 50 days of sowing. Results indicated that all concentrations and treatments reduced the severity of rust disease caused by *P. recondita* f. sp. *tritici* in wheat leaves

during 45 days of inoculations. Disease incidence was decreased significantly in MJ-treated plants as seed soaking plus foliar spray with 20 and 30 mM when compared to 10 mM MJ or control plants. The study revealed that, with increasing concentrations of MJ, the secondary metabolites were greatly increased. Endogenous levels of both free and conjugated putrescine, spermidine and spermine increased in response to the elicitor. Activities of polyamine biosynthetic enzymes of ornithine decarboxylase (ODC) and polyamine oxidase (PAO) displayed up to threefold increases relative to untreated control. Moreover, significant increases in activities of plant defense-related protein, enzymes as peroxidase and chitinase as well as free and conjugated phenols contents were recorded in treated plants compared with untreated and infected plants.

In isozymatic analysis of peroxidase, polyphenoloxidase and catalase enzymes polymorphic banding pattern was observed in some isolates of *Pythium aphanidermatum* causing rhizome rot of ginger indicating variation in the pathogen (Shalini *et al*., 2009).

Polyphenol oxidase and peroxidase activities in diseased fruit of banana, under the pathogenesis of *Macrophomina phaseolina, Fusarium oxysporum and Nigrospora oryzae* underwent significant changes. An increase in polyphenol oxidase and peroxidase activity was recorded up to 8th day of inoculation in Cavendish variety, while in other three varieties, an increase in enzyme activity was recorded up to 6th day. The increase was more significant in *M. phaseolina* infected fruits followed by *F. oxysporum* and *N. oryzae.* In Rasthali and Poovan varieties the increase in polyphenol oxidase and peroxidase was recorded upto 6th day of inoculation in course of infection by three fruit-rot fungi under study (Sarkar *et al*., 2010).

2.3.8 Histology of slow leaf rusters

Stubs and Plotnikova, (1972) observed differences in spore germination and germ tube penetration of race 60 of *P. striiformis* on wheat varieties investigated *T. spelta* L. var. Album was the most resistant, strongly inhibiting spore germination and retarding germ tube penetration. On the hexaploid wheat varieties the germ tube penetrated through the stomata, wheareas on the tetraploid varieties it did so at the junction of two epidermal cells. There was no correlation between density or length of hairs on the leaves and the rate of spore germination and germ tube penetration.

Heath (1981) suggested that the mechanisms, presented before the formation of the first haustorium are those of the strongest impact. After the fungus invades the tissues of an incompatible leaf, some adverse effects on growth or the appearance of sub-stomal bladder and infection hypha become evident. Although this is more common in non-hosts, it is also reported for resistant cultivars (Leath and Rowell, 1966). In all the genotypes with *P. triticina* evaluated, haustoria formation was not observed; there was, however, formation of colonies, which according to Bushnell (1972) is probably due to the fact that forming of haustorium occurs immediately after penetration; but this moment is not visible because of the different compounds, that are released with the breaking of the cell wall. Rubiales and Niks (1995) indicated that in the infection process of leaf rust, partial resistance conferred by the gene *Lr34* was based on a diminution of haustoria formation rate at the early stage of infection, associated to little or total absence of vegetal cell necrosis, which could explain why haustoria were not observed in the genotypes having this gene.

Poyntz and Hyde (1987) reported differences among wheat varieties, resistant and susceptible to leaf rust in germination and germinative tube length; the lowest percentage in both types corresponded to the susceptible varieties and in the infection process the largest colonization was presented by the susceptible varieties. Chang and Line (1983) reported that there are no significant differences in appresorium formation among susceptible genotypes and with partial resistance in wheat. The differences in percentage of appresorium formation between the apex and the central part of the leaf are relating all differences in environmental conditions (Broers and Jacobs, 1989).

Hu and Rijkenberg (1998) studied the morphology of infection structure development of *P. recondita* f. sp. *tritici* on and in susceptible and resistant wheat lines inoculated with urediospores was examined by SEM. The germ-tube extends over the leaf surface and elongates perpendicularly to the long axis of the leaf. When the germ-tube encounters the stomatal lip, an appressorium forms over the stoma and the pore is entered by an infection peg produced on the surface of the appressorium in contact with the host leaf. At 6 h post-inoculation (hpi), infection pegs develop terminally substomatal vesicles (SSVs) in the substomatal chambers of all wheat lines. A septum separates each SSV from its interconnective tube. A primary infection hypha forms terminally from the elongated SSV either parallel to the long axis of the stomatal slit or perpendicular to the leaf surface. When a primary

infection hypha attaches to a host cell, a septum forms cutting off the tip of the hypha, delimiting a terminal haustorium mother cell (HMC) by 12 hpi. Secondary infection hyphae arise from a position proximal to and in the proximity of, the HMC septum. Additional HMCs are formed when a secondary hypha or a tertiary hypha adheres to a plant cell. Infection sites with HMCs were observed at 24 hpi and at subsequent sampling stages. There were no significant differences between the infection processes on the three wheat lines examined.

Garcia-Lara *et al.* (2007) studied the infection process with partial resistance. The genes *Lr34*, *Lr46* and one gene (Gene 1) not yet named confer to prehaustorial resistance, Jupateco 73R+*Lr34*, Avocet+*Lr34*, Lalbahadur+*Lr46* and Lalbahadur+Gene 1 and in genotypes that do not contain the gene: Jupateco 73S-*Lr34*, Avocet-*Lr34* and Lalbahadur. In the percentage of urediospore germination, there were no significant differences in multiple comparison of means ($p>0.05$; DMS). They were found that Lalbahadur+*Lr46* and Lalbahadur+*Lr34* showed higher percentage of reduction ($p\leq0.05$) in the uredospore germination percentage. With respect to the formation of appresoria, penetration and substomatal bladder formation, significant differences ($p\leq0.05$) between the genotypes Lalbahadur+*Lr46* and Lalbahadur+*Lr34* were noticed. Besides, papillae were detected in Jupateco 73R 40 h after inoculation. From the results obtained it is found that *Lr34*, *Lr46* and Gene1 confer prehaustorial resistance, or in other words, before the haustorium of the fungus, which is very similar to the resistance of the non-host, that is of the durable type.

2.4 Evaluation of identified slow leaf rusters for quality traits

Until the recent past rust resistance and plant yield are the prime area where the wheat breeders all over the world were concentrating. When the humankind realized that it is not only the energy which satisfies the human diet but also there is a need of nutritious food. Realizing the need of nutritious food from the last decade the interest of breeders slowly moving towards quality parameters in that micronutrient like Iron, Zinc, Manganese, Copper and Magnesium concentrations are of prime importance. The research in this area is still in infant stage. The available literature regarding this studies are cited below,

Dyck and Lukow (1988) explained that resistant backcross lines with either *Lr29* or *Lr*VPM had higher kernel protein levels than did susceptible sister lines under both rust and rust-free conditions. Although this higher protein content was associated with weaker dough mixing properties, the remix loaf volume remained constant. Leaf rust infection had a detrimental effect on grain yield and kernel weight and on wheat quality as shown by decreased kernel protein content and farinograph absorption. Dough mixing strength was higher for the rust infected lines than the rust resistant lines.

The quality and productivity of wheat is often reduced because of leaf rust caused by *Puccinia recondita* f. sp. *tritici*. The extent of yield losses caused by leaf rust depends on the nature and number of leaf rust resistance genes in the cultivars and the composition of virulence in the respective geographical region (McIntosh *et al.,* 1995).

Herrman *et al.* (1996) evaluated the impact of leaf rust on physical grain quality, milling properties, flour protein content, absorption and peak mixing time, changes in grain characteristics and flour properties for the fungicide treatment during 1991 and 1992 whereas, extraction per cent and test weight did not respond significantly ($P>0.05$). Difference between control and fungicide treated wheat were consistent between years (during 1991 and 1992) except for single grain size standard deviation.

Mineral elements play essential roles in the biochemical and physiological functions of any biological system. In plants, appropriate mineral availability is essential for almost every aspect of development, from seed germination and seedling development (Welch, 1999) to yield formation and mineral deposition in grains (Yilmaz *et al*., 1998; Welch, 1999). Mineral elements are also essential nutrients for animal and human well-being. It is estimated that over three billion people suffer from micronutrient malnutrition worldwide (Bouis, 2003; Welch and Graham, 2004; White and Broadley, 2009), resulting in overall poor health, anemia, increased morbidity and mortality rates and low worker productivity (Holtz and Brown, 2004; Sanchez and Swaminathan, 2005; Cakmak, 2008).

Everts *et al*. (2001) observed the changes in milling and baking quality of soft red winter wheat can have a large economic impact on flour mills. To determine the relationship between early-season powdery mildew and late-season leaf rust on flour yield, flour protein, alkaline water retention capacity and kernel texture. A regression model was developed to describe the relationship between the log of the area under the disease progress curves and adjusted flour yield (AFY). The AFY of

Saluda was reduced in the presence of powdery mildew such that %AFY = 103.96 – 0.92 (log AUMPC).

Martin *et al.* (2003) studied the contribution of leaf rust resistance and awns to agronomic and grain quality performance in winter wheat. They reported that for the average effects of *Lr* 41 and *Lr*42, grain yield increased by 63 and 26 per cent, test weight increased by 5 and 3 per cent and kernel weight increased by 14 and 9 per cent. They showed that leaf rust resistance also improved milling quality by increasing flour yield and kernel diameter, independent of the presence or absence of awns.

O'Brien *et al*. (1990) reported that flour from stripe rust affected grains resulted in weaker dough as measured by shorter dough development time. According to Czuchajowska and Pasczyñska, (1996), bread volume increases by 46 to 65 cm^3 for each 1% increase of wet gluten content. Wet gluten ranked the second most desired test to assess field testing of wheat functional quality and end-use characteristics (Chinnaswamy *et al*., 2005).

Ortiz-Monestrio *et al.* (2007) a wide range of germplasm was studied at CIMMYT and reported the variability ranging from 28.8 to 56.5 mg per kg for iron and 25.2 to 53.3 mg per kg for Zn and also showed that among all wheat germplasm studied the species *T. dicoccum* had the highest concentration and he also noted the presence of positive correlation between Zn and Fe.

Calderini and Ortiz-Monestrio (2003) measured biomass, grain yield, micronutrient and macronutrient content in hexaploids with two cultivars and one synthetic hexaploid. The synthetic hexaploids showed higher concentrations of selected micro and macronutrients in grains between 25 and 30 per cent more for iron, magnesium and zinc. These lines also showed higher nutrient uptake of potassium and phosphate and better distribution of micronutrients to the edible grain portions of the wheat, all with yields similar to the non-synthetic hexaploid lines. From these findings, the researchers suggest that synthetic hexaploids would be a valuable source of germplasm for increasing micronutrients in wheat

Cakmak *et al.* (2004) studied the large number of accessions of wild wheat and its relatives which were collected from fertile crescent and screened for Fe and Zn concentrations as well as other mineral nutrients. Among wild wheat, collections of wild emmer wheat, *T. turgidum* sp. *dicoccoides* showed impressive variation and highest concentration of micronutrients.

Kumar and Raghavaiah (2004) Near-isogenic lines carrying the *Lr28* gene developed in five genetic backgrounds were tested for 2 years with and without fungicide treatment. The *Lr28* gene increased grain yield, 1000-grain weight and number of effective tillers per plant under heavy leaf rust infection with no negative effects on yield and bread-making quality in rust-free plots. Although a reduction in dough development time was found to be associated with *Lr*28, it can still be used extensively in wheat breeding programmes.

Wheat (*T. aestivum*) flour has the ability to form dough with unique properties that can be used to produce leavened bread and other food products. This ability is largely determined by glutenins and gliadins, which are two important subfractions of gluten proteins (Singh and Khatkar, 2005).

Ozkan *et al.* (2005) accessed the variation for seed micronutrient content in 54 accessions of einkorn wheat (*T. monococcum*). The result showed the existence of large genotypic variation in content of micronutrients. The contents of Zn and Fe varied from 0.21 to 2.16 mg /seed for Zn with average of 1.19 mg/seed and from 0.54 to 3.09 mg/seed for Fe with average of 1.19 mg/seed and also showed the presence of positive relationship between Fe and Zn, the results of the four traits showed that a major QTL which is common to all four micronutrients explaining from 10 to 30 per cent observed on chromosome-5.

Welch *et al.* (2005) investigated 28 genotypes of *Triticum sps* among the grain Zn concentration differed significantly across the genotypes and ranged from 33 to 149 mg per kg and Fe varied four fold from 80 to 368 mg per kg and showed the presence of positive correlation between the two characters.

Tiwari *et al.* (2005) Analyzed grains of 80 accessions of wild *Triticum* and seven *Aegilops* species along with 15 semi-dwarf bread and durum wheat cultivars for iron and zinc content. The bread wheat and durum cultivars had very low content and limited variability for iron and zinc content. The *Aegilops* species showed up to 2-3 fold higher grain iron and zinc content than the cultivars.

Yusuf *et al.* (2005) shown that a strategy that exploits genetic variability to breed staple crops with enhanced to fortify themselves with micronutrient offers sustainable, cost effective and alternative to conventional supplementation and fortification programs which is more likely to reach most in need.

Chhuneja *et al.* (2006) analyzed *Aegilops kotschyi* and *A. tauschi* for Fe and Zn content in grains and showed that S and SD genome species accumulates significantly higher iron and zinc in the grains than that cultivated wheats observed that one of the CIMMYT synthetics also had significantly higher Fe and Zn in the grain as compared with the cultivated wheat. The study shows that *Aegilops kotschyi* as a promising source for Iron and Zinc.

Oury *et al.* (2006) studied the grain magnesium (Mg), zinc (Zn) and iron (Fe) concentrations in germplasm collection and elite breeding lines or modern cultivars, of bread wheat (*T. aestivum*) grown in different environments. Zn concentration generally ranged from 15 to 43 ppm, 600 to 1890 ppm of mg and 20 to 88 ppm of Fe and showed that Zn and Mg concentration are positively correlated to each other. They also revealed the presence of significant G X E interaction for micronutrient.

Morgounov *et al.* (2007) Selected sixty-six spring and winter common wheat genotypes from Central Asian breeding Programs and evaluated for grain concentrations of iron (Fe) and zinc (Zn). Iron showed large variation among genotypes, ranging from 25 mg kg^{-1} to 56 mg kg^{-1} (mean 38). Similarly, Zn concentration varied among genotypes, ranging between 20 mg kg^{-1} and 39 mg kg^{-1} (mean 28 mg kg^{-1}). Spring wheat cultivars possessed higher Fe-grain concentrations than winter wheats.

Liu *et al.* (2006) studied eighty six genotypes from different areas of china and studied the phytate zinc concentrations. His studies revealed that Zinc and Iron were positively correlated and the effect of grain position on all these components was significant, generally grains on the third floret of central spikelets had the lowest Zinc and phytate contents compared to other seeds within a spike.

Hailu and Fininsa, (2007) studied the effects of stripe (yellow) rust caused by *P. striiformis* f. sp. *tritici* on grain quality of three bread wheat varieties. Grain quality parameters: hectolitre weight, grain protein content and wet gluten were measured following AACC procedures. The spray intervals significantly differed disease severity. High levels of disease severity and longer epidemic duration at Agarfa increased grain protein content of the susceptible variety to 7% while lower levels of severity and shorter epidemic duration at Sinana reduced to 2 to 7%. Similarly, relative gluten content losses of 3 to 13% at Agarfa and 6 to 9% at Sinana were measured in grains of the susceptible variety in 2002. In 2003, wet gluten content loss increased to 21 per cent at Agarfa.

Mobarak *et al*. (2007) studied the rheolegical tests showed increment of water absorption, development time dough stability, extensibility, resistance to extension and dough energy in infected wheat dough compared with protected wheat dough of the same wheat cultivars. Manthey *et al.* (2006) evaluated the rheological and gelatinization properties of durum wheats grown in the USA with Mixolab (Alveoconsistograph), their results showed variability in terms of protein quality and starch pasting properties, which indicated that Mixolab could be used to determine durum wheat quality. Pena *et al.* (2006) found that the Mixolab dough development time, stability and breakdown parameters showed high correlation with the Alveograph W value when testing the whole grain flour. However, the studies related to the utilization of Mixolab to evaluate the bread making quality of flours are limited. Overall results of the present study indicated that Mixolab can be used to predict the bread wheat quality and Mixolab can be used to differentiate wheat genotypes in terms of different quality characteristics.

Mobarak *et al*. (2007) studied the effect of stripe rust disease infection on the quality of physical, chemical and technological characteristics of wheat grain, flours and bread produced from seven Egyptian wheat cultivars (Sakha-8, Sakha-69, Sakha-93, Gemmiza-5, Gemmiza-7, Gemmiza-9 and Giza-168) in compared with uninfected (protected by fungicide) ones. Physical qualities of wheat grain of all wheat cultivars infected with stripe rust disease greatly decreased, where 1000 grain weight, hectoliter weight and flour extraction greatly decreased compared with the same protected wheat cultivars. The infected cultivar, Sakha-8 showed the highest decreased in 1000 grain weight, hectoliter weight and flour extraction rate, which were 24.3 per cent, 13.4 per cent and 22.6 per cent respectively. Meanwhile Gemmiza-9 showed the lowest decrease, which amounted in 0.7 per cent, 0.5 per cent and 0.7 per cent for the above mentioned characteristics respectively, compared with the same uninfected and protected (protected) cultivars. Chemical quality of flour of all wheat cultivars infected with stripe rust improved greatly, where protein, wet gluten, dry gluten ratios greatly increased compared with the same protected cultivars. Baking quality and sensory of balady bread produced from infected wheat cultivars improved greatly compared with balady bread produced from

the same protected cultivars. Where loaf volume and total score of sensory evaluation exhibited the superior values of baking quality and sensory evaluation.

Abebe (2008) Studied for concentrations of grain Fe, Zn and other minerals in two locations. The combined analysis of variance showed significant variation in concentrations of grain minerals among inbred lines in each trial, which was always greater than the variation caused by locations and line X location interactions. The line X location interaction had no significant effect on concentrations of Fe, Zn, Cu, Mg and P. Utilizing leaf rust effective resistant genes in wheat breeding programs and growing resistant cultivars on a large scale would most likely decrease leaf rust related yield and quality losses (Akin *et al*., 2008).

Cakmak (2008) shown that the cultivated wheats contain very low levels of Zn and shows narrow genetic variation for Zn as compared to cultivated wheat. Wild and primitive wheat represents a better and more promising genetic source for Zn among wild collections emmer wheat, *Triticum turgidum* sp *dicoccoides* had the highest genetic variation and highest concentration.

Peleg *et al.* (2008) reported that a new wild emmer wheat accessions have been identified showing simultaneously very high concentration for both Zn and Fe (up to 139 mg per kg for Zn and 88 mg per kg for iron) for protein also it has shown upto 380 mg per kg in seeds and also high tolerance to drought stress and Zn deficiency in the soil.

Ficco *et al.* (2009) studied 84 Italian durum wheat cultivars of old and new germplasm in two locations and showed that content of Zn is ranged from 28.5 to 46.3 mg per kg with average of 37.4 mg per kg and Fe ranged from 33.6 to 65.6 mg per kg with average of 49.6 mg per kg grain, Phosphorus content was 0.46 to 0.76 mg per g showing positive correlation with all minerals except Cu and Zn also showed the significance G x E interaction.

Zhao *et al*. (2009) Observed substantial variation among 175 lines existed in grain Fe, Zn and Se concentrations. Spelt, einkorn and emmer wheat appeared to contain higher Se concentration in grain than bread and durum wheat. Significant differences between bread wheat genotypes were found for grain Fe and Zn, but not Se concentration, Both grain Zn and Fe concentrations also correlated positively and significantly with grain protein content and P concentration, but the correlations with kernel size, kernel weight or bran yield were weak.

The 265 genotypes displayed a large variation for all mineral elements investigated including Fe and Zn, ranging from 28.0 to 65.4 mg kg-1 and 21.4 to 58.2 mg kg-1 for Fe and Zn, with mean values of 39.2 and 32.3 mg kg-1, respectively. Jimai 26, Henong 326 and Jingdong 8 displayed high Fe and Zn concentrations. Jimai 26 and Henong 326 also displayed high concentrations of Cu, Mg, K, P and protein content (Zhang *et al*., 2010).

Makarska *et al.* (2010) revealed that difference in the amount of mineral components in triticale hybrids depends on genotypes of parental forms and also shown that hybrids are having higher nutrient content compared to both the parents.

Ferney *et al.* (2010) studied 19 wild emmer wheat genotypes and the largest variation was observed in Mn concentration (13-87 mg/kg). Accessions with higher nutrient concentration had also shown higher grain yield, analysis of variance showed that significant for environmental variation *i.e*. Up to 44 per cent but genotypic effect was also important for Mg, Zn, Mn and S.

Merav *et al*. (2010) showed the presence of wide genetic diversity among the wild emmer accessions for all grain nutrients. The concentrations of grain zinc, iron and protein in wild accessions are about two-fold greater than in the domesticated genotypes. Concentrations of these compounds are positively correlated with one another, with no clear association with plant productivity, suggesting that all three nutrients can be improved concurrently with no yield penalty. A subset of 12 populations also revealed significant genetic variation between and within populations for all minerals. Association between soil characteristics at the site of collection and grain nutrient concentrations showed negative associations between soil clay content and grain protein and between soil-extractable zinc and grain zinc, the latter suggesting that the greatest potential for grain nutrient minerals lies in populations from micronutrient-deficient soils.

Kwasniewska-Karolak *et al*., (2011) relations between grain hardness and starch damage in the obtained flour were analyzed. Grain hardness, total content of nitrogen compounds and raw protein and the degree of starch damage in flour were studied. The obtained results were verified using statistical analysis. It was shown that the degree of starch damage shows a high positive correlation with grain hardness ($r = 0.7$), which depends mostly on wheat genotype. Weather

conditions during wheat growth in central Poland did not have a significant effect on grain hardness, although the grain of spring wheat cultivars was more sensitive to changeable weather conditions than the grain of winter wheat cultivars.

Suchowilska *et al.* (2012) investigated the whole grain of spring lines of emmer, einkorn, spelt and two common wheat cultivars, all grown under identical environmental conditions. He showed *Triticum* species differed significantly with respect to the concentrations of P, Mg, Zn, Fe, Mn, Na, Cu, Sr, Rb and Mo. The grain of all hulled wheats, compared with common wheat, contained significantly more Zn (from 34 to 54 per cent), Fe (from 31 to 33 per cent) and Cu (from 3 to 28 per cent). In the majority of cases, there were no relationships between the concentrations of the analyzed elements, except for significant positive correlations between the levels of Fe, Zn and Mn, in particular in *T. monococcum* and *T. dicoccum* and also showed that a significant discrimination in concentrations of the investigated elements are a species-specific character. A strong correlation between Zn, Fe and Mn are important implications for wheat quality breeding.

2.4.1 Economic yield and thousand grain weight loss assessment

Wheat cultivars that are susceptible to leaf rust regularly suffer yield reductions of 5–15 per cent or greater, depending on the stage of crop growth when the initial rust infections occur. The economic and social upheals resulting from crop loss following epidemic have been the dominant influence on the research activities directed on wheat rusts. Roelfs (1978) compiled an over view of losses due to the cereal rusts in the United States of America from 1918 to 1976, noting state wide yield reduction of 50 per cent or more in the epidemic years due to black and leaf rust. In Australia, sequence of severe stem and leaf rust epidemics in 1980's resulted in the establishment of State Department of Agriculture in New South Wales and Victoria (Rees and Platz, 1975).

Occurrence of rust epidemic in India has been chronologically provided by Nagarajan and Joshi (1975) and Joshi *et al.* (1980). Severe rust epidemics occurred in central provinces in 1832 and 1879. In recent past, rust epidemics reported are of 1946-47, 1971-72, 1972-73 and 1978-79. The 'Sonalika epidemic' of leaf rust swept over entire Uttar Pradesh and part of Bihar in 1980 (Joshi *et al.,* 1984).

The global crop loss caused by three rusts of wheat analysed by Saari and Prescott (1985), indicate more serious losses in South Asia due to leaf rust incidence. Milus (1994) reported that leaf rust and septoria leaf blotch are the most serious foliar fungal diseases of wheat in Arkansas. Field experiments were conducted on three wheat cultivars during two growing seasons at two locations. For each cultivar, foliar fungicides caused differences in leaf rust and leaf blotch severities, yield and test weight. Regression was used to determine the relationship of leaf rust and leaf blotch severities to yield and test weight losses. Average yield losses for cultivars Florida 302 and Rosen were 0.30 and 0.25 per cent for each 1 per cent increase in rust severity, respectively. The average test weight loss was 0.08 per cent on Florida 302 and 0.03 per cent on Rosen for each 1 per cent increase in rust severity. Results of this study support the use of foliar fungicides on wheat in Arkansas to control leaf rust and leaf blotch and to protect yield and test weight potential. Estimates of rates of yield and test weight losses should be useful for making disease management decisions.

Khan *et al.* (1997) reported that out of 10 wheat varieties evaluated for slow leaf rusting, the varieties Chenab-70, WL-711 and Pak-81 were fast rusting cultivars suffering from 11.22, 19.73 and 13.88 per cent grain yield losses, respectively. The cultivars LU-26, V-87094 and V-8829 were moderately slow rusters with 8.10, 9.28 and 5.19 per cent yield losses, respectively and Paven, FSD-85 and INQ-91 were slow rusters. Although the cultivar SH-88 was fast rusting, it responded as rust tolerant with respect to yield loss. Fischer, (2001) reported that, yield losses due to leaf rust were mostly attributed to a reduction in TGW.

Sayre *et al*. (1998) found that leaf rust caused losses irrespective of the level of resistance possessed by the cultivars. Smedegaard-Petersen and Tolstrup (1985) observed that powdery mildew resistance in barley, highly resistant plants do not show any visible disease symptoms after inoculation does not mean that the plants are not affected.

The yield reduction depends on the stage of plant growth in which infection occurs, the degree of resistance of cultivars being used and disease severity which in turn depends on weather conditions (Casulli and Pasquini 1993, 1998; Pasquini and Zitelli 1984; Pasquini *et al*., 2003, 2005). Leaf rust caused by the biotrophic species *P. triticina* is one of the most common diseases of wheat, causing yield loss and decreasing grain quality (Sumikova and Hanzalova 2010).

Herrera-Foessel *et al*. (2006) ten durum wheat lines with race-specific resistance, 18 with slow-rusting resistance and 2 susceptible were included in two yield loss trials sown on different planting dates in Mexico with and without fungicide protection under high disease pressure. Eight genotypes with race-specific resistance were immune to leaf rust. Durum wheat lines with slow-rusting resistance displayed a range of severity responses indicating phenotypic diversity. Mean yield losses for susceptible, race-specific and slow-rusting genotypes were 51, 5 and 26 per cent, respectively, in the normal sowing date trial and 71, 11 and 44 per cent when sown late. Yield losses were associated mainly with a reduction in biomass, harvest index and kernels per square meter. Slow-rusting durum wheat lines with low disease levels and low yield losses, as well as genotypes with low yield losses despite moderate disease levels, were identified. Such genotypes can be used for breeding durum wheat genotypes with higher levels of resistance and negligible yield losses by using strategies that previously have been shown to be successful in bread wheat.

Afzal *et al*. (2007) reported that yield was significantly negatively correlated with the proportion of leaf area affected by stripe rust. The correlation coefficient (-0.67805) depicted highly significant effect of stripe rust in lowering wheat yield. There was varying resistance level among different wheat varieties. The extensively cultivated wheat variety, Inquilab-91 was found to be most resistant with minimum yield loss of 5.77 per cent followed by Wafaq-2001 and Bakhtawar with yield loss of 6.63 per cent and 14.90 per cent respectively.

2.5 Management of leaf rust through chemicals

Barros *et al.* (1982) reported that triademefon and mancozeb were most effective fungicides against stem and leaf rust of wheat. Jalinder (1983) reported that Vigil at 125 g. a.i. per ha. and Bayleton at 100 g. a.i. per ha. were highly effective in reducing the disease incidence in the field and in preventing the loss followed by Plantvax and Vigil at 75 g. a. i. per ha. against black stem rust of wheat.

Polityko *et al.* (1983) reported that in field experiment on wheat rust, Zineb completely inhibited uredospore germination while triadimefon had little effect. He also found that treatment with triadimefon, increased the incubation period and reduced the period of uredospore formation.

Nargund (1989) reported that maximum 1000 grain weight was noticed in propiconazole treatment (25.04 g) followed by diclobutrazol (23.20 g) and triadimefon (23.20 g) and also obtained similar trend in grain yield.

Brahma *et al.* (1991) evaluated efficiency of Tilt (propiconazole) on different cultivars during 1985-86 and 1986-87. Propiconazole (0.1 %) was applied to seedlings at the first appearance of rust. The fungicide was found effective against all rusts.

Sajid *et al.* (1995) studied the comparative effects of neem products and baytan against leaf rust of wheat in the laboratory. Neem oil and Baytan (Triademinol) completely inhibited germination of *P. recondita* f.sp. *tritici* uredospores. In the field neem oil at 4 per cent concentration checked leaf rust on wheat after four applications but Baytan at 0.1 per cent showed excellent rust control and best improvement in yield.

Flaishman *et al.* (1996) reported that strain BK8661 of *Pseudomonas putida* produces siderophores, antibiotics and low levels of hydrogen cyanide (HCN), suppresses growth of *Septoria tritici* and *P. recondita* f.sp. *tritici in vitro* and on wheat leaves. In the absence of siderophores and antibiotics, HCN production by the overproducing bacterial strains resulted in a small but statistically significant increase in the suppression of symptoms caused by *S. tritici* and *P.recondita* f. sp. *tritici* on wheat seedling leaves.

Khan and Ilyas (1996) tested propiconazole (Tilt®) and tebuconazole (Folicur®) for leaf rust. A single application of either Tilt® or Folicur® at growth stage 10 resulted in significant reduction in the rate of leaf rust and spot blotch development and lower AUDPC at FSD-85.

Khan and Trevathan (1997) evaluated five fungicides (the eradicant fungicides, flusilazole, propiconazole and fenconazole and the protectant fungicides mancozeb and triadimefon on four wheat varieties (Coker 9323 and 9733 and Pioneer 2548 and 2555) for control of leaf rust of wheat. Wheat varieties responded differently to the leaf rust fungus during the two seasons. In 1992, most fungicide treatments slowed the rate of disease development and enhanced yield of Coker 9323, 9733, Pioneer 2548 and 2555. In 1993, all fungicide treatments reduced disease severity, AUDPC and enhanced yield of Pioneer 2548 and 2555. Response of rust-tolerant, medium height, stiff-straw varieties to fungicide application was reflected in increased yield.

Kalappanavar and Patil (1998) investigated the ability of fungicides to control *P. recondita* f.ap. *tritici* on wheat. They tested propiconazole (Tilt®), triadimefon (Bayleton®), hexaconazole (Contaf®), cyproconazole (San 619®) and Mancozeb (Dithane M-45®) (Patidar, 2006). They reported that the most effective fungicide was cyproconazole and mancozeb was the least effective but the highest yield was observed on the plot treated with cyproconazole.

Zhang and Zheng (1998) tested 63 chemicals for the control of leaf rust of wheat. They found that out of 63 chemicals only triadimefon and difenoconazole showed 100 per cent control.

Reis *et al*. (2000) showed the effect of leaf rust on wheat grain yield. The treatments imposed on the cultivar Embrapa 16 and OR 1 were *viz.,* cyproconazole, difenoconazole + propiconazole, propiconazole, triadimenol, azoxystrobin, epoxiconazole + carbendazim, metconazole and tebuconazole. The damage coefficients obtained from the regression analysis equations with R2>more or =>0.8 at different phonological plant stages allowed the economic damage threshold to be calculated to control the disease with fungicides.

Bertelsen *et al.* (2001) showed that the effects of the fungicides azoxystrobin (a strobilurin) and epoxiconazole (a sterol biosynthesis inhibitor) on phyllosphere fungi, senescence and yield were studied in winter wheat in field trials. Inoculation in a glasshouse experiment with the saprophytic fungi *Alternaria alternata* and *Cladosporium macrocarpum* accelerated wheat senescence. Both fungicides reduced *A. alternate* induced papilla formation in wheat leaves, with epoxiconazole being more effective. Inoculation with either of the two saprophytes did not significantly increase wheat leaf respiration, in contrast to inoculation with the nonhost pathogen *Erysiphe graminis* f.sp. *hordei*. It is proposed that the greater inhibition of infection attempts from *Mycosphaerella spp.* by azoxystrobin, compared with epoxiconazole, may account for the greater yield given by azoxystrobin in field plots.

Boshoff *et al.,* (2002) studied the impact of wheat leaf rust on spring wheat. The various fungicides used were *viz*., bromuconazole, epoxiconazole/carbendazim, flusilazole/carbendazi, flutriafol, propiconazle, cyproconazole, tebuconazole and tebuconazole/ carbendazim. The protein content, over the fungicides applied, varied from 12.5 per cent for the combined seven and flag leaf treatments to 12.8 per cent for the seven leaf treatments.

Kalappanavar *et al*. (2008) reported that among the 13 treatments imposed fungicide propiconazole performed best followed by triadimefon and hexaconazole. However, in different categories, neem leaf extract, *Trichoderma harzianum* and Panchgavya were the succeeding treatments effective against leaf rust of wheat. The yield of propiconazole, triadimefon and hexaconazole sprayed plots were significantly superior over control indicating marked influence of the leaf rust on yield. The 1000-grain weight was also significant in the above said treated plots compared to other treatments.

MATERIAL AND METHODS

The experiments were conducted during *rabi* 2011-2012 and 2012-13. The details of materials used and the techniques adopted for the collection, analysis and interpretation of data are described in this chapter.

3.1 Experimental site

The present investigation was carried out at Dr Sanjay Rajaram Wheat Laboratory, All India Coordinated Wheat Improvement Project, Main Agricultural Research Station (MARS), University of Agricultural Sciences, Dharwad (UASD) and Directorate of Wheat Research (DWR), Regional Station, Flowerdale, Shimla, Himachal Pradesh, India. Dharwad is situated in northern transitional tract of Karnataka with 15° 26' N latitude and 76° 07' E longitude an altitude of 678 m above mean sea level (AMSL). The Flowerdale, Shimla is situated at 2054 AMSL with 31° 05' 12.7 N latitude and 77° 11' 01.7 E longitude.

3.2 Survey, surveillance and race identification of leaf rust in wheat growing region of Karnataka

The study was carried out from three wheat growing seasons as off season and normal season grown wheat, during 2010-11 to 2012-13.

3.2.1 Off season survey

An intensive roving survey was conducted during July and August months of 2010-11 and 2011-2012 at Chikmagaluru, and Chitradurga districts of Karnataka to record leaf rust incidence.

3.2.2 Normal Season survey

All the three cultivated species of wheat *viz*., bread wheat (*Triticum aestivum* L.), durum wheat (*T. durum* Desf.) and dicoccum wheat (*T. dicoccum* Shrank) are being grown in northern parts of Karnataka under both rainfed and irrigated conditions. An intensive survey was carried out during the wheat growing season of the year 2010-11 to 2012-13.

The wheat growing areas of different taluk *viz*., Athani, Gokak, Hukkeri, Ramdurg and Saudatti (district Belgaum); Bijapur, Indi and Jamakhandi (district Bijapur); Bhagalkot, Mudhol and Badami (district Bhagalkot), Dharwad, Hubli and Navalgund (district Dharwad); Gulbarga (district Gulbarga), Shaapur and Surupur (district Yadgiri) Gadag and Nargund (district Gadag) were selected for survey. In addition to the farmer's field, wheat disease trap nursery sown at Agriculture Research Station, Kalloli (during *rabi* 2010-11 and 2011-12) and Ugar-Khurd (during *rabi* 2012-13) and breeding trials were also included. The roving survey method was followed. The scale proposed by Loegering scale (Joshi *et al*., 1988) and Modified Cobb scale (Peterson *et al.,* 1948) were used for recording infection type and disease severity respectively (Table 1).

3.2.3 Race identification

The leaf rust affected leaf samples were collected from different parts of Karnataka and brought to the laboratory with all necessary information. These samples were shade dried and pressed carefully. Next day leaf samples were cut into 2-3 cm length and same were sent to DWR, Regional Station, Flowerdale, Himachal Pradesh, India for race identification. For production of large quantity of inoculum, single pustule from each sample was inoculated on universal susceptible variety Agra-local (Stakman *et al.,* 1962). The inoculum in the form of uredospores was inoculated on latest set of differentials (O, A and B) having *Lr* isogenic lines to identify the new races (Nagarajan *et al.,* 1983; Nayar *et al.,* 1997; Bhardwaj, 2011a). The composition of these sets is as follows:

Set-O	Set-A	Set-B
IWP-94	*Lr14a*	Loros (*Lr2c*)
Kharchia Mutant	*Lr24*	Webster (*Lr2a*)
Raj 3765	*Lr18*	Democrat (*Lr3*)
PBW 343	*Lr13*	Thew (*Lr20*)
UP 2338	*Lr17*	Malakoff (*Lr26*)

K 8804	*Lr15*	HP 1633 (*Lr9+*)
Raj 1555	*Lr10*	
HD 2189	*Lr19*	
Agra Local	*Lr28*	

Frequency and per cent distribution of detected races were calculated (Kiyar, 2002).

3.3 Study of genetic diversity in *Puccinia triticina* population of Karnataka through molecular techniques

3.3.1 Collection of *P. triticina* isolates

During *rabi* 2012-13 *P. triticina* isolates were collected from wheat growing areas of Northern Karnataka at 20 locations and five phenotypical known races collected from Directorate of Wheat Research, Regional Station, Flowerdale, Himachal Pradesh, India (Table 2) were used for genetic diversity study.

3.3.2 DNA Extraction

The DNA was extracted as per the method developed by Moghaddam *et al*. (2004) with little alterations. The DNA was extracted from 30 to 50 mg of uredospores of *P. triticina* from each isolate. The method explained below as follows:

Protocol

1. Initially 50 mg of uredospore was measured and put in a 1.5 ml micro-centrifuge tube
2. The above tubes were placed on a preheated heating block at 95 ℃ specially designed for holding the tubes, with caps open.
3. One ml of hot extraction buffer heated to 65-70℃ containing 100mM Tris-HCl (pH8.0) and, 10mM EDTA (pH8.0), 1 M KCl was added to each tube and the cap was closed.
4. The mixture was incubated for 10 min with intermittent vortexing of the tubes once in every 2 min. the tubes were then transferred into an icebox filled with ice flakes for 2 min.
5. The cooled samples were centrifuged at 10,000 rpm for 10 min at 4℃.
6. The aqueous phase was transferred to another tube and centrifuged again in the same condition for 5 min.
7. Approximately 0.6 equivalent of the volume of the supernatant (500-600 µl) of cold isopropanol (-20℃) was added to each sample, placed on microtube rock and mixed by gentle rocking for 5 min to precipitate DNA and centrifuged at 10,000 rpm for 5 min. The supernatant was discarded.
8. The DNA pellet was allowed to suspend in water by adding 300 µl of sterile water.
9. The samples were heated for 2 min at 50℃ in a hot water both and the DNA was dispersed by intermittent flicking of the tubes.
10. The DNA was re-precipitated by adding 15 µl of 3 M sodium acetate (pH 4.8) and 300 µl of 95per cent cold ethyl alcohol (-20℃) to each tube for 5 min and centrifuged at 10,000 rpm for 5 min.
11. The supernatant was removed and the DNA pellet was washed by adding 200 µl of 70 per cent cold ethyl alcohol for centrifuged at 10,000 rpm for 3 min.
12. The dried DNA pellet was re-suspended in 50 µl of sterile water and stored in a refrigerator at 4℃ as stock DNA.

3.3.3 Selection of primers

The Expressed Sequence Tag - Simple Sequence Repeat primers (EST-SSR) primers (Wang *et al*., 2010) were selected to study the genetic diversity of *P. triticina* (Table 3) and were obtained from integrated DNA technologies supplied by Sigma Industrial and Laboratory Equipments Inc., Bangalore, India.

Table 1. Infection type, response value and description for calculating average coefficient of infection

Infection Types	Response Value	Description
0	0.0	Immune. No visible infection on plants.
0;	0.0	Nearly Immune. Yellow flecks on plants
R	0.2	Resistant. Necrotic areas, with or without minute uredia.
MR	0.4	Moderately resistant, Small uredia surrounded by necrotic areas.
X	0.6	Mesothetic/Heterogeneous. Variable sized uredia, some with necrosis or chlorosis.
MS	0.8	Moderately Susceptible. Medium uredia with no necrosis but possibly some distinct chlorosis.
S	1.0	Susceptible. Large uredia with little or no necrosis but possibly some distinct chlorosis

Joshi *et al*. (1988)

Table 2: *Puccinia triticina* isolates collected from different taluk of Northern Karnataka

Isolate No.	Place	Taluka	District	Variety
1	UAS-Dharwad	Dharwad	Dharwad	Amruth (DW)
2	Amminabhavi	Dharwad	Dharwad	DWR-162 (BW)
3	Mulmuthla	Dharwad	Dharwad	DWR-162 (OT)
4	Saunshi	Kundagol	Dharwad	Amruth
5	Hubli	Hubli	Dharwad	Amruth
6	M. K. Hubli	Bailhongal	Belgaum	DWR-162 (OT)
7	U. Khanapur	Hukkeri	Belgaum	Bread Wheat
8	Shiruguppi	Athani	Belgaum	Bread Wheat
9	Ugar-Khurd	Athani	Belgaum	Local Red (DW)
10	Bijapur	Bijapur	Bijapur	Bread Wheat
11	Muddapur	Mudhol	Bagalkhot	Bread Wheat
12	Kulageri	Badami	Gadag	Bread Wheat
13	Nargund	Nargund	Gadag	Bread Wheat
14	Halakusugal	Navalgund	Dharwad	Amruth
15	Garag	Dharwad	Dharwad	DWR-1006 (OT)
16	Anigol	Bailhongal	Belgaum	DWR-162 (OT)
17	Badakondri	Hukkeri	Belgaum	Bread Wheat
18	Ugar-Khurd	Athani	Belgaum	Dicoccum Wheat
19	Siddapur	Jamakhandi	Bagalkhot	Bread Wheat
20	77-5*	-	-	-
21	77-6*	-	-	-
22	104-2*	-	-	-
23	162-2*	-	-	-
24	12-4*	-	-	-
25	UAS-Dharwad	Dharwad	Dharwad	UAS-195

Note: BW=Bread Wheat, DW=Durrum Wheat and OT=Off type

***** Phonotypical known predominant races in India collected from Directorate of Wheat Research (DWR), Regional Station, Flowerdale, Himachal Pradesh, India

Table 3: List of expressed sequence tag simple sequence repeats (EST-SSR) used for genetic diversity study of *P. triticina*

Name of Primer (Locus)	Primer sequence		Annealing Temp. (℃)
	Forward	Reverse	
Ptssr0083	5'-ATGGATTTGGAGACCAGTCG-3'	5'-GTTGAAAGATCTGGGGGTGA-3'	60
Ptssr6981	5'-ACGTGGTGAGGTTTCTGCTC-3'	5'-TTCCGTTTTTGAAAGCAAGC-3'	59
Ptssr5649	5'-CAGACGACCATCAACATTCG-3'	5'-CATGAACCAAACAAACAGCTTC-3'	60
Ptssr0085	5'-CCAAAATTATCCCGCCCTAT-3'	5'-GCGAGGGGGTAGGAAGTAAT-3'	60
Ptssr6259	5'-GTTCAACACATTGCGCTGTT-3'	5'-ATGGGTTGTGCAGATCGAGT-3'	59
Ptssr2948	5'-CACACACCACACAAAACCAA-3'	5'-CCCAACAAGCTCGTGTCTTT-3'	59
Ptssr0536	5'-TGTTGCGAATTGATGGTACG-3'	5'-GAAGTTCTGCTCTGCTGTCG-3'	60
Ptssr5594	5'-CGGACCAAACACAAAGGAAA-3'	5'-CCCTGCGTTTAACACCTTGT-3'	60
Ptssr0189	5'-TCTCAACCAAAAATCAATCTACG-3'	5'-CTTCCACGAAGACGAAGCAC-3'	58
Ptssr0243	5'-CTCACTCGCTCGCTTGTTCT-3'	5'-GACGAAAAGATCGGGTTTGA-3'	60
Ptssr0125	5'-ATCGTGTCATGCAACCAAAA-3'	5'-AGAGAGGGACGTGAGGGATA-3'	59
Ptssr0481	5'-CCACAATCCTCCGTTCTGAT-3'	5'-CGAAAGCAAAACACATGAGG-3'	60
Ptssr3145	5'-TAGGTGCGTGGTTTTCATCA-3'	5'-CAAATGAGAGCGACGAACAA-3'	60
Ptssr6542	5'-TGTGATCTCGCCCGTACATA-3'	5'-TGGGAATGATGGACACACAC-3'	60
Ptssr0182	5'-CGAATCCCTTGTCTTTTGCT-3'	5'-TGTAGAGAGCGGGAGAAGAAA-3'	59

3.3.4 *Taq DNA polymerase*

Taq DNA polymerase and 10x *Taq* buffer were obtained from New England Biolab (NEB).

3.3.5 Polymerase chain reaction (PCR)

DNA concentration was determined using nanodrop readings and a working solution of 30 ng/µl was made. PCR was carried out in a 20 µl reaction volume containing 1 × PCR buffer (NEB), 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 2.5 mM forward and reverse primers, 0.5 U *Taq polymerase* (NEB) and 30 ng template DNA. Amplifications were performed in a thermal cycler (C1000^{TM} Thermal Cycler, Biorad, Germany) using the following temperature profile: initial denaturation step at 95 ℃ for 2 minutes, then 35 cycles at 95 ℃ for 30 s, 60 ℃ for 30 s and 72 ℃ for 1 minute, followed by a final extension step at 72 ℃ 5 minutes (Wang *et al*., 2010). PCR products were analyzed in a 6% polyacrylamide gel and visualized by silver staining (Chalhoub *et al*., 2007). Alleles were scored based on previous report (Wang *et al*., 2010).

3.3.6 Scoring the amplified fragments

The amplified fragments was scored as '1' for the presence and '0' for the absence of a band generating the 0 and 1 matrix.

3.3.7 Data analysis

The genetic similarity co-efficient was estimated using NTSYS PC-2.0 Software programme (Nei and Li, 1979). The clustering was done and dendrograms were generated by following unweighted pair group using arithmetic mean algorithm (UPGMA) routine available in the above programme.

3.4 Identification of slow leaf rusters

The experiment on slow leaf rusters was carried out during *rabi* 2011-12 and 2012-13. The field experiment was conducted in a randomized block design. Forty one (during 2011-12) and 59 (during 2012-13) bread wheat genotypes having diverse genetic resistance to leaf rust were collected from different wheat improvement centers across India (Table 4) and sown in a plot size of 1.15 m^2 (1m length of 5 rows with 0.23 cm width) area during 2011-12 and 1.6 m^2 (1m length of 8 rows with 0.20 cm width) area with two replication during 2012-13. Susceptible checks were planted all around the experimental plots using the universal susceptible varieties like Lal Bahadur, Agra Local, and Local Red. At boot leaf stage of the crop, a suspension of mixture of pathotypes of leaf rust was sprayed on the genotypes.

Five plants were randomly selected in each plot and tagged. Infection types and disease severity of leaf rust was recorded at an interval of seven days by following the scale given in the section survey, surveillance and race identification of leaf rust in wheat growing region of Karnataka. Observations on the following parameters were recorded.

3.4.1 Average coefficient of infection

Average coefficient of infection (ACI) was calculated by multiplying the per cent infection and response value, assigned to each infection type, as per Loegering scale (Joshi *et al.* 1988). The response values were considered as 0.0 to 1.0 using the notations given in Table 1.

The ACI for each variety was computed for five observations at an interval of seven days.

3.4.2 Rate of infection ('r')

The rate of leaf rust infection (units day^{-1}) was computed as described by Van der Plank (1968). The apparent rate of infection ('r') at different intervals was calculated by using the formula given by Van der Plank (1968).

$$r = \frac{2.3}{t_2 - t_1} \log_{10} \frac{X_2}{X_1}$$

Where,

r = apparent rate of growth of disease or rate of infection (units day^{-1})

X_1 = per cent disease severity at t_1 date,

X_2 = per cent disease severity at date t_2

3.4.3 Area under disease progress curve (AUDPC)

The "Area Under Disease Progress Curve" (AUDPC) was calculated by using the formula suggested by Wilcoxson *et al.* (1975).

$$\text{AUDPC value} = \sum_{i=1}^{k} \tfrac{1}{2}(Si + Si\text{-}1) \times d$$

Where,

Si = Disease severity at the end of time I,

k = Number of evaluations of disease and,

d = Interval between two evaluations.

3.4.4 Latent period

The latent period (number of days from inoculation to the appearance of 50% of the uredinia) was calculated according to the following formula (Das *et al*., 1993).

Latent Period = $t_1 + [(F/2\text{-}nt_1)\ (t_2\text{-}t_1) / (nt_2\text{-}nt_1)]$

Where, F = Final number of uredinia,

t_1 = Day before 50% uredinia erupted,

t_2 = Day after 50% uredinia erupted,

nt_1 = Number of uredinia erupted at t_1 and

nt_2 = Number of uredinia erupted at t_2.

3.4.5 Pustule size

The average length and breadth of pustule on the central two third of the leaf was estimated by using differential interference contrast (DIC) microscope at a magnification of 40X (calibrated) in mm at the end of cropping season (Ohm and Shaner, 1976). When the number of uredinia had become stable, the length and width of five randomly chosen uredinia on each tiller were measured with a micrometer. The average of the five measurements taken on the same main tiller. The uredinium size was calculated according to Lee & Shaner's (1985) formula: Uredinium size = (length (mm) × width (mm) × Π /4.

3.4.6 Pustule density

Number of pustules per square centimeter of leaf area were estimated for basal, mid and top portions of each five leaf as described by Peterson *et al.* (1948) and averaged at the end of cropping season.

3.4.7 Yield per plot

The obtained grain yield per plot was recorded and converted into yield as q ha^{-1}.

3.4.8 Thousand grain weight

A total of 200 random seeds were counted from each plot and weighed in grams and computed to 1000-grain weight.

3.4.9 Seedling reaction test and gene postulation

This work was carried out at Flowerdale, Shimla during 2012-13. Forty one bread wheat genotypes (Table 4) including susceptible checks Lal Bahadur and Agra Local and 13 pathotypes of *P. triticina* were used for this study along with a set of tester having known *Lr* genes.

The seedlings were grown in aluminium bread pans (29cm long X 12 cm wide X 7 cm deep size) having a mixture of fine loam and farmyard manure (3:1) that had been sterilized by autoclaving (60°C) for one hour. These trays were sufficiently large to accommodate 18 wheat genotypes, including universal susceptible check Agra Local so as to compare and confirm higher infection types.

For each genotype 5-6 seeds were sown in hills. The seedlings were raised in spore proof chambers (indoors) at 22±2°C, 50-70 per cent relative humidity and 12-hour daylight.

When the seedlings were one week old with fully expanded primary leaves, they were inoculated using a glass atomizer that contained 10 mg spores of pathotypes of *P. triticina* suspended in 2 ml light grade mineral oil (Soltral 170)® (Chevron Phillips Chemicals Asia Pvt. Ltd., Singapore). The oil was allowed to evaporate 30 min. Plants were then sprayed with a fine mist of water and placed overnight at 20±2°C in humidified chamber (locally made) to ensure maximum infection. The plants were then transferred to a glass house; the controlled conditions and method for raising seedlings under glasshouse were followed as explained by Bhardwaj *et al.* (2011a).

Infection types were recorded 14 days after inoculation following method of Stakman *et al.* (1962) with modifications (Nayar *et al*., 1997) where infection types 0 to 2 (small hypersensitive flecks to small-medium uredia surrounded by chlorotic area) were considered resistant and infection types of 3 to 3+ (moderate to large uredial pustules without chlorosis) were considered susceptible. Infection types 33+ classified where both 3 and 3+ pustules were found together. The experiment was performed twice.

The existence of *Lr* genes in the 41 wheat genotypes could be assumed by applying the gene-matching technique using multipathotype data (Browder, 1973). The presences of *Lr* gene(s) using different pathotype were postulated using 13 different pathotypes of *P. triticina*.

3.4.10 Genetics and molecular characterization of bread wheat varieties for durable/slow leaf rust resistance

Molecular characterization was done to confirm presence of slow leaf rust resistant genes (*Lr34*, *Lr46* and *Lr67*) in 59 genotypes (Table 4) by using STS and SSR molecular markers.

3.4.10.1 DNA Extraction

The DNA was extracted from the bread wheat genotypes by following CTAB (N, N, N, N, -Cetyl Trimethyl Ammonium Bromide) extraction method with few modifications as described below.

1. 0.1g of fresh leaves from 6-8 days old seedlings were taken. The sample was grinded to fine powder in liquid nitrogen with micro pestle in centrifuge tube.
2. 1ml of extraction buffer (10% CTAB, 1M Tris base, 4M NaCl) added to the powdered sample and mixed properly.
3. Samples were incubated for 30min at 65°C in water bath with intermittent mixing.
4. Equal volume of chloroform iso amyl alcohol (24:1v/v) was added and gently agitated for 10 min to form an emulsion.
5. The tubes were centrifuged for 10min at 10,000 rpm at room temperature.
6. The supernatant was transferred to sterile tubes and 1ml of chilled isopropanol was added to each of tube, mixed by inverting and incubated at -20°C over night.
7. The contents were centrifuged again for 10 min with 10,000 rpm at 4°C and the pellet was retained by discarding the supernatant.
8. The DNA pellet obtained was washed with 70% ethanol and tubes were inverted on blotting paper to dry the pellet.
9. Later DNA was suspended in 50µl TE (10mM Tris HCl, 1mM EDTA) buffer/sterile distilled water and stored at -20°C.

3.4.10.2 DNA quantity and quality estimation

The concentration of DNA was assessed spectrophotometrically and also by gel electrophorosis on 0.8% agarose gel with known concentrations of uncut DNA.

To test the quality of DNA, samples were run on 0.8% agarose gel in 1x TAE buffer stained with ethidium bromide and checked for contamination by RNA (Which usually runs ahead) and the DNA was evaluated by comparing it with a standard undigested DNA sample.

Table 4: Bread wheat genotypes used for identification of slow leaf ruster

Sl. No.	Genotypes	Pedigree	Origin
1	Agra local	-	LV-Uttar Pradesh
2	AKAW-4627	WH 147/SUNSTAR*/C 80.1 (SELECTION FROM VIMAL)	PDKV, Akola
3	C-306	REGENT 1974/3*CHZ//*2C591/3/P 19/C281	CCSHAU, Hisar
4	DBW-16	RAJ 3765/WR 484//HUW 468	DWR, Karnal
5	DBW-17	CMH 79A.95/3*CNO 79//RAJ 3777	DWR, Karnal
6	DWR-162	KVZ/BUHO//KAL/BB	UAS, Dharwad
7	GW-322	PBW 173/GW 196	SDAU, Vijapur
8	GW-432	BORL 95/SEPT//J 429/CC 558/3/GW 324	SDAU, Vijapur
9	HD-2189	HD 1963/HD 1931	IARI, N. Delhi
10	HD-2733	ATTILA/3/TUI/CARC//CHEN/CHTO/4/ATTILA	IARI, N. Delhi
11	HD-2864	DL 509-2/ DL 377-8	IARI, N. Delhi
12	HD--2888	C 306/T.SPHAEROCOCCUM//HW 2004	IARI, N. Delhi
13	HD-2932	KAUZ/STAR//HD 2643	IARI, N. Delhi
14	HD-3091	PICUS /3/KAUZ*2/BOW//KAUZ/4/TILHI	IARI, N. Delhi
15	HD-3093	NW 1012/HUW 453	IARI, N. Delhi
16	HD-3098	1455/2* PASTOR	IARI, N. Delhi
17	HI-1500	HW 2002*2/STREMPALLI/PNC 5	IARI, RRS, Indore
18	HI-1544	HINDI 62/BOBWHITE/CPAN 2099	IARI, RRS, Indore
19	HI-1563	MACS2496*2/MC 10	IARI, RRS, Indore
20	HI-1584	MGSN 74/HD2820	IARI, RRS, Indore
21	HI-977	GLL/AVST 1161 157//CNO/NO/3/KAL/BB	IARI, RRS, Indore
22	HS-240	AU/KAL/BB/3/WOP/PAVON	IARI, RS, Shimla
23	HS-420	KAJ 3302//CMH 73A-497/3*CNO 79	IARI, RS, Shimla
24	HS-533	13th HRWSN-240	IARI, RS, Shimla
25	HW-2004	C 306*7//TR 380-14#7/3 AG 14	IARI, RS, Wellington
26	KRL-210	PBW 65/2*PASTOR	CSSRI, Karnal
27	Lal Bahadur	S 54723* RS 31-1 ML 293 BB*KAL^{2} ML319 CNO-KAL*CD1 (KAL-INIA*INIA-BB) ML328 BB-KAL^{2} ML408 RON-CHA*KAL-NOR67 ML 414 TOB-INIA*KAL	-
28	LOK-1	S 308/S 331	Lok Bharati Institute, Sansora
29	MACS-2496	SERI"S"	ARI, Pune
30	MACS-6145	C 306+*Lr* 28	ARI, Pune
31	NI-1689	-	MPKV, Niphad
32	NI-5439	REMP 80/3*NP 710	MPKV, Niphad
33	NIAW-1415	GW9506/PRL//PRL	MPKV, Niphad
34	NIAW-917	GW 244/BOB WHITE	MPKV, Niphad
35	NW-4091	MILAN/S 8720//HUITES	NDUA&T, Faizabad
36	Parula	FKN/3/2*FRONTANA//KENYA 350 AD.9C.2/GABO 55/4/BLUEBIRD/CHANATE	CIMMYT, Mexico

Contd…

Sl. No.	Genotypes	Pedigree	Origin
37	Pavon-76	VCM//CNO/7C/3/KAL/BB	CIMMYT, Mexico
38	PBW-343	ND/VG1944//KAL/BB/3/YACO“S”/4/VEE#5“S”	PAU, Ludhiana
39	PBW-590	WH 594/RAJ 3814//W485	PAU, Ludhiana
40	PBW-596	PBW 343/DHARWAD DRY//PBW 343	PAU, Ludhiana
41	Raj-4083	PBW 343/UP 2442//WR 258/UP 2425	RAW, Durgapura
42	Raj-4229	HW 2048/ RAJ 4000	RAW, Durgapura
43	Raj-4240	HW 3019/HD2189//HD 2189	RAW, Durgapura
44	Raj-4245	PBW 343/NW 2044/ PBW 343	RAW, Durgapura
45	Raj-4270	PBW480/WH147	RAW, Durgapura
46	RL-6077	Tc*6/PI250413	CIMMYT, Mexico
47	Sonalika	1154.388/AN/3/YT 54/N 10B/LR 64	IARI, N. Delhi
48	UAS-304	SERI/CEP80120//KAUZ / PBW 343	UAS, Dharwad
49	UAS-315	DWR-163/DHARWAD DRY//DWR-225	UAS, Dharwad
50	UAS-326	KAMBARA2 / NI 5439	UAS, Dharwad
51	UP-2825	UP 2565/ PBW 502	GBPUAT, Pantnagar
52	VL-616	SKA/CPAN 1507	VPKAS, Almora
53	VL-829	IBWSN 149/CPAN 2009	VPKAS, Almora
54	VL-907	DYBR1982-83842ABVD50/VW9365//PBW343	VPKAS, Almora
55	VL-920	PARA2//JUP/BJY/3/VEE/JUN/4/2*KAUZ/5/BOW/PRL//BUC	VPKAS, Almora
56	VL-924	PBW373/VL795	VPKAS, Almora
57	VL-943	WR 798/ VL 826	VPKAS, Almora

3.4.10.3 Optimization of Polymerase Chain Reaction (PCR) Template DNA

The purified genomic DNA extracts (30ng) of bread wheat genotypes were used as template DNA per reaction.

3.4.10.4 STS and SSR primers

A total of 12 gene specific primers were used in the study with the sequences (Table 5) to characterize the presence of above mentioned three slow leaf rust resistant genes among 59 bread wheat genotypes.

3.4.10.5 dNTPS

dNTP mix having dATP, dGTP, dCTP, dTTP were obtained from M/S Bangalore Genei, Pvt. Ltd. Bangalore.

3.4.10.6 *Taq DNA polymerase*

Taq DNA polymerase 3 units per µl and 10x Taq buffer were obtained from M/S Bangalore Genei, Pvt. Ltd. Bangalore.

3.4.10.7 PCR mixture and thermo profile for PCR

The reaction mixture and thermo profile procedures for PCR was followed as per the respective earlier reports given in the table to each marker.

3.4.10.8 Separation of amplified products on agarose gel electrophoresis

The amplified products from each tube along with 2 ml of loading dye (bromophenol blue) were separated on 1.2% agarose gel at 70 volts (5 volts per cm 7 gel) using 1x TAE buffer (pH 8.0) containing ethidium bromide. The gels were photographed using gel documentation system of J H BIO Ltd.

3.4.10.9 Analysis of the profile of the amplified fragments

The presence of band to a particular size (bp) with respect to gene as well as marker was confirmed the presence of specific slow rust resistant gene.

3.4.11 Studies on expression of oxidative enzymes (isozymes) in selected bread wheat under the pathogenesis of leaf rust races

This experiment was conducted at Dr Sanjay Rajaram Wheat Laboratory. Identified slow ruster (HD-2189) in comparison with fast ruster (Agra Local) and resistant genotype (NIAW-917) were selected for this study. Two growth stages such as seedling and adult plant stages were considered by inoculating two *P. triticina* pathotypes 77-5 (121R63-1) and 104-2 (21R55) were inoculated in separate sets. For both the stages, uninoculated sets as healthy ones were maintained.

The leaves were cut at seedling stage on 5^{th} and 10^{th} day after inoculation and at adult plant stage leaves were inoculated at boot leaf stage by *P. triticina* pathotypes and leaf samples were collected on 5^{th} and 10^{th} day after inoculation.

3.4.11.1 Peroxidase

In the present investigation existence of isozyme variation among the genotypes was assessed by adopting the Vertical Polyacrylamide Gel Electrophoresis (PAGE) given by Sadasivam and Manickam (1996).

3.4.11.2 General procedure followed for performing PAGE

The glass plates and spacers were thoroughly cleaned and dried in hot air oven at 60 ^{o}C for 15-20 minutes and assembled in proper position.

3.4.11.3 Sample preparation

Leaves were ground to fine powder. The ground 5g leaf sample was put into eppendorf tubes and 15 ml of 0.1M phosphate buffer was added. These suspensions were agitated thoroughly and kept at 8ºC overnight for protein extraction. Then the suspensions were centrifuged at 10, 000 rpm for 30 minutes. The clear supernatant was collected. These samples were used for loading.

Table 5: Gene specific primers used to characterize slow leaf rust resistant genes

Primer Name	Primer sequence		Marker Type	Target Gene	References
	Forward	Reverse			
csLV34	5'-GTTGGTTAAGACTGGTGATGG-3'	5'-TGCTTGCTATTGCTGAATAGT-3'	STS	*Lr34*	Lagudah *et al*., 2006
XGwm130	5'-AGCTCTGCTTCACGAGGAAG-3'	5'-CTCCTCTTTATATCGCGTCCC-3'	SSR	*Lr34*	Priyamvada *et al*., 2009
XGwm295	5'-GTGAAGCAGACCCACAACAC-3'	5'-GACGGCTGCGACGTAGAG-3'	SSR	*Lr34*	Priyamvada *et al*., 2009
cdo475	5'-GACACATTGACCGCATCTTA-3'	5'-CCTTCACCTCGCTCCCTACC-3'	SSR	*Lr34*	Priyamvada *et al*., 2009
Xwmc44	5'GGTCTTCTGGGCTTTGATCCTG-3'	5'-TGTTGCTAGGGACCCGTAGTGG-3'	SSR	*Lr46*	Lilemo *et al*., 2008
Xwmc719	5'-TTGTGGGAATCTACATCAGAAGG-3'	5'-AACAGCCACGCTCTATCTTCAGT-3'	SSR	*Lr46*	Lilemo *et al*., 2008
Xcfd23-4D	5'-TAGCAGTAGCAGCAGCAGGA-3'	5'-GCAAGGAAGAGTGTTCAGCC-3'	SSR	*Lr67*	Sybil *et al*., 2011
Xbarc98	5'-CCGTCCTATTCGCAAACCAGATT-3'	5'-GCGGATATGTTCTCTAACTCAAGCAATG-3'	SSR	*Lr67*	Sybil *et al*., 2011
Xcfd71-4D	5'-CAATAAGTAGGCCGGGACAA-3'	5'-TGTGCCAGTTGAGTTTGCTC-3'	SSR	*Lr67*	Sybil *et al*., 2011
Xbarc288	5'-GGGTTTTGCTTGGTTGACA-3'	5'-CGGGACGATTTTATTTAGGAGT-3'	SSR	*Lr67*	Sybil *et al*., 2011
Xwmc48	5'-GAGGGTTCTGAAATGTTTTGCC-3'	5'-ACGTGCTAGGGAGGTATCTTGC-3'	SSR	*Lr67*	Sybil *et al*., 2011
Xwmc89-4D	5'-ATGTCCACGTGCTAGGGAGGTA-3'	5'-TTGCCTCCCAAGACGAAATAAC-3'	SSR	*Lr67*	Sybil *et al*., 2011

3.4.11.4 Electrophoresis

Electrophoresis was performed according to Davis (1964). The following stock solutions were prepared.

Solution A

1 N Hydrochloric acid 48.00 ml

Tris (Hydroxyl methyl amine) 36.60 g

Tetra Methyl ethylene diamine (TEMED) 0.23 ml

Tris (Hydroxyl methyl amine) 36.60 g was dissolved in distilled water and 0.23 ml of Tetra Methyl ethylene diamine (TEMED) was added to it. The pH was adjusted to 8.9 and final volume was made up to 100 ml with distilled water.

Solution B

Acrylamide 28.00 g

N, N' – Bis acrylamide 0.74 g

Acrylamide 28.00 g was dissolved in distilled water and then N, N' – Bis acrylamide 0.74 g was added, final volume was made upto 100 ml with distilled water.

Solution C

100 mg of Ammonium per sulphate was dissolved in one ml of distilled water to have 10 per cent solution and it was prepared freshly each time before its use.

3.4.11.5 Electrophoretic buffer (stock buffer)

Tris - 3.00 g

Glycine – 14.00 g

Electrode buffer was prepared by dissolving Tris - 3.00 gm, Glycine – 14.00 g and 1 g sodium dodecyl sulphate and pH was adjusted to 8.3 and final volume was made up to 1000 ml. The separation gel (7.5% acrylamide) was prepared by mixing stocks A: B: C: Water in the ratio of 1:2:4:1.

The solutions were mixed gently and carefully and poured in the chamber between glass plates. Top portion is covered with thin layer of water to hasten the process of polymerization. Since oxygen affects the process, the gel was allowed to set for 1 hour. After polymerization of gel the comb was removed without distorting the shape of the well.

Each well washed with distilled water and is removed by using blotter paper. Thus obtained sandwiched plates were then set in the electrophoresis unit. Both upper and lower tanks of the unit were filled with tank buffer slowly avoiding bubbles. Fifty µl of enzyme extract was loaded in the wells. Twenty five µl of 0.01 per cent bromophenol blue was placed in one of the wells as a tracking dye. Electrodes were placed in the tank buffer in their proper position and connected to AC power through power pack apparatus. The gel was run at 30 mA for five hours at 5^0C. After the run, the gel between the plate was removed and distance travelled by the dye was measured and recorded. Then the gel was incubated in enzyme specific staining solutions for specific period of time. Position of bands were also measured and recorded.

3.4.11.6 Staining of peroxidase

The isozyme bands of peroxidase were localized by incubating the gel in guaiacol (0.25%) for 30 min followed by incubation in 0.3 per cent hydrogen peroxide for 15 min which show the appearance of reddish brown bands of peroxidase enzyme.

3.4.11.7 Polyphenol oxidase

The isozymes of polyphenol oxidase were loacalised on poly acrylamide gels as per the procedure suggested by Park *et al*. (1980). The enzyme source preparation and electrophoretic technique was essentially the same as in case of peroxidase isozyme studies, except the staining procedure. The isozymes of polyphenol oxidase were localized by incubating the gels for 30 min.in 0.1 per cent polyphenyldiamine in 0.1 M potassium phosphate buffer pH (7.00) followed by 10 mM catechol in the same buffer.

3.4.12 Histology of slow leaf rusters

The method explained by Lee and Shaner (1984) was adopted with little modifications as follows:

Histological studies of infection on 14 genotypes of bread wheat by *P. triticina* were conducted. Of these genotypes used susceptible checks (Agra Local, Lal Bahadur, Sonalika, DWR-162 and C-306), slow rusting genotypes (NI-5439, HS-240, HS-420, Pavon-76 and HD-2189) and resistant genotypes (GW-322, UAS-304, NIAW-917 and PBW-343). Plants were grown under polyhouse condition.

3.4.12.1 Inoculation

When plants were reached at boot leaf stage, inoculated with uredineospores of two predominant pathotypes of *P. triticina viz*., 77-5 and 104-2 separately collected from DWR, RS, Shimla. Each inoculation with sufficient inocula were applied with dual action hand sprayer (Mercury®) by maintaining 4 x 10^5 spore/ml load (Kloppers and Pretorius, 1997). After inoculation, plants were sprayed lightly with distilled water and humidity was created over night to get uredospore germination. The humidity and mean temperature in polyhouse were maintained by spraying water regularly up to symptoms appeared on susceptible check.

3.4.12.2 Collection of samples

Inoculated leaf samples from each genotype were collected at an interval of 24, 48, 72, 96 and 192 hours after inoculation (hai). Collected leaf samples were cut (3 cm length) and fixed to the thermo coal sheets. A semisolid solution was prepared by adding thermo coal pieces to xylene solution. This was applied on adaxial leaf surface arranged on thermocol sheets then the samples were dried overnight under room temperature. The thin layer was obtained on the leaf surface which was peeled by holding leaf sample between fingers and examined under Differential Interference Contrast (DIC) microscope (Zeiss®) at 400X.

3.4.12.3 Observations recorded

The observations were made on the following phases of infection process: uredospore germination and appressorial formation.Ten randomly selected uredospore on samples were examined to determine germination of uredinospores and appressorial formation in each sample. Later it was converted to percent germination and appressorial formation.

3.5 Evaluation of identified slow leaf rusters for quality traits

The selected bread wheat genotypes from the identification of slow ruster experiment of four different groups *viz*., 5 resistant genotype, 5 slow rusters, 5 medium slow ruster and 5 susceptible genotypes in comparison with fungicide protected by spray of 0.1 per cent propiconazole (Tilt®) at an interval of 15 days till the maturity of the crop (Brahma and Asir, 1988) were used to study the following quality parameter by using standard instrument as well as yield loss estimation. The layout, planting, inoculation of uredospores and observations on leaf rust were similar as explained under identification of slow ruster. For this study, grains of individual genotypes were milled using a laboratory mill (Foss, Hillerod, Denmark) into flour.

3.5.1 Protein content

The protein content of grain was analyzed by non destructive method using NIRsystems Infratech 1241 grain analyzer (Foss, Hillerod, Denmark) (Hruskova and Famera, 2003; Osborne, 2006; Silva *et al*., 2008; Surma *et al*., 2012).

3.5.2 Sedimentation value

The sedimentation value were determined as per the procedure given by Mishra and Gupta (1995) and expressed in ml.

3.5.3 Damaged starch

The degree of the damage of starch isolated from flour obtained in constant laboratory conditions was studied. Damaged starch contents of the flour samples were determined by one gram of samples using an amperometric method (SD-Matic, Chopin Technologies, Villeneuve la Garenne, France) which provides results in AACC units (American Association of Cereal Chemists). Replicate measurements were carried out for the analysis and the results were averaged (Dhaka *et al*., 2012).

3.5.4 Gluten analysis

Ten gram flour samples of all the wheat varieties were analyzed for wet gluten (WG), dry gluten (DG), and gluten index (GI). These parameters were determined according to standard AACC methods. It was done by using gluten washer (Erkaya, GW 2200®, Ankara, Turkey).

3.5.5 Alveoconsistograph

Alveograph characteristics of three bread wheat genotypes *viz*., NIAW 917 (resistant), HD 2189 (slow leaf ruster) and Agra local (susceptible) grown under protected and unprotected conditions were selected to determined Alveograph characteristics according to AACCI Method No: 54- 30 (AACC International 2000). The Alveograph characteristics were automatically recorded by the Alveolink-NG software (Chopin Technologies, Villeneuve La Garenne, France) including maximum over-pressure (P) needed to blow the dough bubble, the average abscissa (L) at bubble rupture, swelling index (G), the deformation energy (W) relates to the surface under the curve indicating the necessary work input needed to inflate the dough, and the P/L ratio. P, G and W are the indices of resistance to extension, dough extensibility and dough strength, respectively.

3.5.6 Micronutrients

The samples were digested using diacid mixture of nitric acid (HNO_3) and perchloric acid ($HClO_4$) in 9:4 ratio. One gram of grain flour was taken and 5ml of HNO_3 is added and left overnight for pre digestion. Then the samples were digested with 15-20ml of di-acid mixture till all the $HClO_4$ evaporated and snow white residue was obtained. The solution was made by adding 5N HCl and then filtered through Whatman No. 41 filter papers and volume made up to 100ml with distilled water. The amounts of micronutrients were determined by using double beam atomic absorption spectrophotometer (Elico® SL 176, Hyderabad, India).

3.5.7 Yield loss assessment

In this study ACI and AUDPC were correlated with thousand grain weight and net plot yield computed to yield per ha.

To identify the relationship between leaf rust and loss in yield and thousand grain weight, the per cent loss was calculated using the formula:

$$\text{Loss (\%)} = \frac{YP - Y_uP}{YP} \times 100$$

Where,

YP = Yield or 1000 grain weight in protected treatments and

Y_uP = Yield or 1000 grain weight in unprotected treatments

3.6 Management of leaf rust of wheat through chemicals

The *in-vivo* experiment was conducted at the farm of Dr. Sanjay Rajaram Wheat Laboratory, Main Agricultural Research Station, University of Agricultural Sciences, Dharwad during the *rabi* 2012-13. The susceptible bread wheat variety Agra Local was used in a randomized block design of three replications with a plot size of 5 m X 1.60 m (9 m^2). The susceptible varieties like, Agra Local, Local Red, Lal Bahadur, N-59 were planted all around the experimental field to create the sufficient disease pressure in the experimental plots. A mixture of most predominant and virulent races of *P. triticina* (77-5, 104-2 and 12-3) were prepared and sprayed on the susceptible checks and on the experimental plots at an age of one month to create disease pressure. The details of experiment were given in the Table 6.

The treatments which were given significantly superior in yield of two experiments (seed treatment and spray) of first year were combined to the second year. A total of three sprays were imposed at boot leaf stage, flowering stage and dough stage of the crop. The treatments were imposed on 40 days after sowing when the disease appeared in the field and another two sprays were subsequently imposed with an interval of 15 days. Observations on disease severity were recorded after each spray by following the modified Cobb's scale as described earlier in the survey objective for leaf rust. Five randomly selected plants were recorded for per cent disease severity. The Average coefficient of infection (ACI), yield assessment and thousand grain weight were calculated as explained in the identification of slow ruster objective.

Table 6: Details of chemical treatments used for the leaf rust management during *rabi* 2012-13

Trt. No.	Treatment Details	Seed treatment (ml/kg of seed)	Spray Concentration (%)
1	RIL 071/F1 (20%FS) (Seed treatment alone)	1.50	-
2	RIL 071/F1 (20%FS) (Seed treatment alone)	2.00	-
3	Imadachloprid 600FS (Seed treatment alone)	0.77	-
4	T_1 + Propiconazole (Two spray at 45 & 60 DAS)	1.50	0.1
5	T_2 + Propiconazole (Two spray at 45 & 60 DAS)	2.00	0.1
6	T_3 + Propiconazole (Two spray at 45 & 60 DAS)	1.50	0.1
7	Pyraclostrobin 13.3% + Epoxiconazole 5% (Opera 18.3% SE) (Two spray at 45 & 60 DAS)	-	0.1
8	T_3 + T_7	0.77	0.1
9	Chloropyriphos 20% EC (Seed treatment alone)	3.00	-
10	Propiconazole (Two spray at 45 & 60 DAS)	-	0.1
11	Control	-	-

DAS; Days After Sowing

3.7 Statistical analysis

The data collected from the experiments were subjected to various statistical analysis to draw the suitable inference. The details of the statistical procedure followed are given below.

Data were analysed using Window Stat software and Microsoft Office Excel 2007. Means and standard error were derived with Microsoft Office Excel 2007 whereas correlation between various parameters were assessed by Pearson's test (*, ** significant levels at $p<0.01$ and $p<0.05$) in all cases using Window Stat software.

3.7.1 Estimation of correlation coefficient

Across the genotypes, the simple correlation coefficients were calculated to determine the direction and magnitude of association among different characters and tested against table 'r' values (Fisher and Yates, 1963) at (n-2) degree of freedom, both at 0.05 and 0.01 probability levels for their significance. Simple correlations were calculated by using the formula as given by Weber and Moorthy (1952).

$$r = \frac{\text{Cov}(x\ y)}{\sigma_x\ \sigma_y}$$

Where,

Cov (x.y)	=	Covariance of x and y
σ_x	=	Standard deviation of x
σ_y	=	Standard deviation of y

EXPERIMENTAL RESULTS

The results of the investigation on mechanism of slow leaf rusting in bread wheat and variability in *Puccinia triticina* Eriks. are presented here under.

4.1 Survey, surveillance and race identification of leaf rust in wheat growing region of Karnataka

The survey was conducted in two phases, as off-season survey and normal season survey during 2010-11, 2011-12 and 2012-13 (Plate 1).

4.1.1 Off season survey

Survey during *kharif* season revealed the absence of wheat cultivation in all the survey regions. We could observe drastic change in the cropping pattern with the introduction of irrigation facilities. Farmers were growing commercial crops *viz.,* chilli, onion, etc., instead wheat. In the interview and thorough discussion with concerned Govt. department officials, farmers, fertilizer dealers and private company field assistants (Bayer crop science India Ltd. MCF Ltd. and Syngenta India Ltd.). It came to know that, twenty years back farmers were growing off-season wheat on a small scale. But later it was discontinued because Government of Karnataka has banned cultivation of off-season wheat in the area. During the off-season survey there was no wheat crop. Hence, leaf rust infected samples were not available.

4.1.2 Regular season survey

Regular season survey was carried out during *rabi* 2010-11, 2011-12 and 2012-13 in the few taluka of Belgaum, Bidar, Bijapur, Bhagalkot, Dharwad, Gulburga and Gadag (Plate 1).

4.1.2.1 *Rabi* 2010-11

The data on survey and surveillance of 2010-11 are presented in Table 7a and 7b. The severity of leaf rust was higher in Belgaum district, followed by Dharwad and Gadag district. In Belgaum district, Athani and Gokak taluk showed equally higher disease severity ranging from 10MS to 100S but Saudatti taluk recorded less disease severity as compare to other two taluka of the same district. In Dharwad district, Navalgund taluk recorded higher disease severity ranging from 5S to 100S as compared to other taluks of the same district. Gadag taluk of Gadag district showed disease severity ranging from 40S to 80S. The latitude and longitude of the surveyed area located between N15°26'00.0 to N16°3851.7 and E074°49'67.0 to E075°17'60.4. The disease severity among surveyed area was not much related to the latitude and longitude recorded. But trend of survey was more related to host type i.e., with respect to type of wheat variety grown in the locality. Majority of the samples were collected from bread wheat of the Zadoks growth stage between 69 to 83 (Zadoks *et al*., 1974), the varieties *viz.,* Agra Local, Lal Bahadur and DWR-162 showed higher disease severity up to 100S, 100S and 80S respectively. Interestingly, the recently released UAS-415 (durum wheat) resistant variety for leaf rust also showed the susceptible reaction of 5S to10S disease severity range.

4.1.2.1.1 Race Analysis

The collected leaf rust samples were analyzed on new sets of differentials for detecting races of *P. triticina*. The sample-wise leaf rust races detected are presented in Tables 7a, 7b, 8a, 8b, 9a, and 9b for the years 2010-11, 2011-12 and 2012-13 respectively.

In total, 53 samples were collected during *rabi* 2010-11. The race analysis revealed the presence of seven pathotypes (Table 10 and Fig. 1). All seven pathotypes were belonging to pathotype group 77 and 104 further distributed in 48.21 per cent and 51.79 per cent of the sample respectively (Table 11 and Fig. 2). In group 77, the pathotypes 77-5 was most predominant (35.71 %) followed by 77-1 (8.93 %). Similarly, in 104 group, pathotype 104-2 was predominant (28.57 %) followed by 104-3 (14.29 %) and 104B (8.93 per cent) as shown in Table 11. Complete race distribution profile of the surveyed area is summarized in Table 10 and 11 (Fig. 1, 2 and 3).

4.1.2.2 *Rabi* 2011-12

The data on survey and surveillance of 2011-12 are presented in Table 8a and 8b. Among seven districts, disease was observed only in three potential wheat growing districts *viz.,* Belgaum, Dharwad and Gadag with severity and infection type ranges of 10MS-100S, 20MS-100S and 60S-80S respectively. The similar trend of district wise severity of leaf rust was followed as in rabi 2010-11. In

Belgaum district, Gokak taluka showed higher disease severity ranging from 20MS to 100S followed by Saudatti taluk with 10MS to 60S disease severity and Athani taluk recorded less disease severity (20MS to 40S) as compared to other two taluka of the same district. In Dharwad district, Navalgund and Hubli taluka recorded higher disease severity ranging from 20S to 100S as compare to Dharwad taluka which was recorded 20MS to 60S disease severity (Plate 2). Nargund taluk of Gadag district showed disease severity ranging from 10S to 100S. The latitude and longitude of the surveyed area located between N15°11'12.8 to N16°38'51.7 and E074°49'67.0 to E077°24'37.5. Among the different wheat cultivated, bread wheat (variety DWR-162) occupied more than 50% area (data not shown). The disease severity among surveyed area was not much related to the latitude and longitude recorded in this year also. Majority of the samples were collected from bread wheat of the Zadoks growth stage between 20 to 85 (Plate 2).

4.1.2.2.1 Race Analysis

Among 75 samples collected during 2011-12 *rabi* season, we could able to establish only 69 samples on set of host differentials, five samples were not established and only one sample had shown mixture of races so, not detected. Four samples among 69 samples showed two pathotypes infection. A total of 12 pathotypes belonging to four group namely, 77, 104, 162 and 12 were identified and distributed in 67.12 per cent, 21.92 per cent, 6.85 per cent and 4.11 per cent of the sample respectively (Table 10 & 11, Fig. 1 & 2).

Frequency and per cent distribution of leaf rust pathotypes were calculated as shown in Table 11. In case of group 77, 77-5 was predominantly distributed in 58.90 per cent of the sample followed by 77-6 (5 %). In 104 group, 104-2, 104-3 and 104B were distributed in 13.6 per cent, 4.1 per cent and 4.1 per cent of the sample respectively. Whereas, in case of group 162, 162-2 was observed in 6.85 per cent of the sample. Another noticeable observation is that, 93R37 (12-9) and 109R23 (77A-1) recently identified pathotypes were found in each one sample.

4.1.2.3 *Rabi* 2012-13

The severity of leaf rust was comparatively higher and early occurrence than that of *rabi* 2010-11 and 2011-12 (Table 9a & 9b). More number of leaf rust affected samples (105) could be collected as that of 53 and 75 during *rabi* 2010-11 and 2011-12 respectively. The samples were collected from five wheat growing districts. Dharwad (TS to 100S) and Belgaum (10MS to 100S) districts were observed equally higher disease severity followed by Bagalkot (10S to 100S) and Bijapur (10S to 80S) districts (Plate 2). Whereas, Gadag (5S to 60S) district recorded comparatively less disease severity. In Belgaum district, Athani and Hukkeri taluka showed higher disease severity ranging from 10MS to 100S and 20S to 100S respectively followed by Bailhongal (10MS to 100S) and Gokak (10MS to 80S) taluka. Chikkodi and Soudatti talukas recorded less disease severity as compare to other four taluka of the same district. In Dharwad district, Dharwad taluka recorded higher disease severity ranging from TS to 100S as compared to Navalgund and Hubli taluka which was recorded between 60S to 100S disease severity. Nargund taluka of Gadag district showed disease severity ranging from 5S to 60S. The latitude and longitude of the surveyed area located between N15°12'166 to N16°47'718 and E074°31'01.2 to E075°30'45.8. The elevation range of surveyed areas was very high from 531 (Muddapur of Bagalkot district) to 746 m (Bhoregal of Belgaum district). Among the different wheat genotypes cultivated, bread wheat occupied more area than other two cultivated species. The disease severity was very high in Amruth durum variety grown in farmer's field. The disease severity among surveyed area was not much related to the latitude and longitude recorded in this year also. Majority of the samples were collected from of bread wheat grown under irrigated condition of the Zadoks growth stage between 20 to 91 (Plate 2).

4.1.2.3.1 Race Analysis

Among 105 samples collected during 2012-13 *rabi* season, it could able to establish only 85 samples on set of host differentials. Among 85 samples two samples showed mixture of two pathotypes. A total of 16 pathotypes belonging to four group namely, 77, 104, 162 and 12 were identified and distributed in 74.71 per cent, 5.75 per cent, 2.30 per cent and 17.24 per cent of the sample respectively (Fig. 2).

In case of group 77, 77-9 was predominantly distributed in 29.89 per cent of the sample followed by 77-5 (28.74 %). In 104 group, 104, 104-2 and 104B were distributed in 2.30 per cent, 1.15 per cent and 2.30 per cent of the sample respectively. Whereas, in case of group 162, 162 and 162-1 was observed in 1.15 per cent of the sample. Another noticeable observation is that, 125R28 (77-11), 93R37 (12-9) and 109R23 (77A-1) recently identified pathotypes were found in each one sample (Table 9a, 9b and 10).

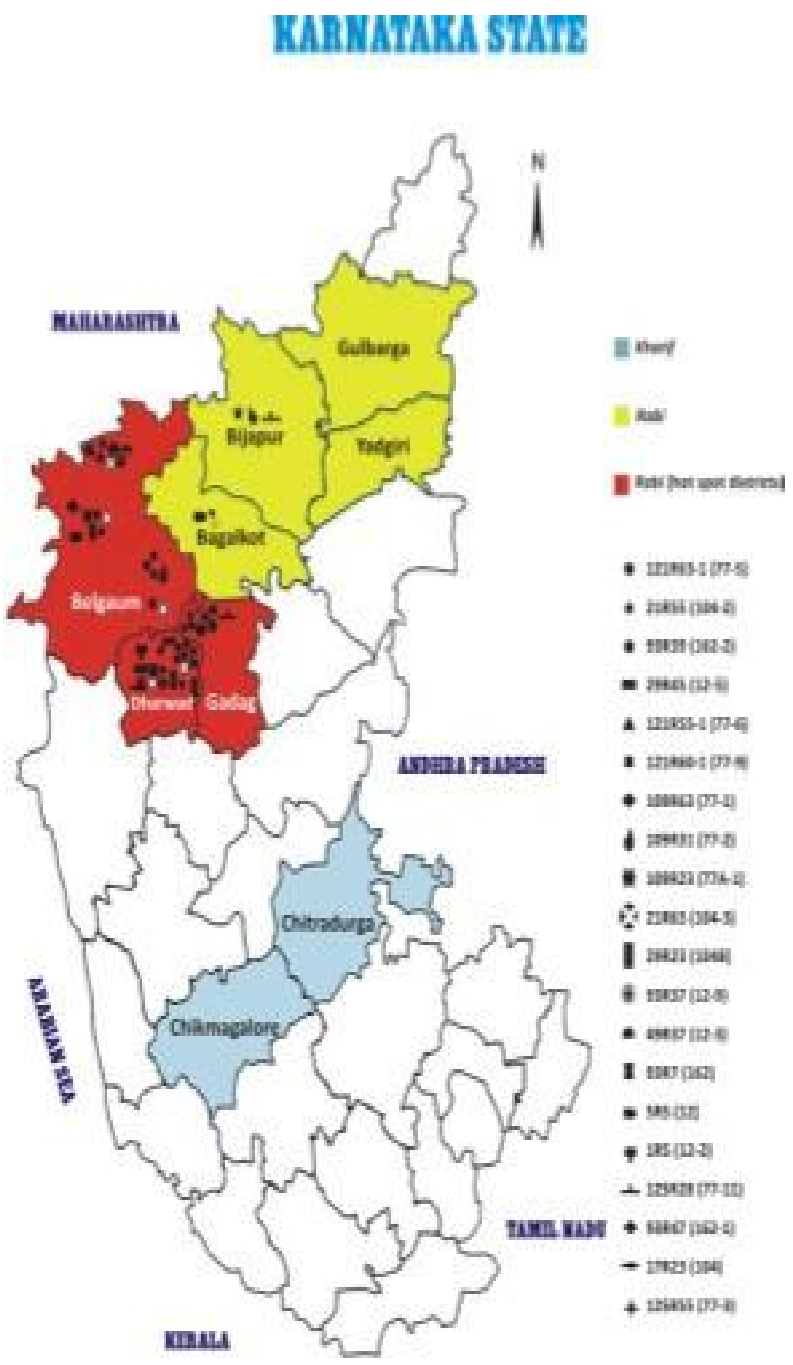

Plate 1: Distribution of wheat leaf rust pathotypes in Karnataka during *rabi* 2010-11 to 2012-13

Table 7a: A detailed survey of leaf rust of wheat caused by *Puccinia triticina* during *rabi* 2010-11

District	Taluka	Place	Latitude (N)	Longitude (E)	Cropping Situation	Disease severity[a]	Genotypes/Variety[b]	Nomenclature of Race Identified	
								Old	New
Belgaum	Saudatti	Benakatti				40S-60S	Bread Wheat	104-3	21R63
		Saudatti	15°47.154'	075°08.286'	IR	30S-40S	Bread Wheat	104-2	21R55
		Hooli	-	-	IR	40S-60S	DWR-162	77-2	109R31
Dharwad	Navalgund	Halekusugal	15°34.593'	075°15.458'	IR	80S-100S	Bread Wheat	77-5	121R63-1
	Dharwad	UAS-Dharwad	15°29.802'	74°58.798'	IR	20MS-30MS	Amrut*	77-5	121R63-1
		Chikoppa S.K.			IR	20S-30S	Bread Wheat	77-5	121R63-1
	Navalgund	Tirlapur	15°33.065'	075°14.530'	IR		Bread Wheat	77-5	121R63-1
		Tirlapur			IR	40S-60S	DWR-162	77-5	121R63-1
		Tirlapur	-	-	IR	30S-40S	Bread Wheat	77-5	121R63-1
		Sangal	-	-	IR	20S-30S	DWR-195	77-6	121R55
		Chikoppa S.K.	-	-	IR		Bread Wheat	77-5	121R63-1
		Hallur	-	-	IR	40S-60S	DWR-162	104-2	21R55
		Chimmad	-	-	IR	30S-40S	Sonalika	77-1	109R63
		Thumchi	-	-	IR	40S-60S	NIAW-917	104-2	21R55
		Chimmad	-	-	IR	5MS-10MS	MACS-6222	77-1	109R63
		Thirmachi	-	-	IR	10S-20S	Bread Wheat	77-5	121R63-1
		Adihodi	-	-	IR	10S-20S	Bread Wheat	77-1	109R63
		Adihodi	-	-	IR	60S-80S	DWR-162	77-5	121R63-1
Gadag	Nargund	Nargund	-	-	IR	10S-20S	Bread Wheat	77-5, 77-1	121R63-1, 109R63
		Alagavadi	15°38.029'	075°17.604'	IR	60S-80S	DWR-162	77-5	121R63-1

[a] Ranges of disease severity and infection type was recorded based on Modified Cobb scale (Peterson *et al.,* 1948) and Loegoring Scale (Loegoring, 1959)

[b] Leaves samples were collected at Zadoks growth stages between 69 to 83. * Durum wheat, remaining all bread wheat varieties. UAS=University of Agricultural Sciences. IR=Irrigatted. Other pests and diseases observed during survey were Aphids, Shoot fly, Spot blotch, Smut. - = Not recorded.

Table 7b: Collection of leaf rust infected samples from breeding trials and trap nursery during *rabi* 2010-11

District	Taluka	Place	Latitude (N)	Longitude (E)	Cropping Situation	Disease severity[a]	Genotypes/Variety[b]	Nomenclature of Race Identified	
								Old	New
Breeding trials:									
Belgaum	Athani	Ugar-khurd	16°37.000'	070°51.000'	IR	10S-20S	Bread wheat	104-3	21R63
						40S-60S	Bread wheat	104-2	21R55
						80S-100S	CHAM 6	104-2, 104-3	21R55, 21R63
						30S-40S	WAXWING*2/CIRCUS	77-5	121R63-1
						40S-60S	DWR-195	104-2	21R55
							DWR-39	104-2	21R55
						10MS-20MS	BABAX/LR42//BABAX/3/ER2000	77-5	121R63-1
							C-306	104-2	21R55
						80S-100S	CROC_1/AE.SQUARROSA (213)// PGO/3/CMH81.38/2*KAUZ/4/BERKUT	104-3	21R63
						60S-80S	MTRWA92.161/PRINIA/5/SERI*3// RL6010/4*YR/3/PASTOR/4/BAV92	77-5	121R63-1
						40MS-60MS	SOKOLL/EXCALIBUR	77-5	121R63-1
						40S-60S	DWR-162	104-2	21R55
						60S-80S	BERKUT/EXCALIBUR	77-1	109R63
						60S-80S	T.TAU.83.2.36/BERKUT	104-3	21R63
						40S-60S	SAAR/2*WAXWING	77-5	121R63-1

Contd…

District	Taluka	Place	Latitude (N)	Longitude (E)	Cropping Situation	Disease severity[a]	Genotypes/Variety[b]	Nomenclature of Race Identified	
								Old	New
						80S-100S	WHEAR/KRONSTAD F2004	77-5	121R63-1
						80S-100S	FD-693/2*FAHAD_4//POLLMER_4/3/ POLLMER_2.1/4/ FARAS/CMH84.4414/6/RHINO_3/ BULL_1-1/5/CMH77.1135/CMH77A.1165// 2*YOGUI_1/3/IBEX/4/JLO 97/CIVET	77-5	121R63-1
	Gokak	Gokak			IR	80S-100S	Lal bahadur	104B	29R23
		ARS-Arabhavi	16°15.579'	074°49.059'	IR	20MS-30MS	MACS-2496	104B	29R23
						5S-10S	UAS-415*	104-3	21R63
Trap nursery:									
	Gokak	ARS-Kallolli	16°15.579'	074°52.351'	IR	60S-80S	WL-711	77-5	121R63-1
						80S-100S	Agra local	104-2	21R55
						80S-100S	MACS-9	104B	29R23
						60S-80S	DWR-195	104-3, 104-2	21R63, 21R55
						40S-60S	Kite	104B	29R23
					IR	60S-80S	Pusa-4	104B	29R23
						60S-80S	Lr-9	104-2	21R55
						40S-60S	Lr-19	104-2	21R55
						20S-30S	HD-2932	104-2	21R55
						60S-80S	MACS-2496	104-2	21R55
						40S-60S	Bread wheat	104-2	21R55
					IR	80S-100S	Agra local	77-5	121R63-1
						30S-40S	C-306	104-3	21R63

[a] Ranges of disease severity and infection type was recorded based on Modified Cobb scale (Peterson *et al.,* 1948) and Loegoring Scale (Loegoring, 1959)

[b] Leaves samples were collected at Zadoks growth stages between 69 to 83. * Durum wheat, remaining all bread wheat varieties. ARS=Agricultural research station, UAS=University of Agricultural Sciences. IR=Irrigatted. Other pests and diseases observed during survey were Aphids, Shoot fly, Spot blotch, Smut. - = Not recorded.

Table 8a: A detailed survey of leaf rust of wheat caused by *Puccinia triticina* during *rabi* 2011-12

District	Taluka	Place	Latitude (N)	Longitude (E)	Disease severity[a]	Genotypes/Variety[b]	Nomenclature of Race Identified	
							New	Old
Belgaum	Gokak	Hirenandi	16°03'35.3	074°57'36.9	60S-80S	DWR-162 (Mixture)	121R63-1	77-5
	Saudatti	Inamhongal	15°38'53.1	075°04'42.5	10S-20S	DWR-162 (Mixture)	121R63-1	77-5
		Saudatti	15°47'15.4	075°08'28.6	10MS-20MS	Bread Wheat	NE	NE
		Jeevapura	15°55'30.7	075°03'20.1	30S-40S	Bread Wheat	X	X
		Jeevapura			10MS-20MS	Bread Wheat	NE	NE
		Yeragatti	15°57'22.0	075°01'48.5	10S-20S	Bread Wheat	121R63-1	77-5
		Renapura			40S-60S	Bread Wheat	21R55	104-2
		Kavarlageri Cross	16°02'44.8	074°58'18.9	20S-30S	Bread Wheat	121R63-1	77-5
Dharwad	Dharwad	UAS-Dharwad	15°29.802'	74°58.798'	20MS-30MS	DW	93R7	162
		Marewad	15°31'31.9	075°02'50.0	20S-30S	DWR-162 (Mixture)	121R63-1, 21R63	77-5, 104-3
		Amminabhavi	15°32'43.0	075°03'41.4	40S-60S	Bread Wheat	121R63-1	77-5
	Hubli	Bandiwada	16°13'35.5	077°24'37.5	40S-60S	Bread Wheat	121R63-1	77-5
		Bandiwada	16°13'35.5	077°24'37.5	20S-30S	Amruth*	29R23	104B
		Shirguppi	15°21'48.3	075°15'43.2	20S-30S	Amruth*	29R23	104B
		Bandiwada	16°13'35.5	077°24'37.5	30S-40S	Amruth*	29R23	104B
			15°29'22.4	074°59'16.2	40S-60S	DWR-1006*	21R63	104-3
		Akkipet	15°11'12.8	075°29'53.0	80S-100S	DWR-162 (Pragati)	121R63-1	77-5
		Murgod			20S-30S	DWR-162	21R55	104-2
		Sulla	15°27'22.1	075°10'00.2	5S-10S	DWR-162 (Mixture)	121R63-1	77-5
		Hebsuru	15°26'59.0	075°18'00.6	60S-80S	DWR-162 (Mixture)	121R63-1	77-5
					40S-60S	Bread Wheat	109R23	77A-1

Contd…

District	Taluka	Place	Latitude (N)	Longitude (E)	Disease severity[a]	Genotypes/Variety[b]	Nomenclature of Race Identified	
							New	Old
	Navalgund	Badrapur	15°29'22.4	074°59'16.2	40S-60S	Amruth*	121R63-1	77-5
					60S-80S	Rasi-72	121R63-1	77-5
		Tirlapur	15°33'06.5	075°14'53.0	80S-100S	DWR-162	121R63-1	77-5
					30S-40S	Bread Wheat	121R63-1	77-5
		Halekusugal	15°34'22.9	075°15'20.4	80S-100S	DWR-162 (Mixture)	121R63-1	77-5
					20S-30S	DWR-162	NE	NE
			15°34'59.3	075°15'45.8	80S-100S	Bread Wheat	121R63-1	77-5
					80S-100S	Bread Wheat	121R63-1	77-5
			15°35'44.2	075°16'28.7	30S-40S	Bread Wheat	121R63-1	77-5
					60S-80S	Amruth*	121R63-1	77-5
		Amargol	15°38'52.9	075°21'48.6	80S-100S	Bread Wheat	121R63-1	77-5
		ARS-Belavatagi	15°34'44.0	075°21'34.5	20S-30S	DWR-162	93R39, 121R63-1	162-2, 77-5
		Mannur	15°31'42.2	075°18'43.8	80S-100S	DWR-162	21R55	104-2
		Karlawada	15°29'53.6	075°18'39.1		DWR-162 (Mixture)	121R63-1	77-5
Gadag	Nargund	Alagavadi	15°38'02.9	075°17'60.4	20S-30S	DWR-162	NE	NE
			15°38'01.9	075°17'45.6	10S-20S	DWR-225* (Mixture BW)	121R63-1	77-5
					60S-80S	Bread Wheat	121R63-1	77-5
			15°38'54.7	075°18'46.6	20S-40S	DWR-225*	121R63-1	77-5
		Hansipura	15°41'27.1	075°21'38.9	80S-100S	DWR-162 (Mixture)	121R63-1	77-5
		Bairanahatti	15°48'12.8	075°27'49.9	20S-30S	Bread Wheat	121R55-1	77-6
		Konnur	15°51'27.3	075°28'46.4	20S-40S	HD-2189	121R63-1	77-5
		ARS-Konnur	15°51'50.0	075°27'58.9	5S-10S	DWR-162 (Mixture)	21R55	104-2
		Konnur	15°49'50.8	075°28'51.7	40S-60S	Bread Wheat	21R55	104-2
		Konnur	15°49'39.6	075°28'48.8	20S-30S	Bread Wheat	21R55	104-2
		Konnur	15°49'39.6	075°28'48.8	60S-80S	DWR-162 (Mixture)	93R39	162-2
		Kalkeri	15°41'14.1	075°22'32.3	80S-100S	Bread Wheat	121R63-1	77-5

[a] Ranges of disease severity and infection type was recorded based on Modified Cobb scale (Peterson *et al.,* 1948) and Loegoring Scale (Loegoring, 1959)

[b] Leaves samples were collected at Zadoks growth stages between 20 to 85

* Durum wheat, remaining all bread wheat varieties.

ARS = Agricultural research station, UAS = University of Agricultural Sciences. NE = Not established. X = mixture of races

Other pests and diseases observed during survey were Aphids, Shoot fly, Spot blotch, Smut. - = Not recorded.

Table 8b: Collection of leaf rust infected samples from breeding trials and trap nursery during *rabi* 2011-12

District	Taluka	Place	Latitude (N)	Longitude (E)	Disease severity[a]	Genotypes/Variety[b]	Nomenclature of Race Identified	
							New	Old
Breeding trials:								
Belgaum	Athani	Ugar-khurd	16°37'00.0	070°51'00.0	20MS-30MS	CSISA-LS	93R39	162-2
					20S-30S	Bread Wheat	21R55	104-2
					20S-40S	Dicoccum SH	93R37	12-9
					10S-20S	Dicoccum SH	49R37	12-3
	Gokak	ARS-Arabhavi	16°13'27.3	074°49'05.9	80S-100S	DWR-162	121R63-1	77-5
					60S-80S	Bread wheat	121R63-1	77-5
					80S-100S	Bread wheat	121R63-1	77-5
					20MS-30MS	AVT-TS (No. 5)	121R63-1	77-5
					80S-100S	DWR-162	121R63-1	77-5
					80S-100S	DWR-162	121R63-1	77-5
		ARS-Kallalli	16°15'57.9	074°52'35.1	10S-20S	Bread Wheat	121R63-1	77-5
					5MS-10MS	Dicoccum Wheat	21R55	104-2
					20S-40S	Bread wheat	121R63-1	77-5
					20MS-30MS	AVT-LS(No. 5)	121R63-1	77-5
Trap nursery:								
	Gokak	ARS-Kallolli	16°15'57.9	074°52'35.1	40S-60S	Local Red	121R55-1	77-6
					20S-40S	Agra Local	121R63-1	77-5
					40S-60S	Pusa-4	121R55-1, 121R60-1	77-6, 77-9
					80S-100S	Agra Local	21R55, 121R55-1	104-2, 77-6
					20MS-30MS	Karchia Mutant	NE	NE
					20MS	WH-147	121R63-1	77-5
					60S	Lal Bahadur	21R63	104-3
					80S	Agra Local	121R63-1	77-5
					60S-80S	C-306	21R55	104-2
					40S-60S	Khite	121R63-1	77-5

[a] Ranges of disease severity and infection type was recorded based on Modified Cobb scale (Peterson *et al.,* 1948) and Loegoring Scale (Loegoring, 1959)

[b] Leaves samples were collected at Zadoks growth stages between 20 to 85

* Durum wheat, remaining all bread wheat varieties.

ARS = Agricultural research station, UAS = University of Agricultural Sciences. NE = Not established. X = mixture of races

Other pests and diseases observed during survey were Aphids, Shoot fly, Spot blotch, Smut.

Table 9a: A detailed survey of leaf rust of wheat caused by *Puccinia triticina* during *rabi* 2012-13.

District	Taluka	Village	Latitude (N)	Longitude (E)	Elevation (m)	Growth stage[b]	Disease severity[a]	Variety/Type of Wheat	Cropping Situation	Nomenclature of Race Identified	
										New	Old
Bagalkot	Badami	Kulageri	15°55.289'	75°30.458'	587	81	60S-80S	Bread Wheat (OT)	IR	5R5	12
	Jamakhandi	Kavatadi Cross	16°34.735'	75°25.789'	548	81	10S-20S	Bread Wheat (OT)	IR	1R5	12-2
		Siddapur	16°25.634'	75°16.767'	591	77	20S-40S	Bread Wheat (OT)	IR	121R60-1	77-9
	Mudhol	Mudhol	16°20.525'	75°16.13'	545	85	20S-40S	Bread Wheat (OT)	IR	5R5	12
Belgaum	Athani	Athani	16°44.069'	75°01.634'	546	83	10S-20S	Bread Wheat (OT)	IR	5R5	12
			16°44.045'	75°01.634'	547	83	10S-20S	Bread Wheat (OT)	IR	121R60-1	77-9
		Shiruguppi	16°36.926'	74°42.566'	537	85	60S-80S	Bread Wheat (OT)	IR	1R5	12-2
	Bailahongal	Anigol	15°47.002'	74°52.246'	655	83	60S-80S	DWR-162 (OT)	IR	121R63-1	77-5
						85	60S-80S	DWR-162	IR	121R60-1	77-9
		Mallur	-	-	-	81	80S	Bread Wheat	IR	121R63-1	77-5
		Mukabasava			-	81	80S	Bread Wheat (off type)	IR	121R60-1	77-9
		Halingali			-	77	30S	Bread Wheat local	IR	121R63-1	77-5
		M. K. Hubli	15°43.051'	74°41.99'	643	77	80S-100S	DWR-162(OT)	IR	-	-
	Chikkodi	Bhoregal	16°20.353'	74°32.426'	746	81	20S-30S	Bread Wheat (OT)	IR	121R60-1	77-9
	Gokak	Kalloli	16°15.945'	74°52.76'	563	77	10MS	Dicoccum Wheat	IR	121R63-1 +93R7	77-5 +162
			16°15.016'	74°51.481'	571	81	80S	Pragathi Mixture	IR	121R60-1	77-9
	Hukkeri	U.Khanapura	16°08.915'	74°31.012'	683	83	40S-60S	Bread Wheat (OT)	IR	29R23	104B
		Badakodri	16°11.096'	74°34.384'	628	83	100S	Bread Wheat (OT)	IR	121R63-1	77-5
						87	20S	DWR-162	IR	121R63-1	77-5
	Soudatti	Tavagere Cross	16°02.749'	74°58.315'	618	89	20S	DWR-162	IR	121R60-1	77-9
Bijapur	Bijapur	Bijapur	16°47.718'	75°41.658'	624	87	60S-80S	Bread Wheat (OT)	IR	109R31	77-2
		Yakkundi	16°37.82'	75°32.525'	577	83	10S-20S	DWR-162(OT)	IR	125R28	77-11
	Jamakhandi	Navalagi	-	-	-	81	20S	Bread Wheat local	IR	21R55	104-2
Dharwad	Dharwad	UAS-Dharwad	15°29.802'	74°59.032'	691	83	10S-20S	Agra local	RF	109R63	77-1
						87	30S-40S	Amruth*	RF	121R63-1	77-5
		Amminabhavi	-	-	-	83	80S-100S	DWR-162	RI	121R60-1	77-9
		Dharwad				69	40MS-60MS	Durrum Wheat	RI	109R63	77-1
		Mangalagatti	15°31.815'	74°57.767'	675	83	10S-20S	Bread Wheat	RI	121R63-1	77-5
		Mulmuthla	15°33.128'	74°58.435'	671	27	10S-20S	Bread Wheat	RI	5R5	12

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District	Taluka	Village	Latitude (N)	Longitude (E)	Elevation (m)	Growth stage[b]	Disease severity[a]	Variety/Type of Wheat	Cropping Situation	Nomenclature of Race Identified	
										New	Old
			15°33.147'	74°58.425'	673	81	80S-100S	DWR-162 (OT)	RI	121R60-1	77-9
		Mulmuthla	15°33.128'	74°58.435'	671	81	60S-80S	Bread Wheat	RI	93R37	12-9
			15°33.593'	74°58.839'	664	83	80S-100S	DWR-162 (OT)	RI	125R55	77-3
			15°33.678'	74°58.269'	684	87	5S-10S	DWR-2006 (mixture)	RI	17R23	104
		Narendra	15°30.712'	74°58.537'	684	75	10S-20S	DWR-2006 (OT)	RI	121R63-1	77-5
		Narendra	15°30.712'	74°58.537'	684	83	40S-60S	Bijaga Yellow	RI	121R63-1	77-5
		Narendra	-	-	-	81	20S	Bread Wheat	IR	121R60-1	77-9
			15°33.678'	74°58.269'	684	81	30S-40S	Durrum Wheat (BW mixture)	RI	29R23	104B
			15°33.678'	74°58.269'	684	81	40S-60S	Bread Wheat	RI	1R5	12-2
		Maradagi	15°27.948'	75°06.11'	654	87	5S-10S	Bread Wheat (OT)	IR	109R23	77A-1
		Govinakoppa	15°27.825'	75°03.284'	663	81	5S-10S	Bread Wheat (OT)	IR	109R63	77-1
		Dharwad	-	-	-	20	10S-20S	Bread Wheat	IR	121R60-1	77-9
						87	80S	NIVT-2-21	IR	121R63-1	77-5
						81	20S	NIVT-2-32	IR	121R63-1	77-5
						83	20S	NIVT-2-19	IR	121R60-1	77-9
		Dharwad	15°29.802'	74°58.798'	689	81	40S	AVT-BW-93	IR	5R5	12
						83	60S	AVT-BW-99	IR	17R23	104
						87	60S	AVT-BW-270	IR	125R55	77-3
						87	40S	AVT-BW-226	IR	125R55	77-3
						87	80S	AVT-BW-212	IR	121R60-1	77-9
						87	60S	NIVT-BW-168	IR	121R60-1	77-9
						83	40S	NIVT-BW-63	IR	121R60-1	77-9
						81	40S	NIVT-BW-279	IR	121R63-1	77-5
						87	40s	NIVT-BW-299	IR	121R63-1	77-5
	Hubli	Hubli	15°21.365'	75°10.642'	631	81	60S-80S	Amruth*	IR	93R47	162-1
	Kundagal	Shamshi	15°12.166'	75°18.57'	654	27	10S-20S	Amruth*	IR	121R63-1	77-5
	Navalgund	Halakusugal	15°35.46'	75°16.263'	591	81	80S-100S	Amruth*	IR	121R60-1	77-9
Gadag	Nargund	Alagavadi	15°38.401'	75°18.065'	597	83	20S-40S	Bread Wheat (OT)	IR	121R63-1	77-5
		Bhairanatti	15°46.696'	75°26.835'	599	81	5S-10S	Bread Wheat (OT)	IR	125R28	77-11
					599	81	5S-10S	Bread Wheat (OT)	IR	125R55	77-3
		Nargund	15°42.339'	75°22.335'	580	83	40S-60S	Bread Wheat (OT)	IR	121R63-1	77-5

[a] Ranges of disease severity and infection type was recorded based on Modified Cobb scale (Peterson *et al.,* 1948) and Loegoring Scale (Loegoring, 1959)

[b] Leaves samples were collected at Zadoks growth stages between 20 to 91

* Durum wheat, remaining all bread wheat varieties. OT: Off Type

ARS=Agricultural research station, UAS=University of Agricultural Sciences. IR=Irrigated. RI=Restricted irrigated. RF=Rainfed.

Other pests and diseases observed during survey were Aphids, Shoot fly, Spot blotch, Smut. - Not recorded.

Table 9b: Collection of leaf rust infected samples from breeding trials and trap nursery during *rabi* 2012-13

District	Taluka	Village	Latitude (N)	Longitude (E)	Elevation (m)	Growth stage[b]	Disease severity[a]	Variety/Type of Wheat	Cropping Situation	Nomenclature of Race Identified	
										New	Old
Breeding trials:											
Belgaum		Ugar-Khurd	16°38.517'	74°49.67'	535	91	60S	45th IBWSN-1112	IR	121R60-1	77-9
						85	40S	AVT (LS) PZ-LS-06	IR	121R63-1	77-5
						83	40S	AVT (LS) PZ-LS-12	IR	121R63-1	77-5
						91	20S	HTTT-2-LS Pl. No. 02	IR	121R60-1	77-9
						89	20S	HTTT-2-LS Pl. No. 10 (G-1114)	IR	121R63-1	77-5
						87	20S	32nd SAWSN-No. 89	IR	121R63-1	77-5
						24	80S	GCP Parent No. 20	IR	121R60-1	77-9
						83	80S	GCP-F4(Bulk) No. 28	IR	125R55	77-3
						81	60S	31st SAWSN- No. 3163	IR	121R60-1	77-9
						89	40S	EHTN - 5	IR	121R60-1	77-9
						89	20S	EHTN (BZ) - 4	IR	121R63-1	77-5
						89	80S	29th SAWSN - 3120	IR	121R60-1	77-9
		Bailahongal	15°49.516'	74°51.369'	692	87	40S-60S	AVT-RI-PZ05	IR	121R63-1 +125R55	77-5+ 77-3
						87	10MS	AVT-RI-PZ04	IR	93R5	93R5
						87	20S	AVT-RI-PZ08	IR	121R63-1	77-5
Dharwad		Dharwad	15°29.802'	74°58.798'	689	87	80S	NIVT-2-21	IR	121R63-1	77-5
						81	20S	NIVT-2-32	IR	121R63-1	77-5
						83	20S	NIVT-2-19	IR	121R60-1	77-9
						81	40S	AVT-BW-93	IR	5R5	12
						83	60S	AVT-BW-99	IR	17R23	104
						87	60S	AVT-BW-270	IR	125R55	77-3
						87	40S	AVT-BW-226	IR	125R55	77-3
						87	80S	AVT-BW-212	IR	121R60-1	77-9
						87	60S	NIVT-BW-168	IR	121R60-1	77-9

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District	Taluka	Village	Latitude (N)	Longitude (E)	Elevation (m)	Growth stage[b]	Disease severity[a]	Variety/Type of Wheat	Cropping Situation	Nomenclature of Race Identified	
										New	Old
						83	40S	NIVT-BW-63	IR	121R60-1	77-9
						81	40S	NIVT-BW-279	IR	121R63-1	77-5
						87	40s	NIVT-BW-299	IR	121R63-1	77-5
Trap nursery:											
Belgaum		Ugar-Khurd	16°38.517'	74°49.67'	535	49	40MS	MACS-9	IR	1R5	12-2
						49	20MS	Kite	IR	109R63	77-1
						47	10MS	Lr-19	IR	121R63-1	77-5
						49	80S-100S	Local Red*	IR	5R5	12
						59	30S	Kite	IR	109R63	77-1
						59	60S	MACS-9	IR	1R5	12-2
						61	20S	Pusa-4	IR	5R5	12
						69	20S	Kharchia Mutant	IR	121R60-1	77-9

[a] Ranges of disease severity and infection type was recorded based on Modified Cobb scale (Peterson *et al.,* 1948) and Loegoring Scale (Loegoring, 1959)

[b] Leaves samples were collected at Zadoks growth stages between 20 to 91

* Durum wheat, remaining all bread wheat varieties. OT: Off Type

ARS=Agricultural research station, UAS=University of Agricultural Sciences. IR=Irrigated. RI=Restricted irrigated. RF=Rainfed.

Other pests and diseases observed during survey were Aphids, Shoot fly, Spot blotch, Smut.

- Not recorded.

Table 10: Prevalence of *Puccinia triticina* pathotypes in Karnataka over the seasons

Sl. No.	Races		No. of samples during the year		
	New name	Old name	2010-11	2011-12	2012-13
1	109R63	77-1	5	-	4
2	109R31	77-2	1	-	1
3	125R55	77-3	-	-	6
4	121R63-1	77-5	20	43	25
5	121R55-1	77-6	1	4	-
6	121R60-1	77-9	-	1	26
7	125R28	77-11	-	-	2
8	109R23	77A-1	-	1	1
9	17R23	104	-	-	2
10	21R55	104-2	16	10	1
11	21R63	104-3	8	3	-
12	29R23	104B	5	3	2
13	93R7	162	-	1	1
14	93R47	162-1	-	-	1
15	93R39	162-2	-	4	-
16	29R45	12-5	-	1	-
17	49R37	12-3	-	1	1
18	93R37	12-9	-	1	1
19	5R5	12	-	-	8
20	1R5	12-2	-	-	5
		Total	**56**	**73**	**87**

Table 11: Frequency and per cent distribution of *Puccinia triticina* races prevailed during *rabi* 2010-11 to 2012-13

Sl. No.	Races		Years			
	New name	Old name	2010-11	2011-12	2012-13	Mean
1	5R5	12	-	-	9.19	3.06
2	1R5	12-2	-	-	5.75	1.92
3	49R37	12-3	-	1.37	1.14	0.84
4	29R45	12-5	-	1.37	-	0.46
5	93R37	12-9	-	1.37	1.14	0.84
6	109R63	77-1	8.93	-	4.60	4.51
7	109R31	77-2	1.79	-	1.15	0.98
8	125R55	77-3	-	-	6.90	2.30
9	121R63-1	77-5	35.71	58.9	28.74	41.12
10	121R55-1	77-6	1.76	5.48	-	2.41
11	121R60-1	77-9	-	1.37	29.89	10.42
12	125R28	77-11	-	-	2.30	0.77
13	109R23	77A-1	-	1.37	1.15	0.84
14	17R23	104	-	-	2.30	0.77
15	21R55	104-2	28.57	13.7	1.15	14.47
16	21R63	104-3	14.29	2.74	-	5.68
17	29R23	104B	8.93	4.11	2.30	5.11
18	93R7	162	-	1.37	1.15	0.84
19	93R47	162-1	-	-	1.15	0.38
20	93R39	162-2	-	6.45	-	2.15

Data collection at farmers field of Hubli

Interaction with farmers during Kharif season at shivani(Chikmagalore)

Sample collection at seeding stage (Mulamuttala)

Sample collection at adult plant stage (Jeevapur cross)

Severity of leaf rust on off- type in farmers field

Plate 2: Leaf rust samples collection and severity at formers field

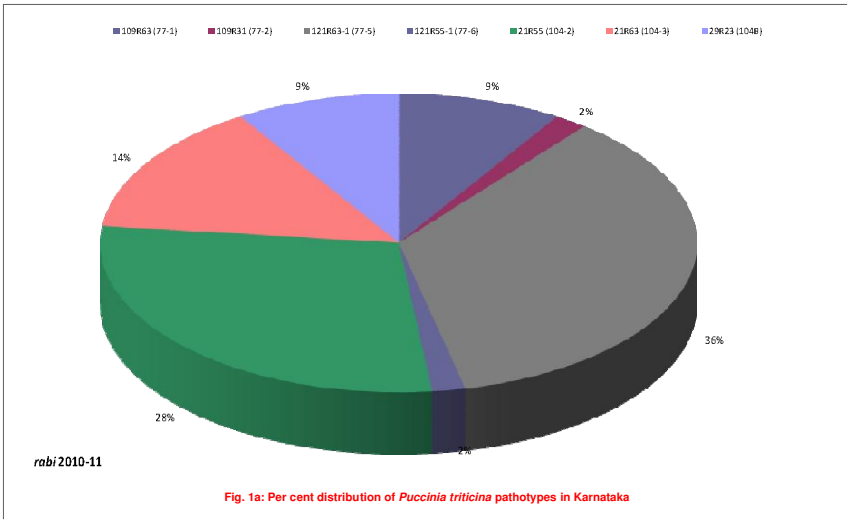


Fig. 1a: Per cent distribution of *Puccinia triticina* pathotypes in Karnataka

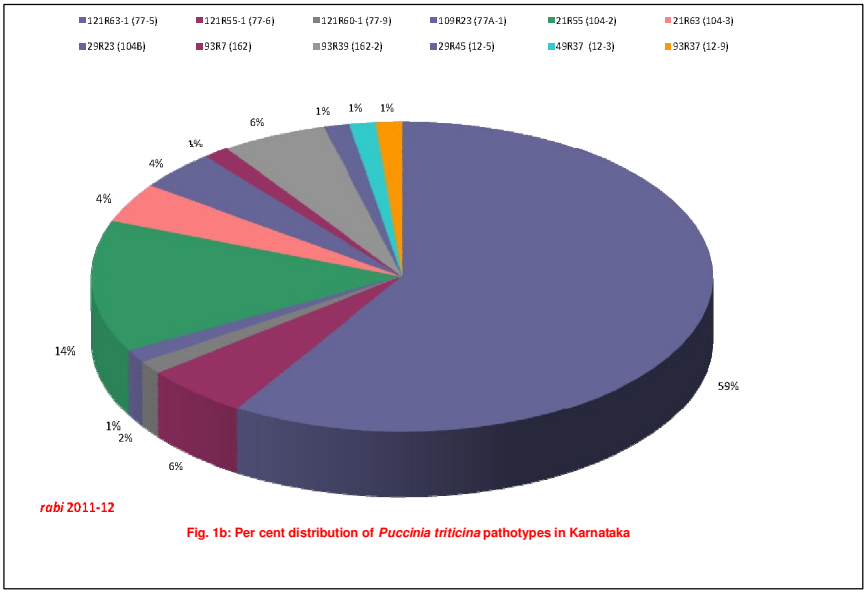


Fig. 1b: Per cent distribution of *Puccinia triticina* pathotypes in Karnataka

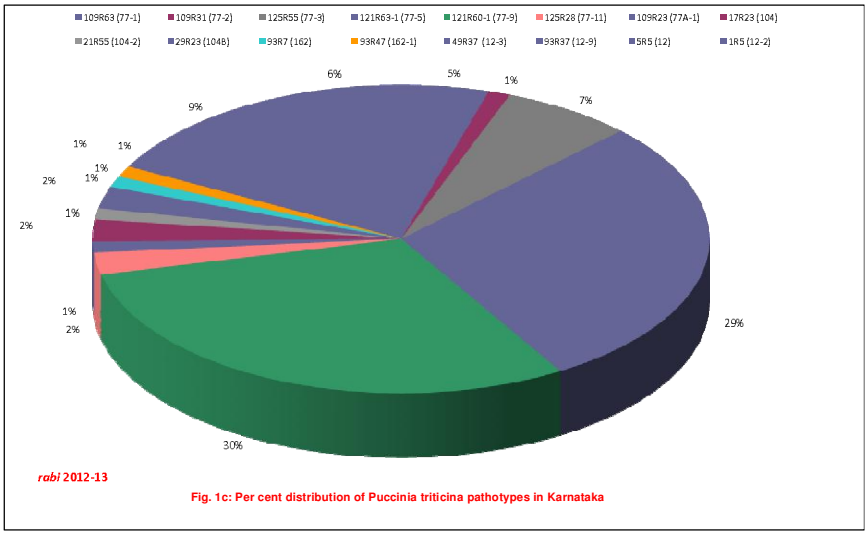


Fig. 1c: Per cent distribution of Puccinia triticina pathotypes in Karnataka

Fig 1: Per cent distribution of *Puccinia triticina* pathotypes in Karnataka

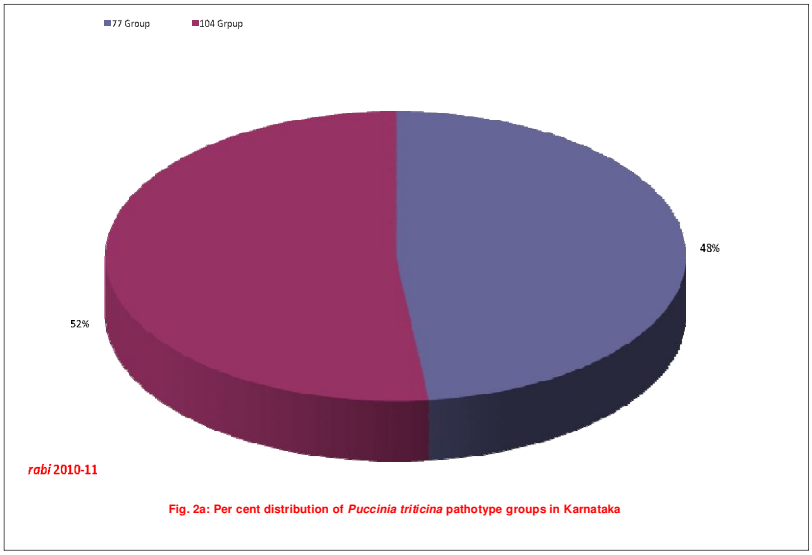


Fig. 2a: Per cent distribution of *Puccinia triticina* pathotype groups in Karnataka

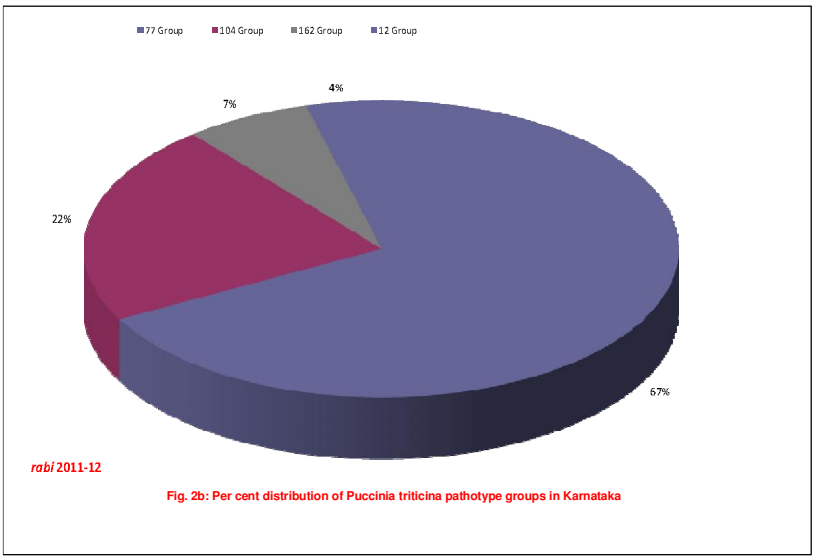


Fig. 2b: Per cent distribution of Puccinia triticina pathotype groups in Karnataka

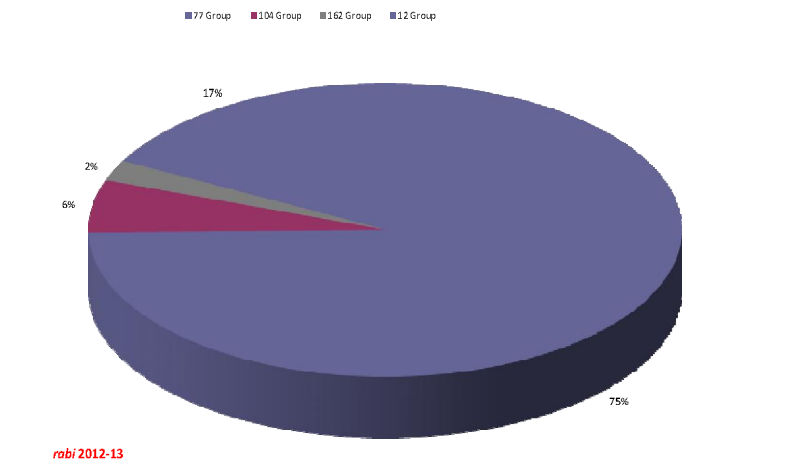


Fig. 2c: Per cent distribution of *Puccinia triticina* pathotype groups in Karnataka

Fig 2: Per cent distribution of *Puccinia triticina* pathotype groups in Karnataka

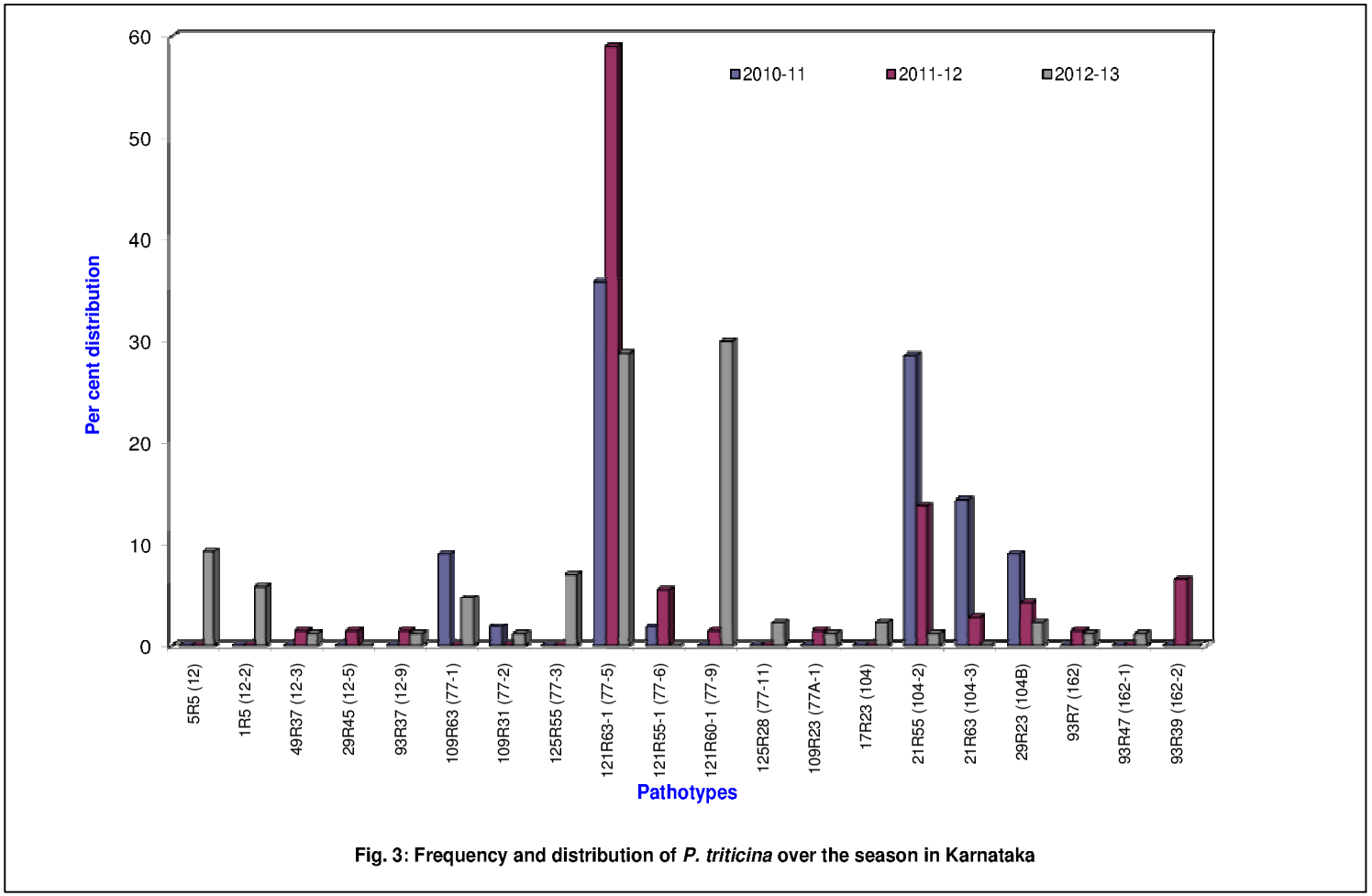


Fig. 3: Frequency and distribution of *P. triticina* over the season in Karnataka

Fig 3: Frequency and distribution of *P. triticina* over the season in Karnataka

4.1.3 Comparison of all the three years survey data

In comparison of all the years it is stated that, during the year 2010-11 only seven pathotypes were identified out of 53 samples collected, which belongs to two pathotype group. Whereas, during the year 2011-12 almost double i.e. 12 pathotypes were identified from 73 samples which includes recently identified two pathotypes *viz.,* 93R37 (12-9) and 109R23 (77A-1). During the year 2012-13 total 16 pathotypes were identified from 85 samples which includes recently identified three pathotypes *viz.,* 125R28 (77-11), 93R37 (12-9) and 109R23 (77A-1). So, it is clearly indicated that, during the year 2012-13 the pathogen variability and distribution was more as compared to that of previous two year observation (Table 10 and 11).

The leaf rust virulence frequency of all the years is presented in Table 11 and Fig. 3. During 2010-11 and 2011-12 pathotype 121 R 63-1 (77-5) showed highest frequency in the surveyed area (35.71 and 58.90 % respectively). Whereas, during 2012-13 pathotype 121R60-1 (77-9) was found in highest frequency (29.89 %).

4.2 Study of genetic diversity in *P. triticina* population through molecular techniques

All the 25 *P. triticina* isolates collected from wheat field selected at 20 locations in districts of Northern Karnataka during *rabi* 2012-13 and five phenotypical known races collected from Directorate of Wheat Research, Regional Station, Flowerdale, Himachal Pradesh, India (Table 12) were subjected to DNA profiling to know the extent of genetic diversity by using Expressed Sequence Tag - Simple Sequence Repeats (EST-SSR) markers. A set of six EST-SSR markers were used for molecular profiling of 25 different isolates to study the genetic diversity of *P. triticina* (Table 3, Plate 3 and 4).

Based on simple matching co-efficients, a genetic similarity matrix was constructed using EST-SSR markers data to assess the genetic relatedness among 25 isolates. Cluster analysis was estimated using NTSYS PC-2.0 Software programme. The clustering was done and dendrograms were generated by following unweighted pair group using arithmetic mean algorithm (UPGMA) routine available in the above programme.

Twenty five isolates were grouped into 14 clusters (Table 12 and Fig. 4). The cluster XIV was largest with 12 isolates including phenotypically known isolates (12-4, 77-6 and 77-5). Remaining 13 isolates formed individual cluster including phenotypically known isolates (104-2 and 162-2). This clearly reveals that, these 13 individual clusters differed 100 per cent with each other and also with cluster XIV. In the cluster XIV, twelve isolates showed similar as they were grouped in the same cluster and these isolates were 100 per cent dissimilar from that of all other 13 isolates.

Similarity co-efficients ranged from 0.0 to 0.99, the minimum genetic relatedness was zero per cent and the maximum genetic relatedness was 99 per cent between clusters (Table 13).

4.3 Identification of slow leaf rusters

An experiment on slow leaf rusters was conducted during *rabi* 2011-12 and 2012-13 at wheat field of Dr. Sanjaya Rajaram Wheat laboratory, MARS, UAS, Dharwad. Host responses on various slow leaf rusting mechanisms / components by 40 and 57 bread wheat genotypes during 2011-12 and 2012-13 respectively are furnished here under. The overall field view at seedling and adult plant stage was given in Plate 5.

4.3.1 Average Coefficient of Infection (ACI)

The leaf rust development was recorded at 7 days interval starting from the onset of the disease till it attained maximum severity. ACI was computed as per the procedure explained under 'Material and Methods' (Plate 6a).

4.3.1.1 *Rabi* 2011-12

The results from the Table 14 revealed that, among 40 bread wheat genotypes, Agra Local (29.00) and Lal Bahadur (27.00) were first to show high leaf rust incidence and later it reached to maximum of 100 and 98.00 ACI respectively. Fifty per cent of (20) genotypes were showed zero ACI. Whereas, ten genotypes were showed medium range of ACI between 0.25 to 22.36. The genotype HD-2189 showed 6.3 ACI at the end. The lowest ACI was observed in HS-420 (0.06) followed by RAJ-4083 (0.20) and HD-2932 (0.50) but genotypes GW-322, PBW-343 and NW-4091 were on par with the ACI of Raj-4083 and HD-2932 (Fig. 5).

The mean values of the ACI over five intervals were revealed that, at the second interval disease score was increased by 2.30, at third by 1.54, at fourth and fifth by 1.13 times of preceding values.

4.3.1.2 *Rabi* 2012-13

During the *rabi* 2012-13 the overall disease development was higher as compared to previous year (2011-12) and it is indicated in the Table 15. Among 57 bread wheat genotypes sown, the first observation at 54 days after sowing on two susceptible checks, Agra Local (27) and Lal Bahadur (16.00) were showed very high leaf rust incidence (ACI) followed by Lok-1(10.40), Sonalika (7.6), HS-240 (7.10) and Pavon-76 (5.92). Agra Local and Lal Bahadur were reached highest final disease score as 100 and 99 ACI respectively. Totally, 21 genotypes showed zero ACI with immune to resistant reaction.

The lowest ACI was observed at 82 days after sowing in RAJ-4083 (0.25) and VL-907 (0.38) followed by VL-943 (0.85) and GW-322 (2.54) which were on par with RAJ-4083 and VL-907. The next lowest ACI at last disease score was recorded by HI-977, UAS-315, KRL-210, NI-5439, DBW-16, HD-2733, HS-420, MACS-2496, HD-3091, Pavon-76, RL-6077 and Parula which were statistically on par with earlier known slow rusting genotype, HD-2189 (9.48 ACI).

The mean values of ACI over five intervals revealed that, at the second interval disease score was jumped by 2.13, at third by 1.62, at fourth by 1.27 and at last by only 1.24 times of preceding values. The mean ACI values of most of the bread wheat genotypes differ significantly with range of 0.0 to 68.0 (Fig. 5 & 6). The trend of jump in mean values over preceding score was as that of last year.

4.3.2 Rate of disease development ('r')

Rate of leaf rust development is an indication of the severity of the disease. The data on the rate of leaf rust development of both the years were presented in the Table 16 and 17. Data revealed a wide variation among different genotypes at different intervals.

4.3.2.1 *Rabi* 2011-12

The results indicated that, maximum 'r' values were noticed in DWR-162 (0.17), Agra Local (0.12) and Lal Bahadur (0.12) units per day between first two observations of the disease development. The HD-2189 showed 0.08 'r' value in first two interval but shown 0.09 and 0.04 units per day at second and third interval subsequently it was reduced to 0.02 unit per day. More or less similar trend was observed by HI-977, NI-5439, GW-322, UAS-315, VL-616 and HS-240. These genotypes were found to have slow leaf rusting resistance, where availability of green tissue was not a bar, even though disease progress showed decline.

The peak average 'r' value was observed in the genotype DWR-162 (0.14) followed by HI-977, LOK-1 and Sonalika with 'r' values 0.12, 0.10 and 0.08 respectively. The least average 'r' value was noticed in the genotype Raj-4083 (0.01) followed by NI-5439 (0.02) and KRL-210 (0.02). Total 21 genotypes were showed zero rate of disease development indicated immune to resistant type of reaction. The mean 'r2' (0.04) value was exactly double of the r3 mean value, later slight increase in r4 to 0.03 units per day was observed.

4.3.2.2 *Rabi* 2012-13

During 2012-13 higher rate of disease development was observed as compared to previous year and it is evidenced in the Table 17. The results revealed that, maximum 'r' value were noticed in KRL-210 (0.32), C-306 (0.27) and PBW-343 (0.18) units per day) between first two observations of the disease development. After r1 there was steady increase in 'r' value observed in HD-2189. Whereas, decrease in 'r' value was also observed in VL-924, C-306, NI-5439, DBW-17, MACS-2496, RL-6077 and Parula.

The peak average 'r' value was observed in the genotype DWR-162 (0.14) followed by HI-977, LOK-1 and Sonalika with 'r' values 0.12, 0.10 and 0.08 respectively. The least average 'r' value was noticed in the genotype RAJ-4083 (0.01) followed by NI-5439 (0.02) and KRL-210 (0.02). Totally, 21 genotypes were showed zero rate of disease development revealed immune to resistant type of reaction. The mean 'r2' (0.04) value was exactly double of the r1 and r3 mean value, later slight increase in r4 to 0.03 units per day was observed.

Table 12: Grouping of *P. triticina* isolates based on genetic dissimilarity clusters

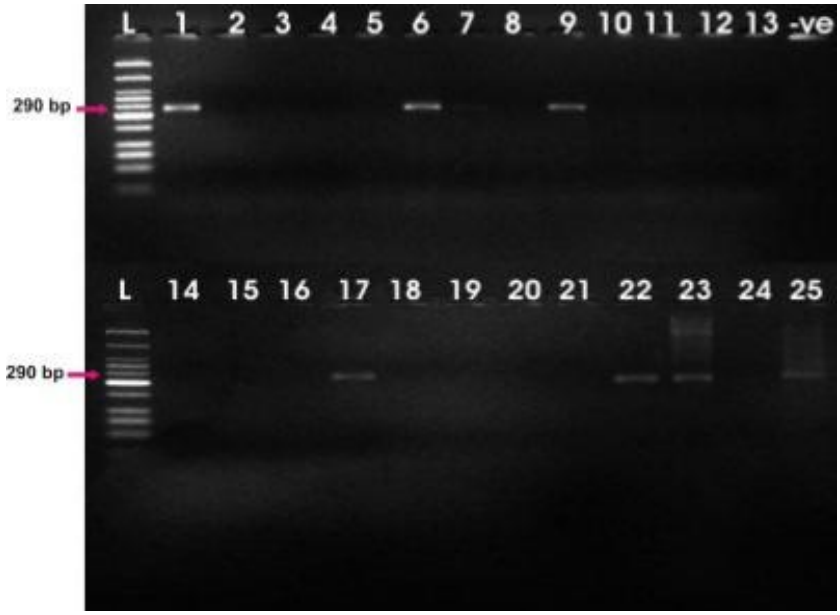
Sl. No.	Cluster No	Isolate No.	Place	Taluka	District	Variety
1	I	1	UAS-Dharwad	Dharwad	Dharwad	Amruth (DW)
2	II	6	M. K. Hubli	Bailhongal	Belgaum	DWR-162 (OT)
3	III	25	UAS-Dharwad	Dharwad	Dharwad	UAS-195
4	IV	22	104-2*	-	-	-
5	V	17	Badakondri	Hukkeri	Belgaum	Bread Wheat
6	VI	7	U. Khanapur	Hukkeri	Belgaum	Bread Wheat
7	VII	23	162-2*	-	-	-
8	VIII	9	Ugar-Khurd	Athani	Belgaum	Local Red (DW)
9	IX	10	Bijapur	Bijapur	Bijapur	Bread Wheat
10	X	3	Mulmuthla	Dharwad	Dharwad	DWR-162 (OT)
11	XI	16	Anigol	Bailhongal	Belgaum	DWR-162 (OT)
12	XII	11	Muddapur	Mudhol	Bagalkhot	Bread Wheat
13	XIII	14	Halakusugal	Navalgund	Dharwad	Amruth
14	XIV	2	Amminabhavi	Dharwad	Dharwad	DWR-162 (BW)
15		4	Saunshi	Kundagol	Dharwad	Amruth
16		5	Hubli	Hubli	Dharwad	Amruth
17		8	Shiruguppi	Athani	Belgaum	Bread Wheat
18		24	12-4*	-	-	-
19		12	Kulageri	Badami	Gadag	Bread Wheat
20		13	Nargund	Nargund	Gadag	Bread Wheat
21		21	77-6*	-	-	-
22		20	77-5*	-	-	-
23		19	Siddapur	Jamakhandi	Bagalkhot	Bread Wheat
24		18	Ugar-Khurd	Athani	Belgaum	Dicoccum Wheat
25		15	Garag	Dharwad	Dharwad	DWR-1006 (OT)

Note: BW=Bread Wheat, DW=Durrum Wheat and OT=Off type
* Phonotypical known predominant races in India collected from Directorate of Wheat Research (DWR), Regional Station, Flowerdale, Himachal Pradesh, India

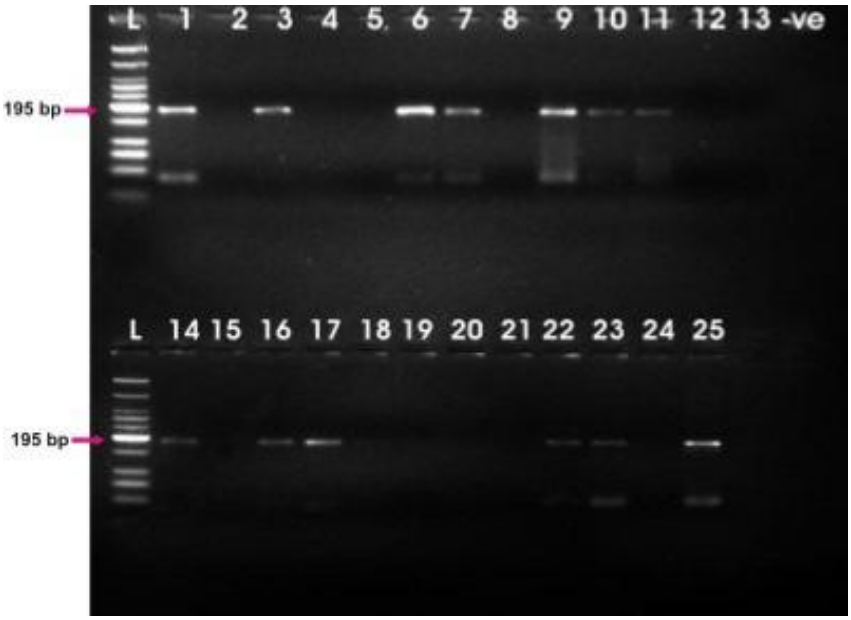
LEGEND

Lane No.	Place	Variety
L	Ladder 25bp	
1	UAS-Dharwad	Amruth (DW)
2	M. K. Hubli	DWR-162 (OT)
3	UAS-Dharwad	UAS-195
4	104-2*	-
5	Badakondri	Bread Wheat
6	U. Khanapur	Bread Wheat
7	162-2*	-
8	Ugar-Khurd	Local Red (DW)
9	Bijapur	Bread Wheat
10	Mulmuthla	DWR-162 (OT)
11	Anigol	DWR-162 (OT)
12	Muddapur	Bread Wheat
13	Halakusugal	Amruth
14	Amminabhavi	DWR-162 (BW)
15	Saunshi	Amruth
16	Hubli	Amruth
17	Shiruguppi	Bread Wheat
18	12-4*	-
19	Kulageri	Bread Wheat
20	Nargund	Bread Wheat
21	77-6*	-
22	77-5*	-
23	Siddapur	Bread Wheat
24	Ugar-Khurd	Dicoccum Wheat
25	Garag	DWR-1006 (OT)
-ve	Control	

Note: BW=Bread Wheat, DW=Durrum Wheat and OT=Off type
* Phonotypical known predominant races in India collected from Directorate of Wheat Research
(DWR), Regional Station, Flowerdale, Himachal Pradesh, India



Ptssr0083



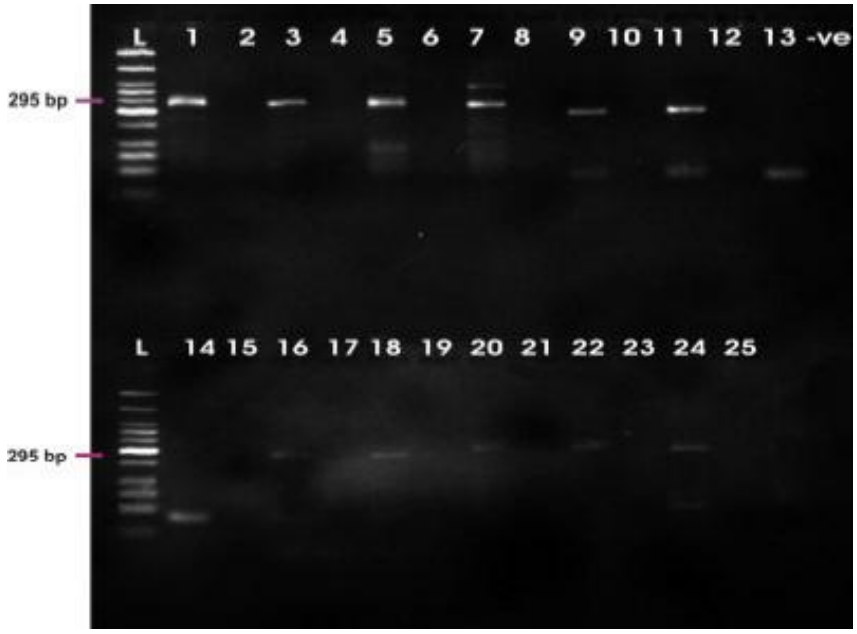
Ptssr5649

Plate 3: Genetic diversity of *Puccinia triticina*

LEGEND

Lane No.	Place	Variety
L	Ladder 25bp (Ptssr0085) and 100bp (Ptssr5594)	
1	UAS-Dharwad	Amruth (DW)
2	M. K. Hubli	DWR-162 (OT)
3	UAS-Dharwad	UAS-195
4	104-2*	-
5	Badakondri	Bread Wheat
6	U. Khanapur	Bread Wheat
7	162-2*	-
8	Ugar-Khurd	Local Red (DW)
9	Bijapur	Bread Wheat
10	Mulmuthla	DWR-162 (OT)
11	Anigol	DWR-162 (OT)
12	Muddapur	Bread Wheat
13	Halakusugal	Amruth
14	Amminabhavi	DWR-162 (BW)
15	Saunshi	Amruth
16	Hubli	Amruth
17	Shiruguppi	Bread Wheat
18	12-4*	-
19	Kulageri	Bread Wheat
20	Nargund	Bread Wheat
21	77-6*	-
22	77-5*	-
23	Siddapur	Bread Wheat
24	Ugar-Khurd	Dicoccum Wheat
25	Garag	DWR-1006 (OT)
-ve	Control	

Note: BW=Bread Wheat, DW=Durrum Wheat and OT=Off type
* Phonotypical known predominant races in India collected from Directorate of Wheat Research
(DWR), Regional Station, Flowerdale, Himachal Pradesh, India



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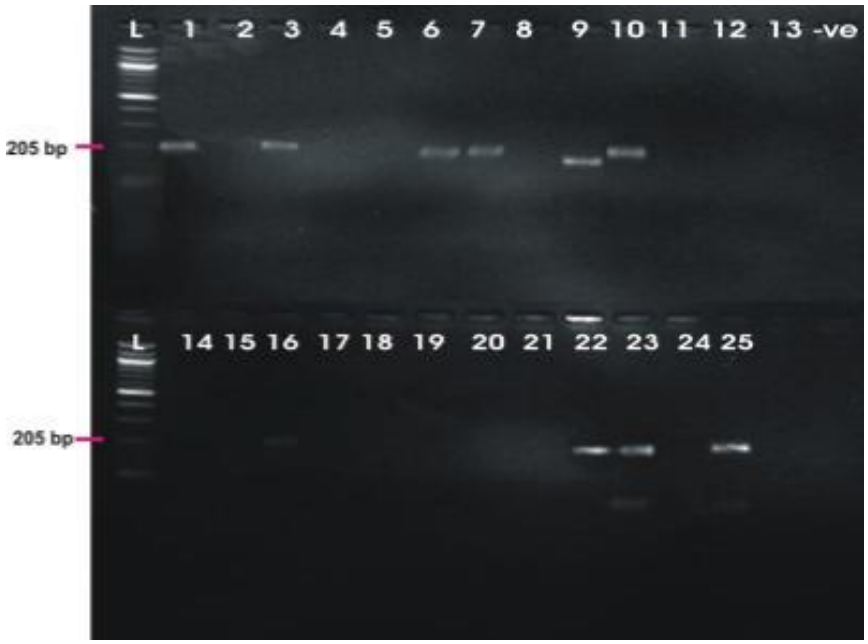


Ptssr5594

Plate 4: Genetic diversity of *Puccinia triticina*

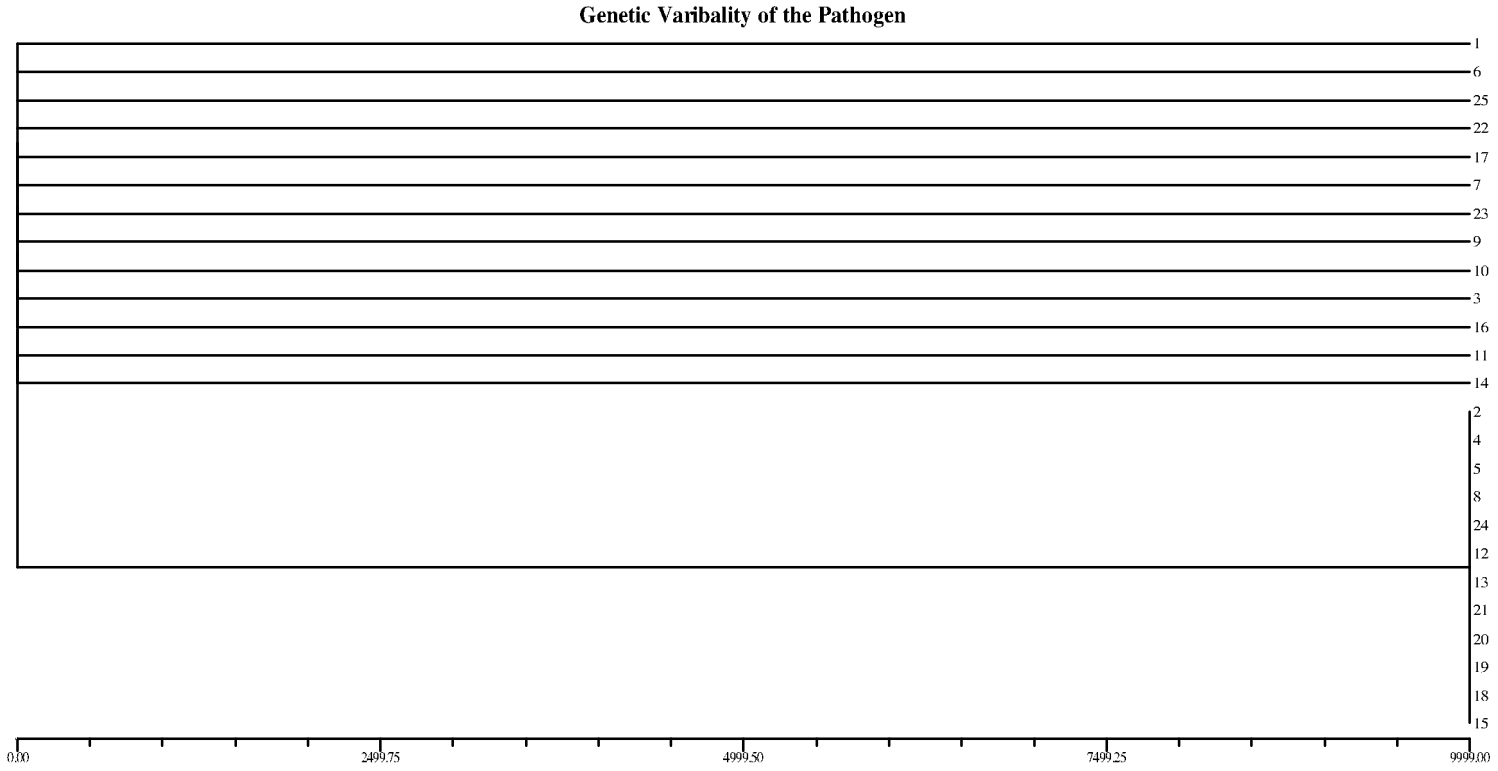


Fig. 4: Genetic diversity of *Puccinia triticina* in

Table 13: Similarity co-efficients of 25 isolates of *P. triticina* collected from different wheat growing areas of Northern Karnataka during *rabi* 2012-13.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
1	1.00																								
2	0.00	1.00																							
3	0.66	0.00	1.00																						
4	0.00	0.99	0.00	1.00																					
5	0.00	0.99	0.00	0.99	1.00																				
6	0.99	0.00	0.66	0.00	0.00	1.00																			
7	0.99	0.00	0.66	0.00	0.00	1.00	1.00																		
8	0.00	0.99	0.00	0.99	0.99	0.00	0.00	1.00																	
9	0.99	0.00	0.66	0.00	0.00	0.99	0.99	0.00	1.00																
10	0.90	0.00	0.75	0.00	0.00	0.90	0.90	0.00	0.90	1.00															
11	0.66	0.00	0.33	0.00	0.00	0.66	0.66	0.00	0.66	0.75	1.00														
12	0.00	0.99	0.00	0.99	0.99	0.00	0.00	0.99	0.00	0.00	0.00	1.00													
13	0.00	0.99	0.00	0.99	0.99	0.00	0.00	0.99	0.00	0.00	0.00	0.99	1.00												
14	0.66	0.00	0.33	0.00	0.00	0.66	0.66	0.00	0.66	0.75	0.99	0.00	0.00	1.00											
15	0.00	0.99	0.00	0.99	0.99	0.00	0.00	0.99	0.00	0.00	0.00	0.99	0.99	0.00	1.00										
16	0.66	0.00	0.66	0.00	0.00	0.66	0.66	0.00	0.66	0.75	0.66	0.00	0.00	0.66	0.00	1.00									
17	0.99	0.00	0.66	0.00	0.00	0.99	0.99	0.00	0.99	0.90	0.66	0.00	0.00	0.66	0.00	0.66	1.00								
18	0.00	0.99	0.00	0.99	0.99	0.00	0.00	0.99	0.00	0.00	0.00	0.99	0.99	0.00	0.99	0.00	0.00	1.00							
19	0.00	0.99	0.00	0.99	0.99	0.00	0.00	0.99	0.00	0.00	0.00	0.99	0.99	0.00	0.99	0.00	0.00	0.99	1.00						
20	0.00	0.99	0.00	0.99	0.99	0.00	0.00	0.99	0.00	0.00	0.00	0.99	0.99	0.00	0.99	0.00	0.00	0.99	0.99	1.00					
21	0.00	0.99	0.00	0.99	0.99	0.00	0.00	0.99	0.00	0.00	0.00	0.99	0.99	0.00	0.99	0.00	0.00	0.99	0.99	0.99	1.00				
22	0.00	0.99	0.90	0.66	0.00	0.00	0.66	0.00	0.66	0.99	0.00	0.00	0.00	0.00	0.99	0.99	0.00	0.66	0.00	0.00	0.99	1.00			
23	0.00	0.99	0.90	0.66	0.00	0.00	0.66	0.00	0.66	0.99	0.00	0.00	0.00	0.00	0.99	0.99	0.00	0.99	0.00	0.99	0.99	0.00	1.00		
24	0.99	0.00	0.00	0.00	0.99	0.99	0.00	0.99	0.00	0.00	0.99	0.99	0.99	0.99	0.00	0.00	0.99	0.99	0.00	0.66	0.00	0.00	0.99	1.00	
25	0.99	0.00	0.66	0.00	0.00	0.99	0.99	0.00	0.99	0.90	0.66	0.00	0.00	0.66	0.00	0.66	0.99	0.00	0.00	0.00	0.00	0.99	0.99	0.00	1.00

Seeding statge

Adult plant stage

Plate 5: Overall view of experimental plot

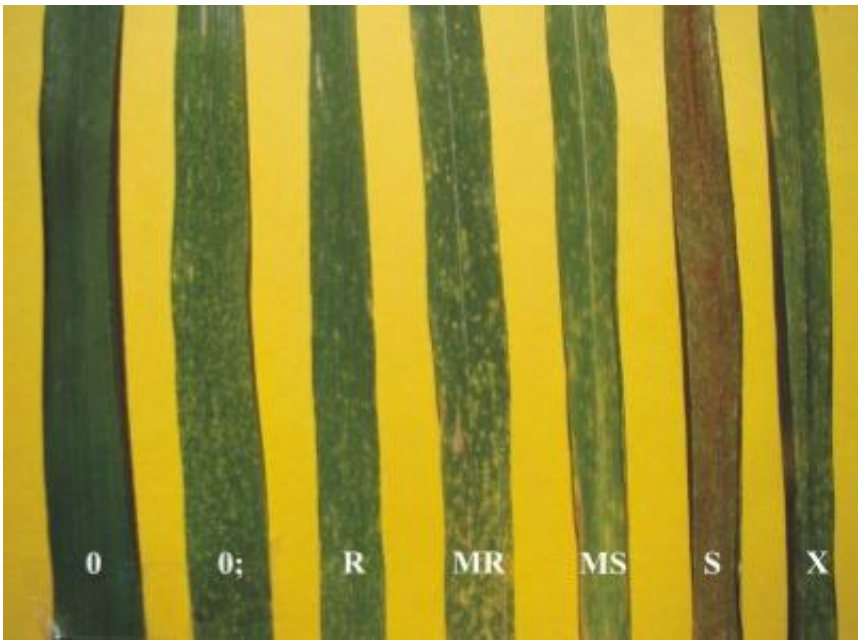


Plate 6a: Infection types of leaf rust disease

Plate 6b: Morphological marker of *Lr34* (leaf tip necrosis) at adult plant stages

Table 14: Average coefficient of infection of leaf rust in selected bread wheat genotypes during *rabi* 2011-12

Sl. No.	Genotype	Days after sowing					
		54	61	68	75	82	Mean
	Susceptible						
1	Agra Local	29.00	68.50	93.50	95.00	100.00	81.20
		(5.48)	(8.34)	(9.72)	(9.80)	(10.05)	(9.07)
2	C-306	1.32	2.48	8.89	15.00	22.36	10.01
		(1.52)	(1.87)	(3.14)	(4.00)	(4.83)	(3.32)
3	DWR-162	0.40	1.20	14.00	15.80	16.54	9.59
		(1.18)	(1.48)	(3.87)	(4.10)	(4.19)	(3.25)
4	HI-977	0.00	0.80	3.50	7.50	13.68	5.10
		(1.00)	(1.34)	(2.12)	(2.92)	(3.83)	(2.47)
5	Lal Bahadur	27.00	63.50	89.00	93.00	98.00	78.10
		(5.29)	(8.03)	(9.49)	(9.70)	(9.95)	(8.89)
	Slow Leaf Ruster						
6	HD-2189	1.29	2.32	4.2	5.5	6.3	3.92
		(1.51)	(1.82)	(2.28)	(2.55)	(2.70)	(2.22)
7	HD-2733	0.50	1.16	3.20	4.15	4.22	2.65
		(1.22)	(1.47)	(2.05)	(2.27)	(2.28)	(1.91)
8	NI-5439	1.00	1.10	1.10	4.56	4.89	2.53
		(1.41)	(1.45)	(1.45)	(2.36)	(2.43)	(1.88)
9	Sonalika	0.85	1.50	2.00	3.10	7.99	3.09
		(1.36)	(1.58)	(1.73)	(2.02)	(3.00)	(2.02)
10	VL-616	2.80	3.46	4.00	5.65	6.83	4.55
		(1.95)	(2.11)	(2.24)	(2.58)	(2.80)	(2.35)
	Resistant						
11	AKAW-4627	0.00	0.00	0.00	0.00	0.00	0.00
		(1.00)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)
12	DBW-16	0.00	0.00	0.00	0.00	0.00	0.00
		(1.00)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)
13	DBW-17	0.00	0.00	0.00	0.00	0.00	0.00
		(1.00)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)
14	GW-322	0.46	0.54	0.64	1.10	1.20	0.79
		(1.21)	(1.24)	(1.28)	(1.45)	(1.48)	(1.34)
15	HD-2888	0.00	0.00	0.00	0.00	0.00	0.00
		(1.00)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)
16	HD-2932	0.00	0.00	0.00	0.00	0.50	0.10
		(1.00)	(1.00)	(1.00)	(1.00)	(1.22)	(1.05)
17	HI-1500	0.00	0.00	0.00	0.00	0.00	0.00
		(1.00)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)
18	HS-240	0.42	0.74	2.05	4.05	4.39	2.33
		(1.19)	(1.32)	(1.75)	(2.25)	(2.32)	(1.82)
19	HS-420	0.00	0.00	0.00	0.00	0.06	0.01
		(1.00)	(1.00)	(1.00)	(1.00)	(1.03)	(1.01)
20	HS-533	0.00	0.00	0.00	0.00	0.00	0.00
		(1.00)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)

Contd…

Sl. No.	Genotype	Days after sowing					
		54	61	68	75	82	Mean
21	HW-2004	0.00	0.00	0.00	0.00	0.00	0.00
		(1.00)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)
22	KRL-210	1.40	1.85	2.05	2.45	2.70	2.09
		(1.55)	(1.69)	(1.75)	(1.86)	(1.92)	(1.76)
23	LOK-1	0.27	0.40	2.10	2.70	3.77	1.85
		(1.13)	(1.18)	(1.76)	(1.92)	(2.18)	(1.69)
24	MACS-6145	0.00	0.00	0.00	0.00	0.00	0.00
		(1.00)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)
25	NIAW-1415	0.00	0.00	0.00	0.00	0.00	0.00
		(1.00)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)
26	NIAW-917	0.00	0.00	0.00	0.00	0.00	0.00
		(1.00)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)
27	NW-4091	0.00	0.00	0.16	0.35	0.58	0.22
		(1.00)	(1.00)	(1.08)	(1.16)	(1.25)	(1.10)
28	PBW-343	0.00	0.06	0.00	0.27	0.90	0.25
		(1.00)	(1.03)	(1.00)	(1.13)	(1.38)	(1.12)
29	PBW-590	0.00	0.00	0.00	0.00	0.00	0.00
		(1.00)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)
30	PBW-596	0.00	0.00	0.00	0.00	0.00	0.00
		(1.00)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)
31	RAJ-4083	0.00	0.00	0.00	0.15	0.20	0.07
		(1.00)	(1.00)	(1.00)	(1.07)	(1.10)	(1.03)
32	RAJ-4270	0.00	0.00	0.00	0.00	0.00	0.00
		(1.00)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)
33	UAS-304	0.00	0.00	0.00	0.00	0.00	0.00
		(1.00)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)
34	UAS-315	0.25	0.50	0.70	1.00	2.60	1.01
		(1.12)	(1.22)	(1.30)	(1.41)	(1.90)	(1.42)
35	UAS-326	0.00	0.00	0.00	0.00	0.00	0.00
		(1.00)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)
36	VL-829	0.00	0.04	0.33	0.00	0.00	0.07
		(1.00)	(1.02)	(1.15)	(1.00)	(1.00)	(1.04)
37	VL-892	0.00	0.00	0.00	0.00	0.00	0.00
		(1.00)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)
38	VL-907	0.00	0.00	0.00	0.00	0.00	0.00
		(1.00)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)
39	VL-924	0.00	0.00	0.00	0.00	0.00	0.00
		(1.00)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)
40	VL-943	0.00	0.00	0.00	0.00	0.00	0.00
		(1.00)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)
	Mean	1.63	3.75	5.70	6.53	7.44	5.03
		(1.62)	(2.18)	(2.60)	(2.74)	(2.91)	(2.46)
	SEm±	0.24	0.38	0.4	0.63	0.51	
	CD at 5%	0.70	1.09	1.13	1.79	1.45	

Data in parenthesis are square root transformed values ($\sqrt{X+1}$).

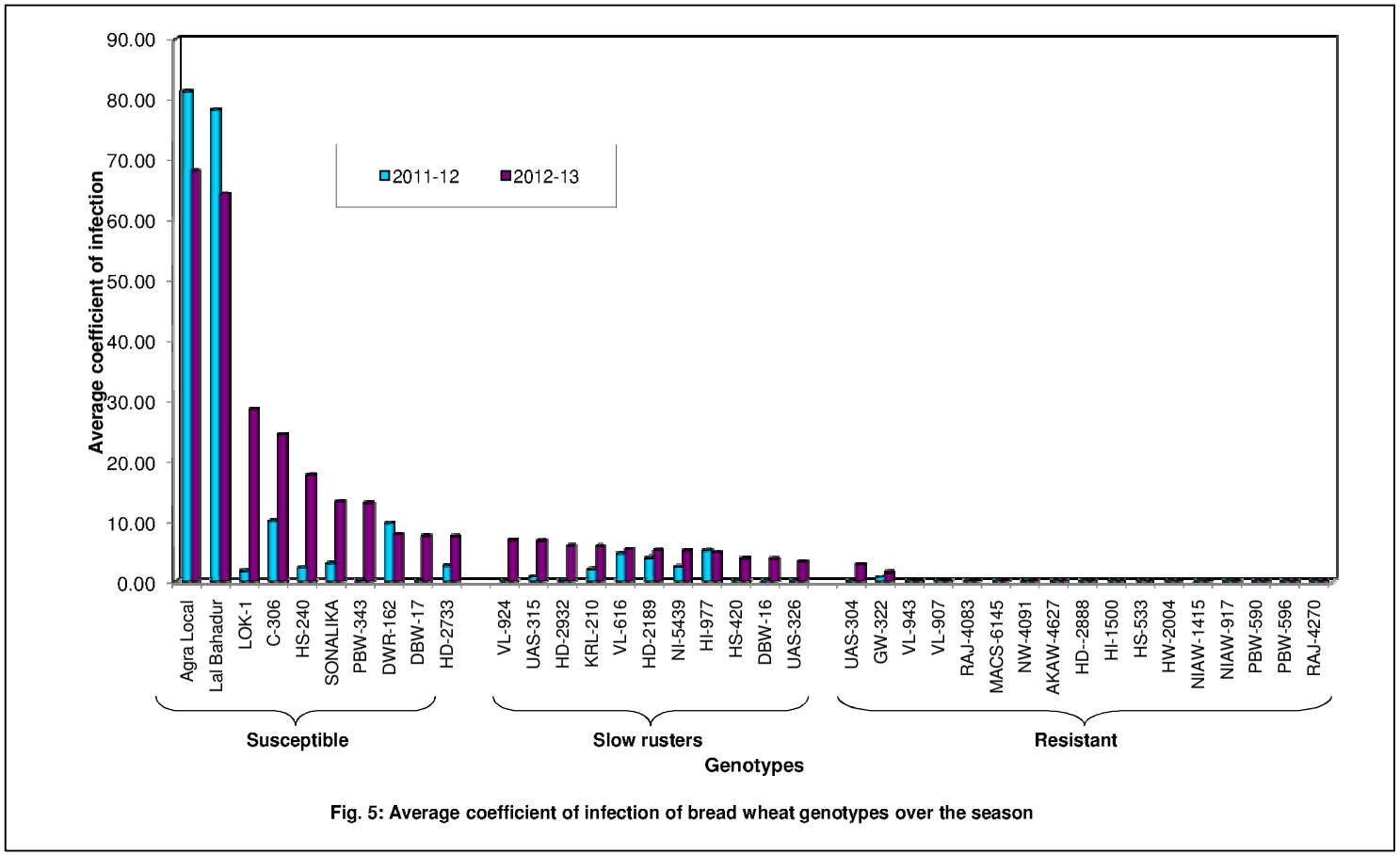


Fig. 5: Average coefficient of infection of bread wheat genotypes over the season

Fig 5: Average coefficient of infection of bread wheat genotypes over the season

Table 15: Average coefficient of infection of leaf rust in selected bread wheat genotypes during *rabi* 2012-13

Sl. No.	Genotype	Days after sowing					
		54	61	68	75	82	Mean
	Susceptible						
1	Agra local	27.00	50.00	77.00	86.00	100.00	68.00
		(5.29)	(7.14)	(8.83)	(9.33)	(10.05)	(8.31)
2	C-306	1.76	11.15	24.60	33.00	51.00	24.30
		(1.66)	(3.49)	(5.06)	(5.83)	(7.21)	(5.03)
3	DBW-17	1.00	3.44	6.56	11.00	16.10	7.62
		(1.41)	(2.11)	(2.75)	(3.46)	(4.14)	(2.94)
4	DWR-162	1.04	2.20	7.30	11.80	17.10	7.89
		(1.43)	(1.79)	(2.88)	(3.58)	(4.25)	(2.98)
5	HD-2733	3.44	5.20	6.00	10.80	12.30	7.55
		(2.11)	(2.49)	(2.65)	(3.44)	(3.65)	(2.92)
6	HD-2932	1.36	1.18	5.10	9.00	14.00	6.13
		(1.54)	(1.48)	(2.47)	(3.16)	(3.87)	(2.67)
7	HS-240	7.10	15.10	15.20	24.00	27.00	17.68
		(2.85)	(4.01)	(4.02)	(5.00)	(5.29)	(4.32)
8	KRL-210	0.56	5.16	4.98	9.20	10.40	6.06
		(1.25)	(2.48)	(2.45)	(3.19)	(3.38)	(2.66)
9	Lal Bahadur	16.00	46.00	74.00	86.00	99.00	64.20
		(4.12)	(6.86)	(8.66)	(9.33)	(10.00)	(8.07)
10	LOK-1	10.40	20.00	36.00	37.00	39.50	28.58
		(3.38)	(4.58)	(6.08)	(6.16)	(6.36)	(5.44)
11	Pavon-76	5.92	5.70	6.46	7.76	8.40	6.85
		(2.63)	(2.59)	(2.73)	(2.96)	(3.07)	(2.80)
12	PBW-343	1.76	6.00	13.30	15.00	29.23	13.06
		(1.66)	(2.65)	(3.78)	(4.00)	(5.50)	(3.75)
13	Sonalika	7.60	10.40	14.40	17.00	17.00	13.28
		(2.93)	(3.38)	(3.92)	(4.24)	(4.24)	(3.78)
14	UAS-315	5.10	5.40	6.20	7.95	9.45	6.82
		(2.47)	(2.53)	(2.68)	(2.99)	(3.23)	(2.80)
15	VL-924	1.84	4.90	8.50	9.50	10.00	6.95
		(1.69)	(2.43)	(3.08)	(3.24)	(3.32)	(2.82)
	Slow Leaf Ruster						
16	DBW-16	2.40	2.76	3.36	4.56	5.70	3.76
		(1.84)	(1.94)	(2.09)	(2.36)	(2.59)	(2.18)
17	HD-2189	1.75	3.60	4.70	6.20	9.48	5.15
		(1.66)	(2.14)	(2.39)	(2.68)	(3.24)	(2.48)
18	HD-3091	0.00	1.08	1.40	4.98	5.90	2.67
		(1.00)	(1.44)	(1.55)	(2.45)	(2.63)	(1.92)
19	HI-977	0.24	0.72	4.80	6.33	11.55	4.73
		(1.11)	(1.31)	(2.41)	(2.71)	(3.54)	(2.39)
20	HS-420	0.56	1.40	2.11	6.70	8.45	3.84
		(1.25)	(1.55)	(1.76)	(2.77)	(3.07)	(2.20)
21	MACS-2496	0.00	1.84	4.30	6.40	8.20	4.15
		(1.00)	(1.69)	(2.30)	(2.72)	(3.030	(2.27)

Contd…

Sl. No.	Genotype	Days after sowing					
		54	61	68	75	82	Mean
22	NI-5439	1.28	2.76	5.20	7.80	8.35	5.08
		(1.51)	(1.94)	(2.49)	(2.97)	(3.06)	(2.47)
23	Parula	0.00	1.52	2.77	4.20	6.10	2.92
		(1.00)	(1.59)	(1.94)	(2.28)	(2.66)	(1.98)
24	RL-6077	0.00	1.08	3.40	5.78	6.45	3.34
		(1.00)	(1.44)	(2.10)	(2.60)	(2.73)	(2.08)
27	UAS-326	0.80	0.80	1.76	4.50	9.00	3.37
		(1.34)	(1.34)	(1.66)	(2.35)	(3.16)	(2.09)
26	VL-616	1.46	4.80	6.00	7.00	7.80	5.41
		(1.57)	(2.41)	(2.65)	(2.83)	(2.97)	(2.53)
	Resistant						
25	AKAW-4627	0.00	0.00	0.00	0.00	0.00	0.00
		(1.00)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)
28	GW-322	0.68	1.08	1.72	2.08	2.54	1.62
		(1.30)	(1.44)	(1.65)	(1.75)	(1.88)	(1.62)
29	GW-432	0.00	0.00	0.00	0.00	0.00	0.00
		(1.00)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)
30	HD-2864	0.00	0.00	0.00	0.00	0.00	0.00
		(1.00)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)
31	HD-2888	0.00	0.00	0.00	0.00	0.00	0.00
		(1.00)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)
32	HD-3093	0.00	0.00	0.00	0.00	0.00	0.00
		(1.00)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)
33	HD-3098	0.00	0.04	0.00	0.00	0.00	0.01
		(1.00)	(1.02)	(1.00)	(1.00)	(1.00)	(1.00)
34	HI-1500	0.00	0.00	0.00	0.00	0.00	0.00
		(1.00)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)
35	HI-1544	0.07	0.00	0.00	0.00	0.00	0.01
		(1.03)	(1.00)	(1.00)	(1.00)	(1.00)	(1.01)
36	HI-1563	0.00	0.00	0.00	0.00	0.00	0.00
		(1.00)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)
37	HI-1584	0.00	0.00	0.00	0.00	0.00	0.00
		(1.00)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)
38	HS-533	0.00	0.00	0.00	0.00	0.00	0.00
		(1.00)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)
39	HW-2004	0.00	0.00	0.00	0.00	0.00	0.00
		(1.00)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)
40	MACS-6145	0.20	0.00	0.00	0.00	0.00	0.04
		(1.10)	(1.00)	(1.00)	(1.00)	(1.00)	(1.02)
41	NI-1689	0.00	0.00	0.00	0.00	0.00	0.00
		(1.00)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)
42	NIAW-1415	0.00	0.00	0.00	0.00	0.00	0.00
		(1.00)	(1.00)	(1.00)	(1.00)	(1.06)	(1.01)
43	NIAW-917	0.00	0.00	0.00	0.00	0.00	0.00
		(1.00)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)
44	NW-4091	0.00	0.00	0.00	0.06	0.00	0.01
		(1.00)	(1.00)	(1.00)	1.03	1.00	1.01

Contd…

45	PBW-590	0.00	0.00	0.00	0.00	0.00	0.00
		(1.00)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)
46	PBW-596	0.00	0.00	0.00	0.00	0.00	0.00
		(1.00)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)
47	RAJ-4083	0.00	0.00	0.00	0.00	0.25	0.05
		(1.00)	(1.00)	(1.00)	(1.00)	(1.12)	(1.02)
48	Raj-4229	0.00	0.00	0.00	0.00	0.00	0.00
		(1.00)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)
49	Raj-4240	0.00	0.00	0.00	0.00	0.00	0.00
		(1.00)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)
50	Raj-4245	0.00	0.00	0.00	0.00	0.00	0.00
		(1.00)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)
51	Raj-4270	0.00	0.00	0.00	0.00	0.00	0.00
		(1.00)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)
52	UAS-304	0.76	1.38	3.58	4.40	4.57	2.94
		(1.33)	(1.54)	(2.14)	(2.32)	(2.36)	(1.98)
53	UP-2825	0.00	0.00	0.00	0.00	0.00	0.00
		(1.00)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)
54	VL-829	0.00	0.00	0.00	0.00	0.00	0.00
		(1.00)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)
55	VL-907	0.00	0.34	0.37	0.37	0.38	0.29
		(1.00)	(1.16)	(1.17)	(1.17)	(1.17)	(1.14)
56	VL-920	0.00	0.00	0.00	0.00	0.00	0.00
		(1.00)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)
57	VL-943	0.00	0.16	0.20	0.48	0.85	0.34
		(1.00)	(1.08)	(1.10)	(1.21)	(1.36)	(1.16)
	Mean	1.79	3.81	6.16	7.84	9.76	5.88
		(1.67)	(2.19)	(2.68)	(2.97)	(3.28)	(2.62)
	SEm±	0.26	0.54	1.03	0.63	1.64	
	CD at 5%	0.74	1.52	2.91	1.79	4.65	

Data in parenthesis are square root transformed values ($\sqrt{X+1}$).

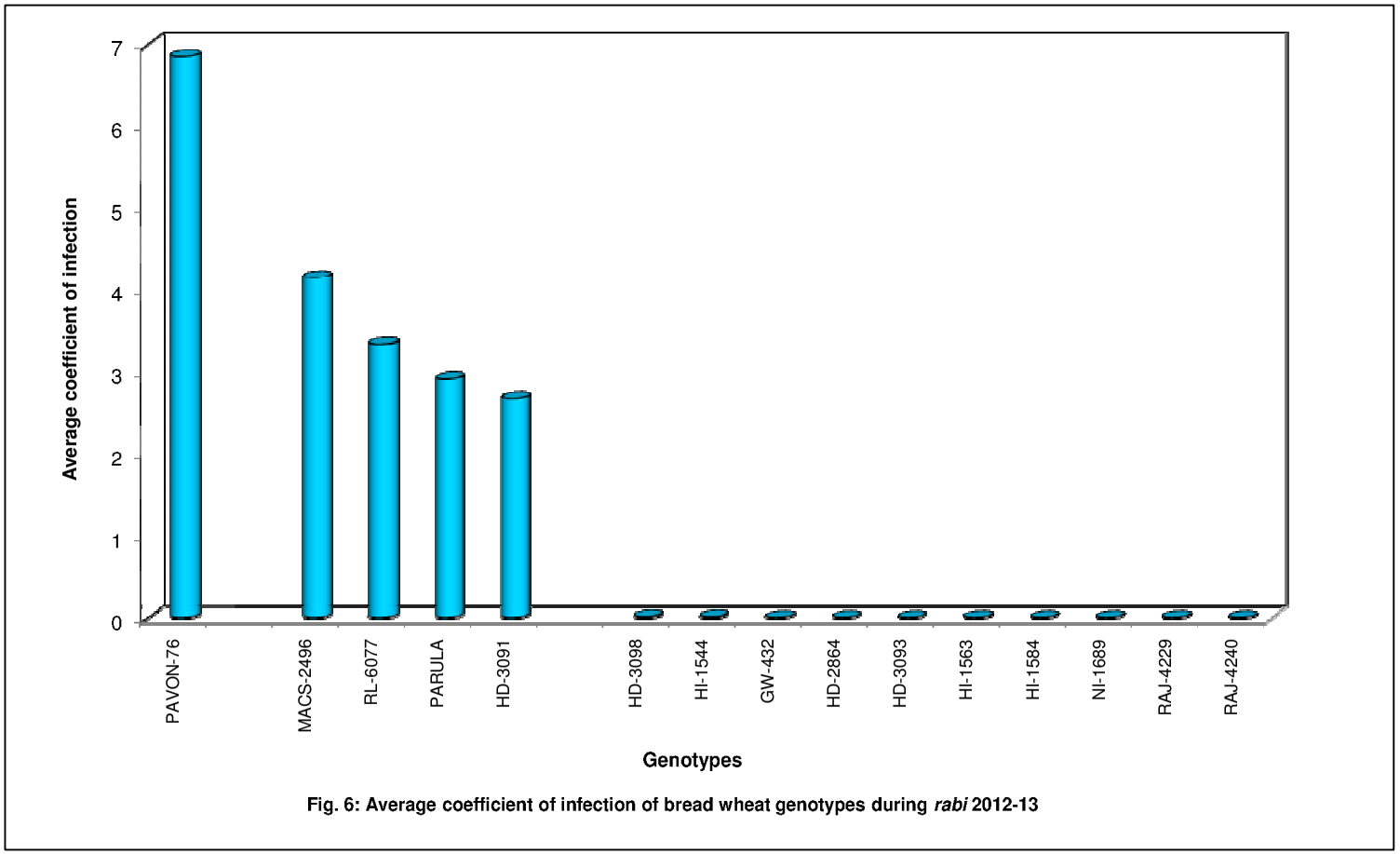


Fig. 6: Average coefficient of infection of bread wheat genotypes during *rabi* 2012-13

Fig 6: Average coefficient of infection of bread wheat genotypes during *rabi* 2012-13

Table 16: Components of slow leaf rusting mechanisms in bread wheat genotypes during *rabi* 2011-12

Sl. No.	Genotype	r1	r2	r3	r4	Mean	AUDPC	LP (Days)	Pustule Density (cm^2)	Yield (q/ha)	TGW (g)
	Susceptible										
1	Agra Local	0.12	0.04	0.00	0.01	0.03	2275.00	8.24	20.00	7.65	26.00
2	C-306	0.06	0.00	0.12	0.04	0.06	436.68	18.44	4.10	27.46	34.36
3	DWR-162	0.17	0.36	0.02	0.01	0.14	296.36	14.45	4.21	21.74	30.44
4	HI-977	0.00	0.27	0.11	0.09	0.12	239.31	12.00	2.67	19.48	30.52
5	Lal Bahadur	0.12	0.05	0.01	0.01	0.03	2135.00	8.56	19.49	6.96	29.71
	Mean	**0.09**	**0.14**	**0.05**	**0.03**	**0.08**	**1,076.47**	**12.34**	**10.09**	**16.66**	**30.21**
	Slow Leaf Ruster										
6	HD-2189	0.08	0.09	0.04	0.02	0.06	132.82	29.32	0.34	25.25	30.10
7	NI-5439	0.01	0.04	0.01	0.01	0.02	120.37	29.00	2.74	25.22	32.20
8	SONALIKA	0.08	0.04	0.05	0.15	0.08	154.70	20.10	1.80	21.22	29.57
9	VL-616	0.03	0.02	0.05	0.03	0.03	168.44	27.50	0.38	9.04	32.06
	Mean	**0.05**	**0.05**	**0.04**	**0.05**	**0.05**	**144.08**	**26.48**	**1.32**	**20.18**	**30.98**
	Resistant										
10	AKAW-4627	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	17.57	37.76
11	DBW-16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	25.57	29.17
12	DBW-17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	33.74	31.13
13	GW-322	0.02	0.02	0.09	0.01	0.04	29.05	32.00	0.04	44.87	35.06
14	HD-2733	0.11	0.15	0.04	0.00	0.08	82.60	26.54	1.01	29.74	33.39
15	HD--2888	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	30.78	37.76
16	HD-2932	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	26.52	33.90
17	HI-1500	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	16.70	37.02
18	HS-240	0.07	0.16	0.10	0.01	0.09	84.18	20.12	6.00	22.43	24.54
19	HS-420	0.00	0.00	0.00	0.00	0.00	1.05	29.86	1.48	28.52	31.61
20	HS-533	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	27.83	35.80
21	HW-2004	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	17.91	37.97

Contd..

Sl. No.	Genotype	r1	r2	r3	r4	Mean	AUDPC	LP (Days)	Pustule Density (cm^2)	Yield (q/ha)	TGW (g)
22	KRL-210	0.04	0.01	0.03	0.01	0.02	71.75	28.12	1.54	19.13	30.69
23	LOK-1	0.06	0.24	0.04	0.05	0.10	70.70	10.45	9.45	17.74	41.67
24	MACS-6145	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	19.65	37.82
25	NIAW-1415	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	12.87	31.32
26	NIAW-917	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	28.00	33.91
27	NW-4091	0.00	0.00	0.03	0.08	0.03	10.06	30.50	0.11	18.09	29.03
28	PBW-343	0.00	0.00	0.00	0.26	0.07	15.66	18.45	1.48	34.26	33.44
29	PBW-590	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	43.65	35.59
30	PBW-596	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	22.26	32.48
31	RAJ-4083	0.00	0.00	0.00	0.05	0.01	3.50	13.65	0.01	27.65	39.03
32	RAJ-4270	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	43.82	42.40
33	UAS-304	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	20.70	30.47
34	UAS-315	0.10	0.00	0.07	0.17	0.08	49.88	30.15	0.54	22.43	32.58
35	UAS-326	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	20.35	32.52
36	VL-829	0.00	0.30	0.00	0.00	0.08	0.00	0.00	0.00	6.09	33.71
37	VL-892	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	27.65	30.32
38	VL-907	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	13.74	28.86
39	VL-924	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	25.74	36.02
40	VL-943	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	17.04	29.97
	Mean	**0.01**	**0.03**	**0.01**	**0.02**	**0.02**	**13.50**	**7.74**	**0.70**	**24.61**	**33.77**
	Grand Mean	**0.02**	**0.04**	**0.02**	**0.03**	**0.03**	**147.50**	**10.19**	**1.93**	**23.18**	**33.05**
	SEm±	0.002	0.004	0.002	0.003		11.95	0.83	0.18	1.64	2.06
	CD at 5%	0.007	0.010	0.006	0.009		34.19	2.37	0.53	4.70	5.90

r: Rate of infection; AUDPC: Area under disease progress curve; LP: Latent period; TGW: Thousand grain weight.

Table 17: Components of slow leaf rusting mechanisms in bread wheat genotypes during *rabi* 2012-13

Sl. No.	Genotype	r1	r2	r3	r4	Mean	AUDPC	LP (Days)	Pustule density (cm^2)	Pustule size (mm^2)	Yield (q/ha)	TGW (g)
	Susceptible											
1	Agra local	0.09	0.06	0.02	0.02	0.05	2222.50	7.32	19.08	0.47	9.33	25.21
2	C-306	0.27	0.11	0.04	0.06	0.12	923.30	19.88	4.22	0.32	35.71	29.22
3	DBW-17	0.17	0.10	0.08	0.05	0.10	299.25	26.07	1.82	0.29	37.71	28.97
4	DWR-162	0.11	0.18	0.07	0.05	0.10	317.45	16.08	3.46	0.22	30.21	31.27
5	HD-2733	0.06	0.02	0.08	0.02	0.05	275.45	27.62	1.48	0.20	34.50	31.90
6	HD-2932	0.01	0.21	0.08	0.06	0.09	268.80	22.75	5.98	0.16	40.13	43.32
7	HI-977	0.03	0.27	0.04	0.09	0.11	206.33	10.14	3.16	0.22	42.04	34.61
8	HS-240	0.11	0.00	0.07	0.02	0.05	596.75	15.47	6.43	0.10	22.71	22.23
9	Lal Bahadur	0.15	0.07	0.02	0.02	0.06	2012.50	8.09	19.78	0.40	12.71	33.55
10	LOK-1	0.09	0.08	0.00	0.01	0.05	873.25	9.67	10.14	0.52	24.08	40.02
11	PAVON-76	0.00	0.02	0.03	0.01	0.01	250.60	29.00	0.44	0.38	19.58	41.39
12	PBW-343	0.18	0.11	0.03	0.10	0.11	542.24	16.24	2.53	0.32	33.71	34.10
13	Sonalika	0.04	0.05	0.02	0.00	0.03	430.50	22.77	0.50	0.46	32.25	32.93
14	UAS-315	0.01	0.02	0.03	0.02	0.02	254.54	28.51	0.64	0.22	31.13	30.05
15	VL-924	0.14	0.08	0.02	0.01	0.06	207.20	16.18	2.69	0.29	38.00	41.58
	Mean	**0.10**	**0.09**	**0.04**	**0.04**	**0.07**	**645.38**	**18.39**	**5.49**	**0.30**	**29.59**	**33.36**
	Slow Leaf Ruster											
16	DBW-16	0.02	0.03	0.05	0.03	0.03	141.75	30.47	0.07	0.15	28.17	28.46
17	HD-2189	0.10	0.03	0.04	0.05	0.06	196.52	28.53	0.57	0.21	24.96	29.96
18	HD-3091	0.00	0.05	0.18	0.03	0.07	103.25	27.50	1.39	0.24	32.54	29.03
19	HS-420	0.13	0.06	0.17	0.03	0.10	157.59	27.38	2.29	0.11	46.04	33.75
20	KRL-210	0.32	0.00	0.09	0.02	0.11	191.80	26.41	1.89	0.18	36.83	37.06

Contd…

Sl. No.	Genotype	r1	r2	r3	r4	Mean	AUDPC	LP (Days)	Pustule density (cm^2)	Pustule size (mm^2)	Yield (q/ha)	TGW (g)
21	MACS-2496	0.00	0.15	0.05	0.04	0.06	143.50	31.05	1.03	0.17	38.75	31.48
22	NI-5439	0.11	0.09	0.06	0.01	0.07	168.53	28.26	2.34	0.28	34.63	28.98
23	Parula	0.00	0.09	0.06	0.05	0.05	106.75	25.18	0.39	0.44	11.38	29.03
24	RL-6077	0.00	0.17	0.08	0.02	0.06	112.88	27.12	0.16	0.32	-	-
25	UAS-326	0.00	0.11	0.14	0.10	0.09	171.50	30.50	1.03	0.15	29.58	33.15
26	VL-616	0.17	0.03	0.02	0.02	0.06	162.05	26.75	0.27	0.27	15.92	28.06
	Mean	**0.08**	**0.07**	**0.09**	**0.04**	**0.07**	**150.56**	**28.10**	**1.04**	**0.23**	**29.88**	**30.90**
	Resistant											
27	AKAW-4627	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	40.83	42.94
28	GW-322	0.06	0.07	0.02	0.03	0.05	56.35	30.94	0.05	0.05	48.96	35.28
29	GW-432	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	41.00	46.25
30	HD-2864	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	48.88	40.33
31	HD—2888	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	38.38	34.88
32	HD-3093	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	49.42	44.67
33	HD-3098	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	33.79	35.49
34	HI-1500	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	19.46	35.28
35	HI-1544	0.00	0.00	0.00	0.00	0.00	1.14	0.00	0.00	0.00	43.04	43.55
36	HI-1563	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	41.21	43.60
37	HI-1584	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	45.96	41.56
38	HS-533	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	27.71	33.15
39	HW-2004	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	24.00	18.38
40	MACS-6145	0.00	0.00	0.00	0.00	0.00	3.50	0.00	0.00	0.00	16.58	33.93
41	NI-1689	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	39.50	48.33
42	NIAW-1415	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	33.42	34.08
43	NIAW-917	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	48.88	34.90

Contd..

44	NW-4091	0.00	0.00	0.00	0.00	0.00	0.00	28.50	0.21	0.15	33.75	35.24
45	PBW-590	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	34.38	36.42
46	PBW-596	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	39.08	38.25
47	Raj-4083	0.00	0.00	0.00	0.00	0.00	4.38	11.00	0.01	0.12	43.29	43.20
48	Raj-4229	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	43.67	42.00
49	Raj-4240	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	41.71	43.44
50	Raj-4245	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	46.42	37.60
51	Raj-4270	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	44.38	45.46
52	UAS-304	0.08	0.15	0.03	0.01	0.07	93.22	18.00	0.33	0.14	35.75	31.69
53	UP-2825	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	49.46	34.86
54	VL-829	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	15.67	44.93
55	VL-907	0.00	0.02	0.00	0.00	0.01	6.65	26.00	0.16	0.17	33.75	29.64
56	VL-920	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	36.58	28.43
57	VL-943	0.00	0.05	0.12	0.09	0.07	14.88	29.63	0.03	0.12	24.88	34.24
	Mean	**0.00**	**0.01**	**0.01**	**0.00**	**0.01**	**5.81**	**4.65**	**0.03**	**0.02**	**37.54**	**37.81**
	Grand Mean	**0.04**	**0.04**	**0.03**	**0.02**	**0.04**	**202.09**	**12.79**	**1.66**	**0.138**	**33.44**	**34.18**
	S.Em±	**0.007**	**0.006**	**0.003**	**0.003**		**19.25**	**0.73**	**0.25**	**0.016**	**2.37**	**3.83**
	CD at 5%	**0.019**	**0.016**	**0.008**	**0.008**		**57.53**	**2.08**	**0.72**	**0.044**	**6.71**	**10.85**

r: Rate of infection; AUDPC: Area under disease progress curve; LP: Latent period; TGW: Thousand grain weight. -: Not flowered.

4.3.3 Area under disease progress curve (AUDPC)

The AUDPC values were calculated for each genotype by using the formula suggested by Wilcoxson *et al.* (1975) and data are presented in the table 16 and 17 separately for the year 2011-12 and 2012-13.

4.3.3.1 *Rabi* 2011-12

The AUDPC values significantly differs with each other among the genotypes, Significantly very higher AUDPC values were observed in the susceptible checks, Agra Local (2275.00) and Lal Bahadur (2135.00) denoting very fast leaf rusting nature. The genotype HD-2189 showed 132.82 AUDPC value. The genotypes VL-616, Sonalika and NI-5439 were on par with the AUDPC value of HD-2189. The lowest value of AUDPC was recorded in the genotype HS-420 (1.05) followed by RAJ-4083 (3.5), NW-4091 (10.06), PBW-343 (15.66) and GW-322 (29.05) (Fig. 7). The AUDPC data states that, the genotypes which showed the lesser values of ACI also showed lesser values of AUDPC and *vice-versa*.

Genotypes HS-420, RAJ-4083, NW-4091, PBW-343 and GW-322 that showed very least AUDPC values which were of resistant types. Whereas, the genotype Agra Local, Lal Bahadur and C-306 with maximum AUDPC values are fast rusters, however, the genotype HD-2189, VL-616, Sonalika and NI-5439 showed the characteristics of slow rusters.

4.3.3.2 *Rabi* 2012-13

The AUDPC values revealed the similar trend as that of previous year but higher values were obtained during 2012-13. As like in the previous year same susceptible checks used and were displayed very high AUDPC values as shown in the Table 17 and Fig. 7.

Except two checks no one genotype exceeded 1000 AUDPC value and the next highest AUDPC value was recorded by C-306 (923.30). The genotypes, VL-616, UAS-326, HD-2189, NI-5439, DBW-16, HS-420, KRL-210, MACS-2496, HD-3091, RL-6077 and Parula were displayed the AUDPC values ranging between 100 to 200 indicated they are falling under slow leaf rusting category. The genotypes VL-924, HD-2932, UAS-315, HI-977 and Pavon-76 also showed slow rusting mechanisms with slightly higher AUDPC values as compared to above category but these were statistically on par with each other (Fig. 8).

The lowest AUDPC values were recorded by the genotype, HI-1544 (1.14) followed by NIAW-1415, MACS-6145, Raj-4083 and VL-907 showed less than 10 AUDPC value as that of resistant types.

4.3.4 Latent period

Latent period was found out in field conditions as described in the material and methods. It has been revealed that appearance of first uridinium correlated highly with LP 50 (days to appearance of 50% uridinia) and hence, latent period was studied in the present investigation.

4.3.4.1 *Rabi* 2011-12

It was noticed that the time required for appearance of pustule after inoculation with the rust pathotypes was significantly very low in the susceptible checks, Agra Local (8.24 days) and Lal Bahadur (8.56) followed by LOK-1 (10.45), HI-977 (12) and DWR-162 (14.45) given in Table 16 and Fig. 9.

Genotypes GW-322 (32.00 days) displayed very longer latent period followed by NW-4091(30.50). Genotype HD-2189 (29.32) also showed longer latent period, VL-616, NI-5439, HS-240 and KRL-210 were on par with the HD-2189. Total 21 genotypes did not showed the infection to the prevailing pathotypes of *P. triticina* indicated that, immune to resistant type of reaction.

4.3.4.2 *Rabi* 2012-13

During 2012-13 the disease pressure was higher to that of previous year as it was already mentioned in the section ACI and AUDPC and also evidenced in Table 17. Similarly, it was reflected in this component that, latent period was reduced as compared to last year among tested genotypes. The lowest latent period was recorded by Agra Local (7.32 days) and Lal Bahadur (8.09) followed by LOK-1 (10.14). Genotypes MACS-2496 (31.05 days), GW-322 (30.94), UAS-326 (30.50), DBW-16 (30.47) were showed very high (>30 days) latent period and were on par with each other. Next highest latent period was recorded by VL-943 (29.63) followed by Pavon-76 (29.00), HD-2189

(28.53), UAS-315 (28.51), NW-4091 (28.50), NI-5439 (28.26), HD-2733 (27.62), HS-420 (27.38), HD-3091 (27.50), RL-6077 (27.12), VL-616 (26.75), KRL-210 (26.41), Parula (25.18) and HD-2932 (22.75) (Fig. 9 &10).

It is generally noticed that the genotypes with the higher average coefficient of infection showed lesser latent period. As explained earlier the genotypes with lesser value of AUDPC are the characteristics of vertical resistance. Hence, the genotype VL-943, Pavon-76, HD-2189, UAS-315, NI-5439, HD-2733, HS-420, NW-4091, HD-3091, RL-6077, VL-616, KRL-210, Parula and HD-2932 were recorded as slow rusters among all genotypes tested.

4.3.5 Pustule density

Pustule density (per cm^2) was recorded at 7 day interval starting from the first appearance of the disease as the number of pustules per square centimeter of bottom, middle and top portion of flag leaf area was counted later it was computed as par the procedure explained under 'Material and Methods'. Among the susceptible genotypes counting number of pustules were difficult at later part of disease development as all the pustules coalesce together and hence, highest number of pustules recorded was used for statistical analysis.

4.3.5.1 *Rabi* 2011-12

From the Table 16 it was observed that, pustule density (number per cm^2) were significantly lower in VL-616 (0.38), GW-322 (0.04), RAJ-4083 (0.01), UAS-315 (0.54), HD-2189 (0.34) and NW-4091 (0.11) and on par with each other followed by HD-2733 (1.01), PBW-343 (1.48), HS-420 (1.48), KRL-210 (1.54) and Sonalika (1.80). The two susceptible checks, Agra Local (20.00) and Lal Bahadur (19.49) were observed very high number of pustules. Remaining 21 genotypes among 40 genotypes were not showed any pustules.

4.3.5.2 *Rabi* 2012-13

During 2012-13 similar trend was observed as of previous year but slightly increase in the pustule number per cm^2 was noticed. The same susceptible checks used previous year were also showed very high number of pustules per cm^2 (19.08 and 19.78 respectively) followed by LOK-1 (10.14), HD-2932(5.98) and C-306(4.22). Lowest pustules per cm^2 were displayed by RAJ-4083 (0.01), VL-943 (0.03), GW-322 (0.05) and DBW-16 (0.07). The next lower pustules cm^{-2} was observed in VL-616 (0.27), UAS-315 (0.64), HD-2189 (0.57), NW-4091 (0.21), VL-907 (0.16), Pavon-76 (0.44), RL-6077 (0.16) and Parula (0.39). In this year also 25 genotypes were not showed pustules. Other slow rusting genotypes, UAS-326 (1.03), KRL-210 (1.89), HS-420 (2.29), HD-3091 (1.39), HD-2733 (1.48) and NI-5439 (2.34) also recorded significantly very less pustule density as compared to susceptible checks (Table 17). The three types of field reactions against leaf rust *viz*., resistant (NIAW 917), slow leaf ruster (HD 2189) and susceptible (Agra local) were given in Plate 7.

4.3.6 Pustule size (40X)

The pustule size was measured only during 2012-13 under Differential Interference Contrast microscope with a magnification of 4X as described under material and methods. The length and breadth of pustules were measured then pustule size was calculated and it is presented in the Table 17. The utmost pustule size was recorded in the genotype LOK-1 (0.52 mm^2) followed by Agra Local (0.47), Sonalika (0.45) and Parula (0.44) whereas, smallest pustule size was observed in GW-322 (0.05). Medium range (0.10 to 0.38 mm^2) of pustule size was observed among slow leaf rusting genotypes, VL-616, UAS-315, UAS-326, HD-2189, HD-2733, HD-3091, HD-2132, NI-5439, HI-977, HS-240, HS-420, KRL-210, Pavon-76 and RL-6077 this has once again proven their slow rusting nature of resistance as they were displayed significantly less pustule size compare to susceptible genotypes (Plate 8).

4.3.7 Yield and thousand grain weight

The yield was recorded per plot and converted into yield per hectare as described in the material and methods. The data on yield and 1000 grain weight presented in the Table 16 and 17 for the year 2011-12 and 2012-13 respectively.

Observations from the table indicated that, during *rabi* 2012-13 more yield was registered as compared to previous year *rabi* 2011-12 by all the genotypes. It was noticed that there was a significant difference among the genotypes in yield and thousand grain weight among the genotypes under high disease severity.

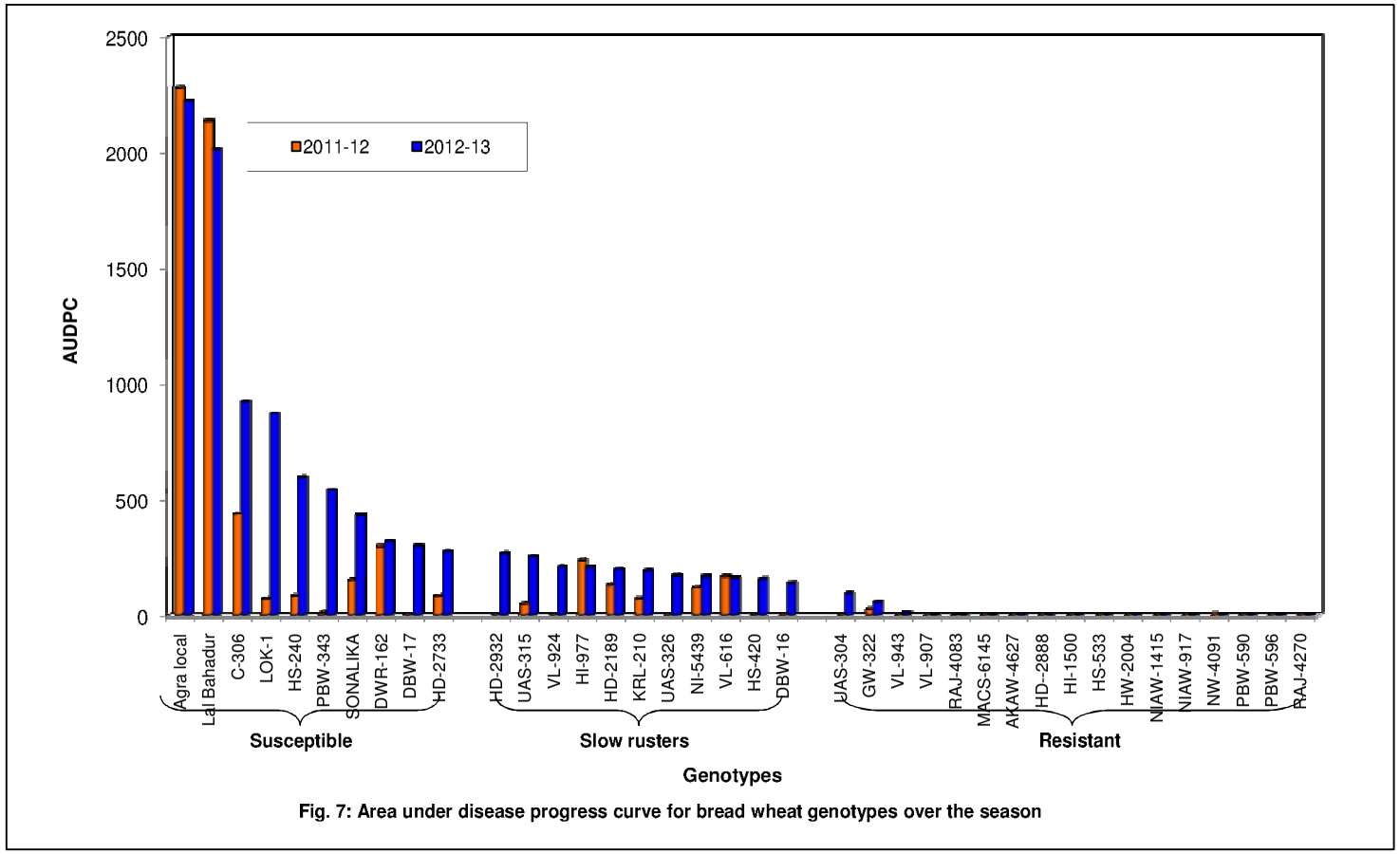


Fig. 7: Area under disease progress curve for bread wheat genotypes over the season

Fig 7: Area under disease progress curve for bread wheat genotypes over the season

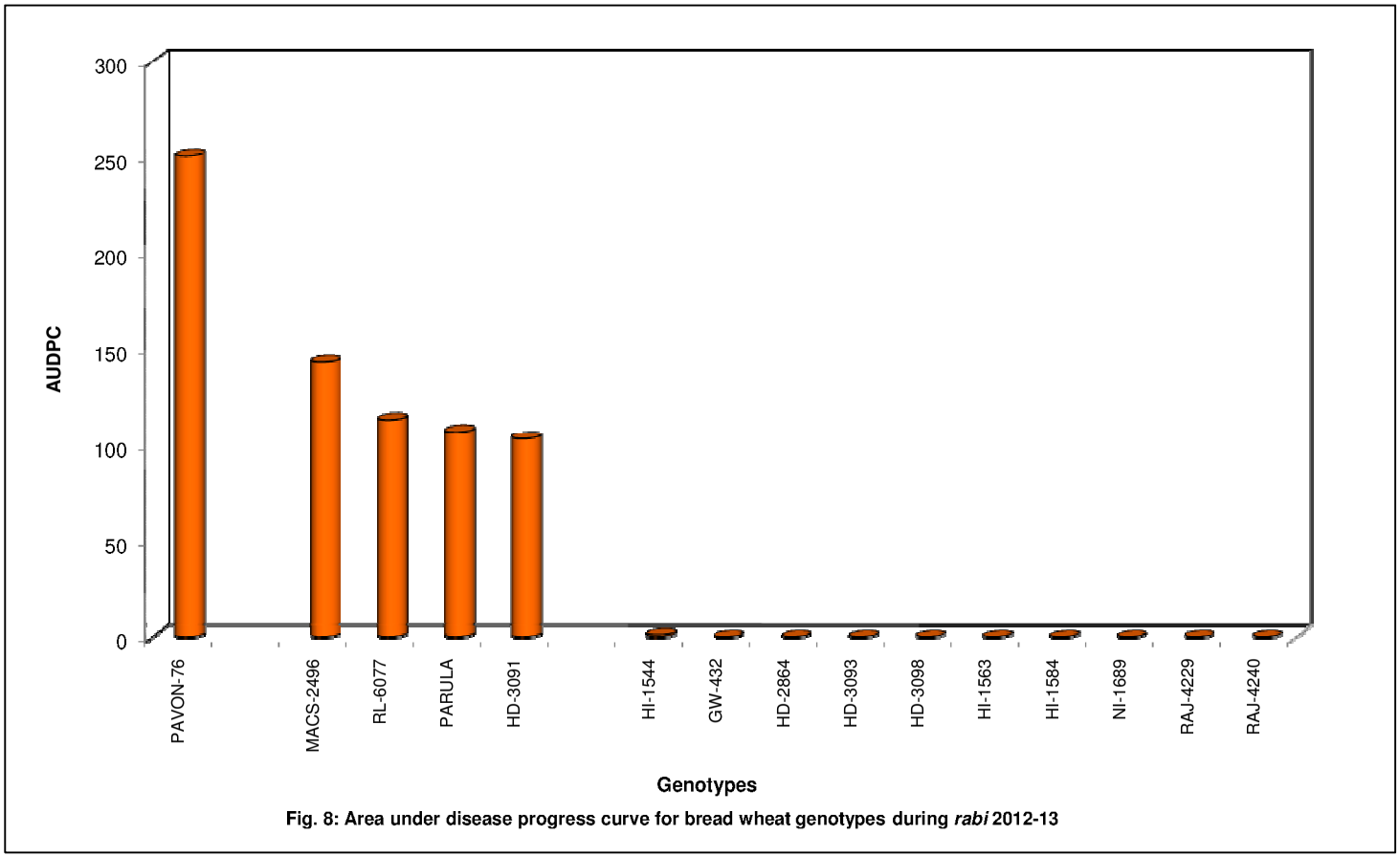


Fig. 8: Area under disease progress curve for bread wheat genotypes during *rabi* 2012-13

Fig 8: Area under disease progress curve for bread wheat genotypes during *rabi* 2012-13

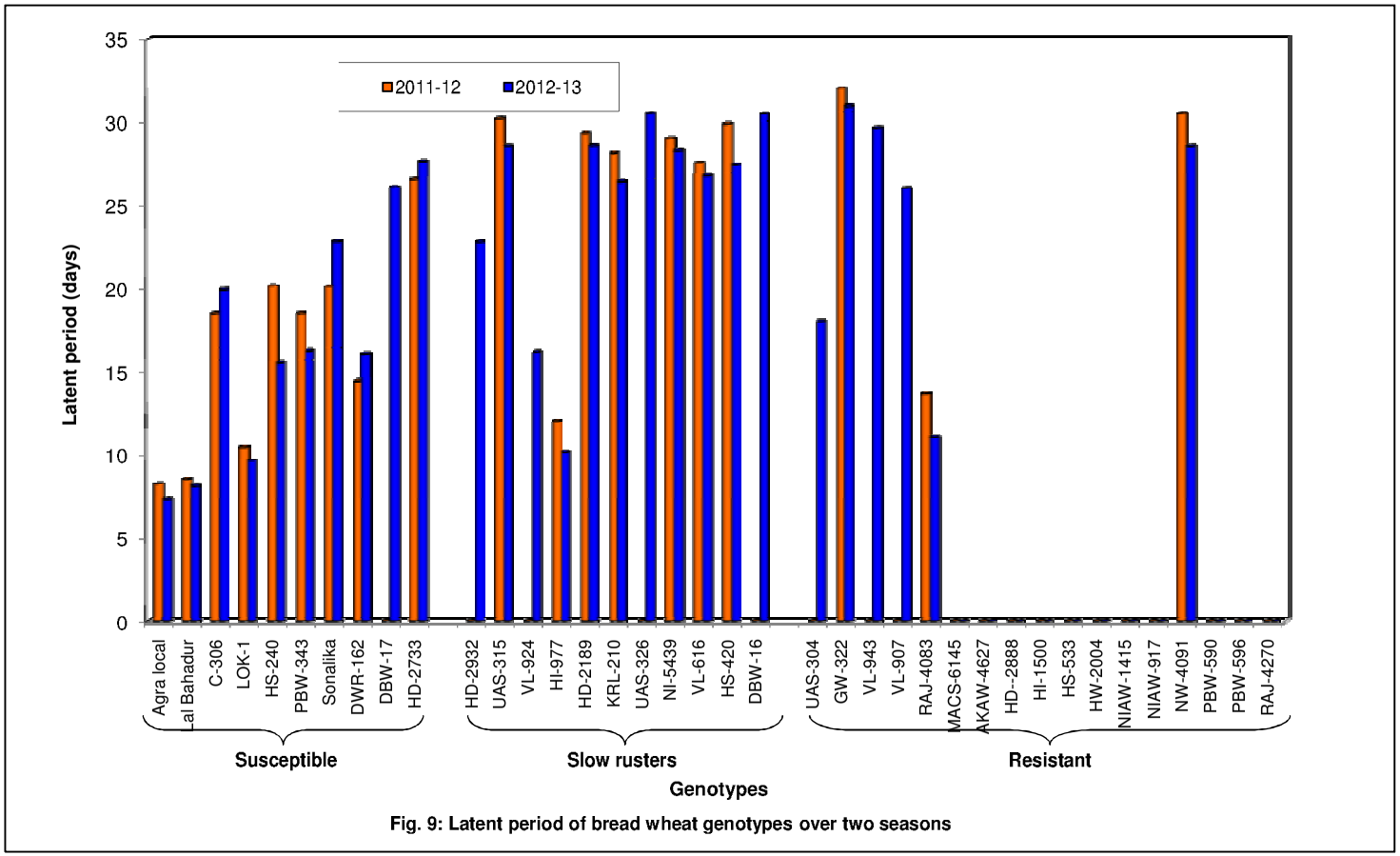


Fig. 9: Latent period of bread wheat genotypes over two seasons

Fig 9: Latent period of bread wheat genotypes over two seasons

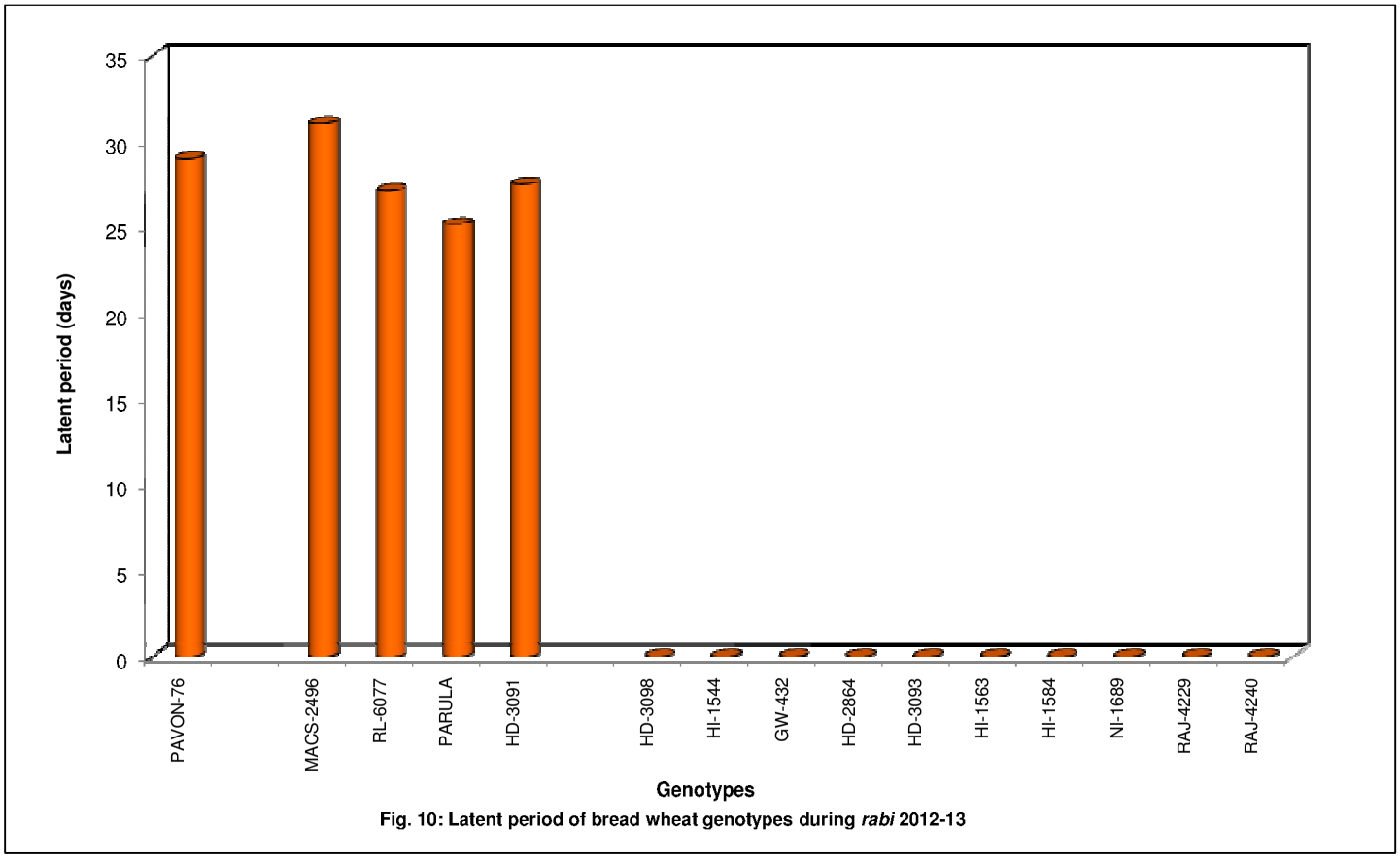


Fig. 10: Latent period of bread wheat genotypes during *rabi* 2012-13

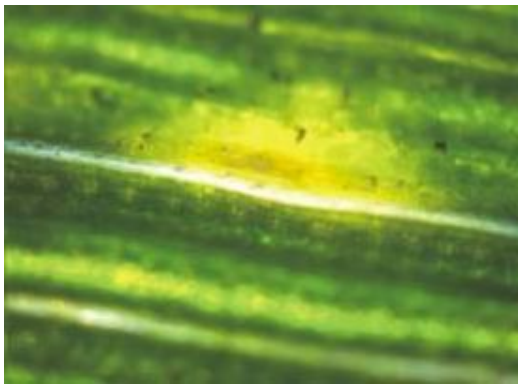
Fig 10: Latent period of bread wheat genotypes over 2012-13

Resistant (NIAW 917)

Slow leaf ruster (HD 2189)

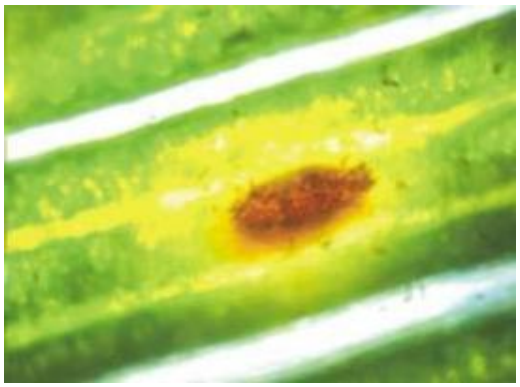
Susceptible (Agra Local)

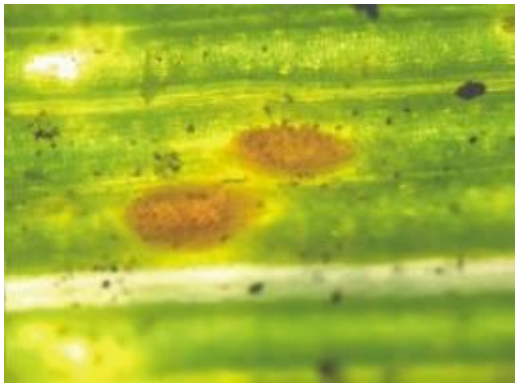
Plate 7: Bread wheat genotypes showing leaf rust reactions



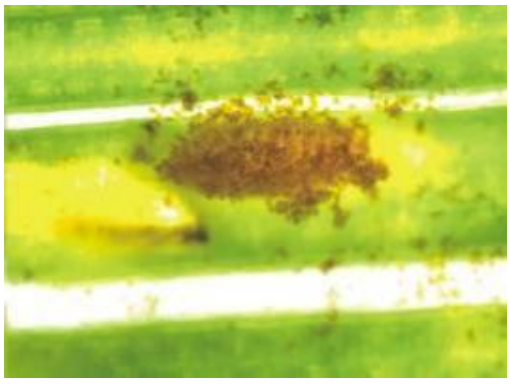
Chlorosis **Necrosis**

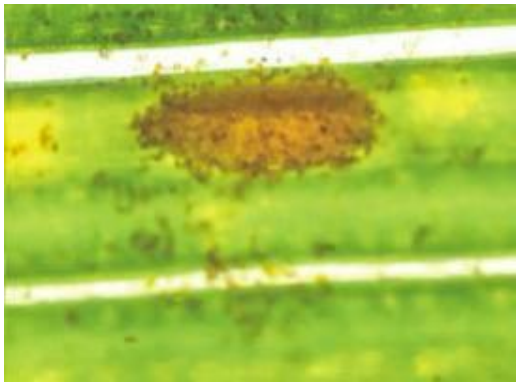
Resistant genotype





Pustules on slow leaf rust genotypes





Pustules on susceptible genotype

Plate 8: Pustule (40X) on three group of bread wheat genotype

4.3.7.1 *Rabi* 2011-12

Maximum yield was recorded in the genotypes GW-322 (44.87 q/ha) followed by RAJ-4270 (43.82 q/ha) and PBW-590 (43.65 q/ha). The lowest yield was recorded in VL-829 (6.09 q/ha) followed by susceptible checks, Lal Bahadur (6.96 q/ha) and Agra Local (7.65 q/ha) under high leaf rust disease pressure (Table 16).

The slow rusting genotypes, VL-616, UAS-315, HD-2189, NI-5439, HI-977, HS-420 and KRL-210 were given medium yield (9.04 to 28.52 q/ha) as compared to the resistant genotypes whereas, compared to the susceptible genotypes which were superior and tolerated high disease pressure under artificial epiphytotic condition then given normal yield.

The highest thousand grain weight was obtained in Raj-4270 (42.40 g) followed by LOK-1 (41.67 g) and Raj-4083 (39.03 g). Least thousand grain weight was recorded in HS-240 (24.54 g) followed by Agra Local (26.00 g) and statistically they are on par with each other. The slow rusting genotype HD-2189 showed 30.10 g of thousand grain weight remaining slow rusters also showed more or less similar results.

4.3.7.2 *Rabi* 2012-13

Genotypes, UP-2825 (49.46 q/ha) and HD-3093 (49.42 q/ha) were obtained with significantly higher yield followed by GW-322 (48.96 q/ha), NIAW-917 (48.88 q/ha), HD-2864 (48.88 q/ha), HS-420 (46.04 q/ha), HI-1584(45.96 q/ha), RAJ-4229 (43.67 q/ha) and RAJ-4245 (46.42 q/ha) which were statistically on par with each other. The lower yield was shown by Agra Local (9.33 q/ha) followed by Parula (11.38 q/ha) and Lal Bahadur (12.71 q/ha). The Mexican genotype RL-6077 did not given any yield.

In this year slow rusters were showed increased yield performance under higher disease severity than previous year as it was already explained. The slow rusting genotypes HD-2189, VL-616, UAS-315, NI-5439, HI-977, HS-420, KRL-210, Pavon-76, HD-2932, HD-3091, VL-924 and DBW-16 showed 24.96, 15.92, 31.13, 34.63, 42.04, 46.06, 36.83, 19.58, 40.13, 32.54, 30.0 and 28.17 q/ha yield respectively (Table 17).

The highest thousand grain weight was obtained in NI-1689 (48.33 g) followed by GW-432 (46.25 g) and Raj-4270 (45.46 g). Least thousand grain weight was recorded in HW-2004 (18.38 g) followed by HS-240 (22.23 g) and statistically they are on par with each other. The slow rusting genotype HD-2189 showed 29.96 g of thousand grain weight remaining slow rusters also showed more or less similar results (Table 17).

4.3.8 Seedling reaction test and gene postulation

This study was carried out in the glasshouse under controlled condition at Regional Station, DWR, Shimla, Himachal Pradesh to know the seedling reaction and genes of selected bread wheat genotypes. The disease scoring scale for leaf rust at seedling stage and view of glasshouse facility at Shimla were given in Plate 9a and 9b respectively.

The infection types (ITs) displayed by the isogenic *Lr* lines and selected bread wheat genotypes when inoculated with 13 pathotypes of *P. triticina* are given in Table 18. The genes *Lr9*, *Lr19* and *Lr28* displayed consistently low ITs with all the tested pathotypes except *Lr28* showed '3+' reaction to 77-10 pathotype. The other 7 *Lr* isogenic lines showed variable effectiveness among the pathotypes. Details of gene postulated among these bread wheat genotypes were presented in Table 18. The bread genotypes *viz*., VL-616, HD-2932, DBW-16, KRL-210, NIAW-917, Lal Bahadur, Local Red and Agra Local did not appear to carry any known and unknown genes. The genes of C-306 and PBW-343 (genotype PBW-343 with gene pyramid by *Lr24+Lr28* seeds were used) could not be detected. A total of 14 genotypes showed equally for single known gene (*Lr2a+, Lr10+, Lr13+, Lr23+, Lr24+, Lr26+* and *Lr28+*) and double known genes with unknown 14 gene combinations. Only 3 genotypes found triple known genes (*Lr23+26+1+)* with unknown gene combination. The reactions of individual gene were also given in the Table 18. The details of seedling reaction test and gene postulation were documented in Plate 10.

4.3.8.1 Seedling resistance and susceptibility of bread wheat genotypes

Seedling resistance and susceptibility of 41 bread wheat genotypes against 13 leaf rust pathotypes were categorized as R, S and X are presented in Table 19. The genotypes *viz*., VL-616 and KRL-210 were found susceptible, in contrast HI-1500, HD-2888, AKAW-4627, PBW-343

(*Lr24*+*Lr28*+) and HW-2004 showed resistant reaction and remaining genotypes displayed variable reaction to the all pathotypes inoculated.

4.3.8.2 *Lr* isogenic lines

At seedling growth stage, 17 *Lr* isogenic lines were inoculated with 13 pathotypes of *P. triticina*. On the basis of infection types, three categaries of reactions *viz*., R, S and X are given in the Table 20. The isogenic lines such as *Lr9* and *Lr19* showed cent per cent effectiveness followed by *Lr28* (92.3) against the pathotypes tested, whereas, four isogenic lines (*Lr10*, Democrat (*Lr3*), *Lr12* and *Lr22a*) found not effective against all the pathotypes tested (Fig. 11).

It was found that all the pathotypes from group 77 had higher percentage of virulence as compare to other groups. Among all the pathotypes 77-5 had highest (82.4%) followed by 77-10 and 104-3 (76.5%) whereas, 12-9 had lowest (47.1%) per cent virulence (Fig. 12). During the course of present investigation under survey and race identification, the race 77-5 had higher frequency and percent distribution over the three seasons. Hence, consistency in the occurrence as well as highest per cent virulence of 77-5 revealed the development of durable resistant varieties against this particular pathotype in the breeding programme will be helpful in getting higher production. The mass multiplication and maintainence of individual pathotypes at Shimla was documented in Plate 11.

4.3.9 Genetics and molecular characterization of bread wheat varieties for durable/slow leaf rusting resistance

Molecular characterization was performed to confirm the presence of slow leaf rust resistant genes (*Lr34*, *Lr46* and *Lr67*) in 57 genotypes (Table 4) by using STS and SSR molecular markers.

A total of 12 gene specific primers were used in this study, details were given in 'Material and Methods' to characterize the presence of above mentioned three slow leaf rust resistant genes among 57 bread wheat genotypes of diverse sources including HD-2189 (*Lr*34), Pavon 76 (*Lr*46), RL6077 (*Lr*67) and Parula (*Lr*68) as reference for their respective slow rusting resistance genes identified worldwide so for. Leaf tip necrosis is the morphological marker for these genes as shown in Plate 6b.

4.3.9.1 Molecular characterization of bread wheat genotypes for slow leaf rust resistant gene *Lr34*

Among four molecular markers of *Lr34*, *csLV34 is* STS marker and remaining three *viz., XGwm130, XGwm295, cdo475* are SSR markers used for this study. Very good results were obtained with bi-allelic STS marker *csLV34*. Whereas, remaining three SSR markers were not consistent in the amplification of target gene.

Molecular characterization of 59 bread wheat genotypes for the presence of *Lr34/Yr18/Pm38* slow rusting pleiotrophic resistant gene using closely linked STS markers, 12 genotypes namely, Raj-4083, DBW-16, VL-892, HS-533, PBW-596, PBW-590, VL-829, VL-907, HI-1563, HI-1584, Raj-4229 and RAJ-4245 showed the presence of 150 bp fragment specific to *Lr34* and rest of the lines amplified 229 bp (non *Lr34* carrying allele) (Plate 12). Exactly 21.1 per cent genotypes carry *Lr34* gene, thus expected to have slow leaf rust resistance. The AUDPC data for all the genotypes were recorded. AUDPC data of genotypes positive for presence of *Lr34* at molecular level are presented in Table 21. Among 12 genotypes positive for *Lr34,* DBW-16 is highly correlated with AUDPC value of 141.75, which indicates the slow rusting association with *Lr34* gene. Remaining 11 genotypes showed 0.0 to 6.65 AUDPC values.

4.3.9.2 Molecular characterization of bread wheat genotypes for slow leaf rust resistant gene *Lr46*

Two QTL molecular markers of *Lr46* namely, *Xwmc44* and *Xwmc719* were used for this study. The results were obtained with *Xwmc44-1B* QTL marker of *Lr46*. Whereas, *Xwmc719* QTL markers was not consistent in the amplification of *Lr46* gene.

Molecular characterization of 57 bread wheat genotypes for the presence of *Lr46/Yr29/Pm39* slow rusting pleiotrophic resistance gene using closely linked QTL markers showed 7 genotypes *viz*., GW-322, HD-2932, Sonalika, HS-240, HS-420, HD-2864 and HD-3093 presence of 242 bp fragment specific to *Lr46* and rest of the lines amplified 200 bp (non *Lr46* carrying allele) (Plate 13). As a positive control of presence of gene *Lr46* carrying by Mexican genotype, Pavon-76 was used. Exactly 12.28 per cent genotypes carry *Lr46* gene, thus expected to have slow leaf rusting resistance. AUDPC data for all the genotypes were recorded. AUDPC data of genotypes positive for presence of *Lr46* at molecular level are presented in Table 21. Among 7 genotypes positive for *Lr46,* HS-420 is highly correlated with AUDPC value of 157.59, followed by HD-2932 showed 268.80 AUDPC values which indicates the slow rusting association with *Lr46* gene. Among remaining five genotypes, GW-

322 showed within 100 AUDPC value, Sonalika and HS-240 showed 430.50 and 596.75 AUDPC values respectively. Genotypes HD-2864 and HD-3093 displayed 0.00 AUDPC value.

4.3.9.3 Molecular characterization of bread wheat genotypes for slow leaf rust resistant gene *Lr67*

Among six SSR molecular markers of *Lr67 (Xcfd23-4D, Xbarc98, Xcfd71-4D, Xbarc288, Xwmc48* and *Xwmc89-4D).* The result was obtained by *Xbarc288* marker closely linked to *Lr67*. Whereas, remaining five SSR markers were not closely linked and also not consistent in the amplification of target gene.

Molecular characterization of 59 bread wheat genotypes for the presence of *Lr*67/*Yr*46 slow rust resistant gene reveled that, none of the selected Indian bread wheat genotypes showed to carry *Lr67* slow rusting gene. A positive control of presence of gene *Lr67* carrying by Mexican genotype, RL-6077 was used which was shown monomarphic amplification at 240 bp fragment specific to *Lr67* (Plate 14). AUDPC data of genotype positive for presence of *Lr67* (RL-6077) at molecular level are presented in Table 21. This genotype had 112.88 AUDPC value.

4.3.9.4 Molecular characterization of bread wheat genotypes for both slow leaf rust resistant genes *Lr34* and *Lr46*

Interestingly, 5 genotypes namely, VL-616, HD-2189, UAS-315, NW-4091 and Raj-4270 carrying both *Lr34/Yr18/Pm38* and *Lr46/Yr29/Pm39* were identified at molecular level for the first time from the selected 57 Indian bread wheat genotypes (Plate 12 & 13) .

AUDPC data of genotypes positive for presence of both *Lr34* and *Lr46* at molecular level are presented in Table 21. Among five genotypes positive for *Lr34* and *Lr46,* Vl-616, HD-2189 and UAS-315 had AUDPC value of 162.05, 196.52 and 254.54 respectively which indicates strong slow rusting mechanisms both at field and molecular level. Remaining two genotypes, NW-4091 and Raj-4270 were showed 0 AUDPC followed by HD-2932 showed 268.80 AUDPC value which indicates the slow rusting association with *Lr46* gene. Among remaining five genotypes, GW-322 showed within 100 AUDPC value, Sonalika and HS-240 showed 430.50 and 596.75 AUDPC values respectively. Genotypes HD-2864 and HD-3093 displayed 0.00 AUDPC value.

The genotypes, VL-924, UAS-326, NI-5439, HI-977, HD-3091, KRL-210 and MACS-2496 (Total 7) were not showed any of the above slow rusting resistance genes but they showed slow rusting mechanisms in the field by recording lower AUDPC value (between 100 to 200) unveiled the presence of unknown slow rusting resistance genes need to be identified.

4.3.10 Studies on expression of oxidative enzymes (isozymes) in selected bread wheat under the pathogenesis of leaf rust races

Isozymes studies were carried out by adopting the Vertical Polyacrylamide Gel Electrophoresis (PAGE) as explained in 'Material and Methods'. Two isozymes such as peroxidase and polyphenol oxidase expression was studied. The well known slow ruster HD-2189 in comparison with fast ruster (Agra Local) and resistant genotype (NIAW-917) were included in this study. Two growth stages such as seedling and adult plant stages were considered by inoculating two predominant *P. triticina* pathotypes, 77-5 (121R63-1) and 104-2 (21R55) in separate sets. For both the stages, uninoculated sets as healthy ones were maintained and also one sample of each genotype from the field at 15 days after inoculation by mixture of races was compared (Plate 15). In the present investigation results pertaining to the existence of isozymes variation among the genotypes was assessed.

4.3.10.1 Peroxidase

It was noticed that, at adult plant stage appearance and expression of peroxidase were prominent than that of respective seedling growth stage (Plate 16).

In the resistant genotype, NIAW-917 showed a expression of peroxidase irrespective of the growth stages for both inoculated *P. triticina* races (77-5 and 104-2) as well as mixture of races under field condition. Whereas, uninoculated conditions did not showed any expression of peroxidase at both the growth stages (Lane 1 & 7). In the slow rusting genotype, HD-2189 showed expression of peroxidase only at adult plant stage for race 104-2 (Lane 10 & 11) as well as in the field condition for mixture of races (Lane 12), but not for race 77-5 at both the stages (Lane 2, 3, 8 & 9). And also it was noticed that, presence of very slight expression of peroxidase at 10^{th} day after inoculation during seedling stage for race 104-2 (Lane 5). Both uninoculated conditions did not showed expression of peroxidase (Plate 16).

Plate 9a: Glasshouse facility for leaf rust study at Shimla

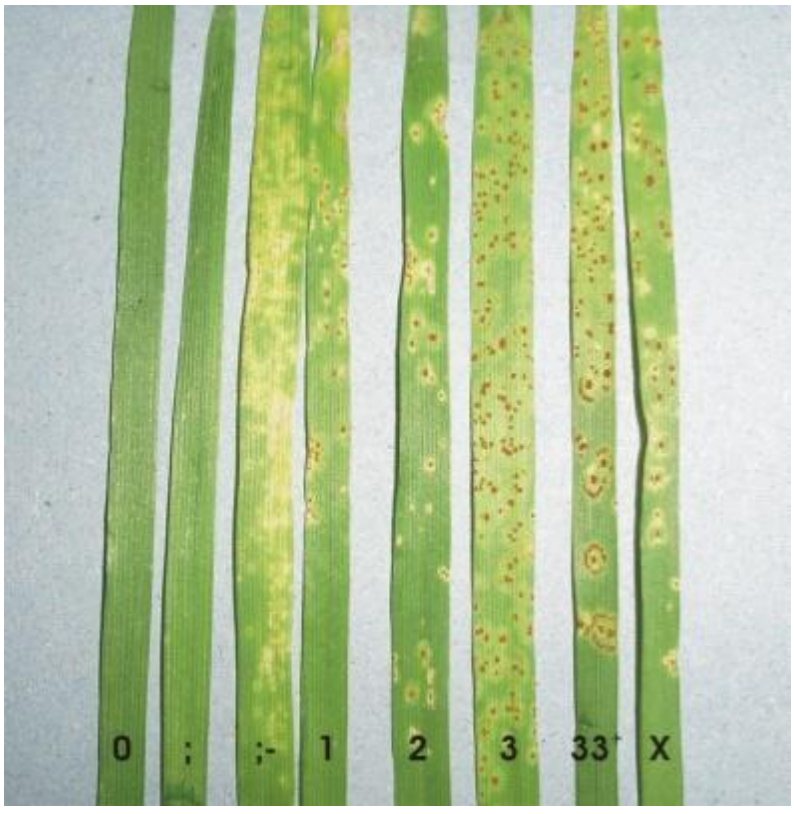


Plate 9b: Infection types for leaf rust of wheat at seedling stage

Table 18: Seedling reaction test and gene postulation of bread wheat genotypes against thirteen pathotypes of *Puccinia triticina*

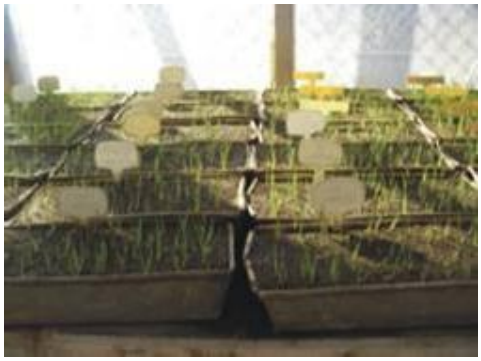
Sl. No.	Genotypes	Reaction against pathotypes													Gene Postulated
		12-4	12-7	12-9	77-1	77-5	77-6	77-10	104-2	104-3	104B	162	162-1	162-2	
1	Agra Local	3+	33+	3+	3+	33+	3+2	3+	33+	3+	33+	3+	33+	2P;2P3	-
2	AKAW-4627	;1	;1	;1	;1	;Sr2	;	0;	;	;1	;	0;	;1	;1	*Lr24+*
3	C-306	0;	0	1P0;1P3+	;	3+ few ;1	;1	;1few3+	2+	3+	0;	0;	121P3+	;1	*?*
4	DBW-16	3+	3+	2+	3	3+	3+	33+	3+	3+	12	3	3+	3+	-
5	DBW-17	0;	01P;1	;	;	2+3	;1	33+few;1	3+	3+	12	0;	;1P3+	;1	*Lr26+23+*
6	DWR-162	0;	0;	3+	;1	3+	3+	;1	3+	33+	0;	0;	12	12	*Lr26+23+*
7	GW-322	;-	;1	;1	3-	X	3+	33+	;1	12	33+	0;	;1	;1P1	*Lr13+10+*
8	HD-2733	0;	0;	0;	12	33+	33+	3	3+	33+	0;	0;	3+	0+	*Lr26+10+*
9	HD--2888	12	;1	12	4P;1P3	;Sr2	;-Sr2	;	1	;1	;1	;1	;1	;	*Lr24+*
10	HD-2932	3+	3+	3+	3+	3	3+	33+	3+	1P3+	33+	1P2+	3+	3+	-
11	HI-1500	12	;1	;1	;-	0;	;-Sr2	0;	2-	;1	1	1	;-	1	*Lr24+*
12	HI-977	0;	0;1P3+	0;	2	3+	3+	12	3+	3+	0;	0;	;1	;1	*Lr23+1+*
13	HS-240	0;	0;	0;	3+	3	33+	3+1P;1	3+	33+	0;	0;	0;	0;	*Lr26+1+*
14	HS-420	0;	0;	0;	3+	0;	3+2	3+	22+	2+3	;1	0;	0;	0;	*Lr26+1+*
15	HS-533	0;	;Sr2	0;	2	3+	0;	12	3+	1P;11P3	0;	0;	0;	0;	*Lr26+23+1+*
16	HW-2004	2-	1P0;1P2+	2+	0;	;1	;-Sr2	0;	;1	;	0;	0;	0;	;Sr2	*Lr24+*
17	KRL-210	33	33+	3+	3+	3+	3+	33+	3+	33+	33	3+	3+	3+	-
18	Lal Bahadur	33+	;1	33+	3+	33+	33+	33+	33+	3+	1P0;1P3	3+1P1	3+	2P;1P3+	-
19	Local Red	3+	33+	3+	3+	3+	3+	3+	33+	33+	2+	3+	33+	3+	-
20	LOK-1	1P;	0;	33+	3+	1P2+1P0;	3	33+	;	3+	33+	;1	3	12	*Lr13+*
21	MACS-6145	0;	0	0;	;-Sr2	0;	0;	33+	0;	;-Sr2	0;	0;	0;	0;	*Lr28+*
22	NI-5439	0;1P2	0	3+	;	2+and ;	;1	;1	3+	3+	0;	0;	3	12	*Lr10+*
23	NIAW-1415	0;	0	0;	;	3	;	0;	;Sr2	;1	0;1;	0;	0;	0;	*Lr26+*
24	NIAW-917	0;	;-Sr2	0;	0;	0;	0;1P3	0;	0;	0;	-	0;	;	0;	-
25	NW-4091	0;	0;	0;	3+	3+	33+	12	23	0;	0;	0;	;1	;11P3	*Lr26+10+*
26	Pavan-76	0;	0;	0;	3+	3+	3+3	3+	2+	12	0;	1P0;	0;	0;	*Lr10+14+*

Contd...

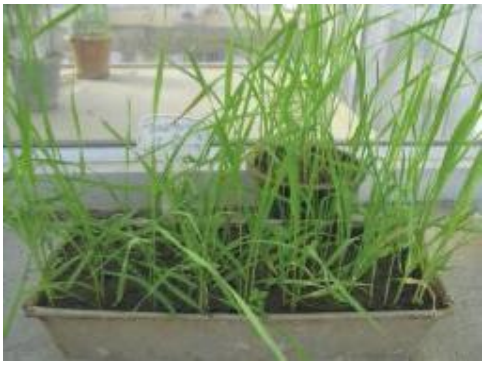
Sl. No.	Genotypes	Reaction against pathotypes													Gene Postulated
		12-4	12-7	12-9	77-1	77-5	77-6	77-10	104-2	104-3	104B	162	162-1	162-2	
27	PBW-343	0;	0;	3+	;1	2+few;1	2	;1	3+	3	0;	0;	;1	X	*Lr26+*
28	PBW-343 (Lr24+Lr28)	0;	0;	0;	0;	0;	;-	0;	0;	;-Sr2	0;	0;	0;	0;	*? Seed*
29	PBW-590	0;	0;	0;	0;	3+	;1	0;	0;	0;	0;	0;	0;Sr2	0;	*Lr26+23+1+*
30	PBW-596	0;	0	2-	;	3	0;	1P;21P3	3	0;	0;	0;	0;	0;	*Lr26+23+*
31	RAJ-4083	;1	1;	X	;1	2+	3+	33+	X	12+	3	0;	;1	;1	*Lr23+*
32	SONALIKA	1P0; 1P33+	1P3+1P;1	;1	3P;12P3+	;1	3P;1P3+	;1P3+	;-	;1	;1	;	;1	3+	*Lr13+*
33	UAS-304	0;	0	1P0;	;1	2+	3	33+	1+	;1	0;	0;	0;	0;	*Lr23+26+1+*
34	UAS-315	0;	0;	0;	3+	3+	3	33+	;1	1P;1	0;1P3	0;1P3	0;	0;	*Lr2a+*
35	UAS-326	2P; 1P3+	3+	3+	3+	33+	3+	33+	3	X	33+	3	3	X	*Lr13+*
36	VL-616	2P2, 1P3	3+	3+	3+	3+	3+	33+	33+	33+	3+	3+	3+	3+	-
37	VL-829	0;	0;	0;	3+	3+	33+	1P20;	3+	2+3	0;	0;	2	;1	*Lr26+23+*
38	VL-892	0;	0;	;1	3+	2	3+	;1	3	3	0;	0;	-	;-	*Lr26+1+*
39	VL-907	0;	0;	0;1P3+	;-Sr2	3	;1	12	23	0;	;	0;	2+	2-	*Lr26+23+*
40	VL-924	;1	2+1P;1	3+	;1	12	;-	3+	;1P3	;1	3+	;	;1	;1	*Lr23+*
41	VL-943	0;	0;	0;	3+	2+	3	1P12	33+	33+	0;	0;	0;	0;	*Lr26+1+*
	Lr1	0;	0;	0;	3+	3	3+	3	3+	3+	0;	01P0;	1P0;	0;	
	Lr2a	2	0;	1P0;	3+	3+	3+	1P0;	3	3	3	3+	3+	3+	
	Lr9	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	
	Lr10	3+	3+	1,3+	3+	3+	3+	3	3+	3	3	3+	1P3	3+	
	Lr13	33+	3	2+	3+	33+	3+	3+	3+	3	3	3+	3+	3+	
	Lr19	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	
	Lr23	3	3	3+	;1	3+	2	33+	3	3+	3	;	33+	2	
	Lr26	3+	3+	3+	3+	1P33+	3+	3	3+	3+	0;	1P0;	3+	3+	
	Lr28	0;	0;	0;	0;	0;	0;	3+	0;	0;	0;	1P0;	0;	0;	
	Lr34	2+	3+	2	3+	3	3+	3	3+	3+	3+	3	3+	3	

P=Plant,?=not detected, - =not known. *Sr2*=adult plant resistance gene for stem rust detected.

Sowing of Seeds for seedling reaction test



Germination of Seeds



Set of differential on 12th day after inoculation

Humidifying chamber to create humidity for infection

Seedling reaction test and gene postulation for race 77-5 and 104-2 at Shimla

Plate 10: Seedling reaction test and gene postulation of selected Indian bread wheat genotypes at Shimla

Table 19: Seedling reaction of bread wheat genotypes against thirteen pathotypes of *Puccinia triticina*

Sl. No.	Genotypes	Reaction against pathotypes												
		12-4	12-7	12-9	77-1	77-5	77-6	77-10	104-2	104-3	104B	162	162-1	162-2
1	Agra Local	S	S	S	S	S	S	S	S	S	S	S	S	X
2	AKAW-4627	R	R	R	R	R	R	R	R	R	R	R	R	R
3	C-306	R	R	S	R	X	R	S	R	S	R	R	X	R
4	DBW-16	S	S	R	S	S	S	S	S	S	R	S	S	S
5	DBW-17	R	R	R	R	S	R	X	S	S	R	R	X	R
6	DWR-162	R	R	S	R	S	S	R	S	S	R	R	R	R
7	GW-322	R	R	R	S	S	S	S	R	R	S	R	R	R
8	HD-2733	R	R	R	R	S	S	S	S	S	R	R	S	R
9	HD--2888	R	R	R	X	R	R	R	R	R	R	R	R	R
10	HD-2932	S	S	S	S	S	S	S	S	S	S	R	S	S
11	HI-1500	R	R	R	R	R	R	R	R	R	R	R	R	R
12	HI-977	R	X	R	R	S	S	R	S	S	R	R	R	R
13	HS-240	R	R	R	S	S	S	X	S	S	R	R	R	R
14	HS-420	R	R	R	S	R	S	S	R	S	R	R	R	R
15	HS-533	R	R	R	R	S	R	R	S	S	R	R	R	R
16	HW-2004	R	R	R	R	R	R	R	R	R	R	R	R	R
17	KRL-210	S	S	S	S	S	S	S	S	S	S	S	S	S
18	Lal Bahadur	S	R	S	S	S	S	S	S	S	X	X	S	X
19	Local Red	S	S	S	S	S	S	S	S	S	R	S	S	S
20	LOK-1	R	S	S	S	R	S	S	R	S	S	R	S	R
21	MACS-6145	R	R	R	R	R	R	S	R	R	R	R	R	R

Contd…

Sl. No.	Genotypes	Reaction against pathotypes												
		12-4	12-7	12-9	77-1	77-5	77-6	77-10	104-2	104-3	104B	162	162-1	162-2
22	NI-5439	R	R	S	R	R	R	R	S	S	R	R	S	R
23	NIAW-1415	R	R	R	R	S	R	R	R	R	R	R	R	R
24	NIAW-917	R	R	R	R	R	S	R	R	R	-	R	R	R
25	NW-4091	R	R	R	S	S	S	R	S	R	R	R	R	X
26	Pavan-76	R	R	R	S	S	S	S	R	R	R	R	R	R
27	PBW-343	R	R	S	R	R	R	R	S	S	R	R	R	X
28	PBW-343 (Lr24+Lr28)	R	R	R	R	R	R	R	R	R	R	R	R	R
29	PBW-590	R	R	R	R	S	R	R	R	R	R	R	R	R
30	PBW-596	R	R	R	R	S	R	X	S	R	R	R	R	R
31	RAJ-4083	R	R	S	R	R	S	S	S	R	S	R	R	R
32	SONALIKA	X	X	X	S	R	X	X	R	R	R	R	R	S
33	UAS-304	R	R	R	R	R	S	S	R	R	R	R	R	R
34	UAS-315	R	R	R	S	S	S	S	R	R	X	X	R	R
35	UAS-326	X	S	S	S	S	S	S	S	S	S	S	S	X
36	VL-616	S	S	S	S	S	S	S	S	S	S	S	S	S
37	VL-829	R	R	R	S	S	S	R	S	S	R	R	R	R
38	VL-892	R	R	R	S	R	S	R	S	S	R	R	-	R
39	VL-907	R	R	S	R	S	R	R	S	R	R	R	R	R
40	VL-924	R	R	S	R	R	R	S	S	R	S	R	R	R
41	VL-943	R	R	R	S	R	S	R	S	S	R	R	R	R

Where, S = Susceptible; R=Resistant; X=Mesothetic; - = Not germinated.

Table 20: Seedling reaction of *Lr* isogenic lines against thirteen pathotypes of *Puccinia triticina*

Sl. No.	Isogenic line	Seedling reaction against pathotypes													Per cent effectiveness
		12-4	12-7	12-9	77-1	77-5	77-6	77-10	104-2	104-3	104B	162	162-1	162-2	
1	Thew *Lr20*	R	R	R	S	S	R	R	R	S	R	R	S	R	69.2
2	Malakoff *Lr1*	R	R	R	S	S	S	S	S	S	R	R	R	R	53.8
3	Bemo *Lr26*	S	S	S	S	S	S	S	S	S	R	R	S	S	15.4
4	*Lr15*	R	R	R	S	S	S	S	R	R	R	R	R	R	69.2
5	*Lr10*	S	S	S	S	S	S	S	S	S	S	S	S	S	0.0
6	*Lr23*	S	S	S	R	S	R	S	S	S	S	S	S	R	23.1
7	Webster (*Lr2a*)	R	R	R	S	S	S	R	S	S	S	S	S	S	30.8
8	Democrat (*Lr3*)	S	S	S	S	S	S	S	S	S	S	S	S	S	0.0
9	Tc *Lr23*	S	S	S	R	S	R	S	S	S	S	R	S	R	30.8
10	Tc *Lr10*	S	S	S	S	S	S	S	S	S	S	S	S	S	0.0
11	Tc *Lr13*	S	S	R	S	S	S	S	S	S	S	S	S	S	7.7
12	*Lr12*	S	S	S	S	S	S	S	S	S	S	S	S	S	0.0
13	*Lr22a*	-	S	S	S	S	S	S	S	S	S	S	S	S	0.0
14	Tc *Lr34*	R	S	R	S	S	S	S	S	S	S	S	S	S	15.4
15	*Lr9*	R	R	R	R	R	R	R	R	R	R	R	R	R	100.0
16	*Lr19*	R	R	R	R	R	R	R	R	R	R	R	R	R	100.0
17	*Lr28*	R	R	R	R	R	R	S	R	R	R	R	R	R	92.3
	Per cent virulence	50.0	58.8	47.1	70.6	82.4	64.7	76.5	70.6	76.5	58.8	52.9	70.6	52.9	

Where, S = Susceptible; R=Resistant; X=Mesothetic; - = Not germinated.

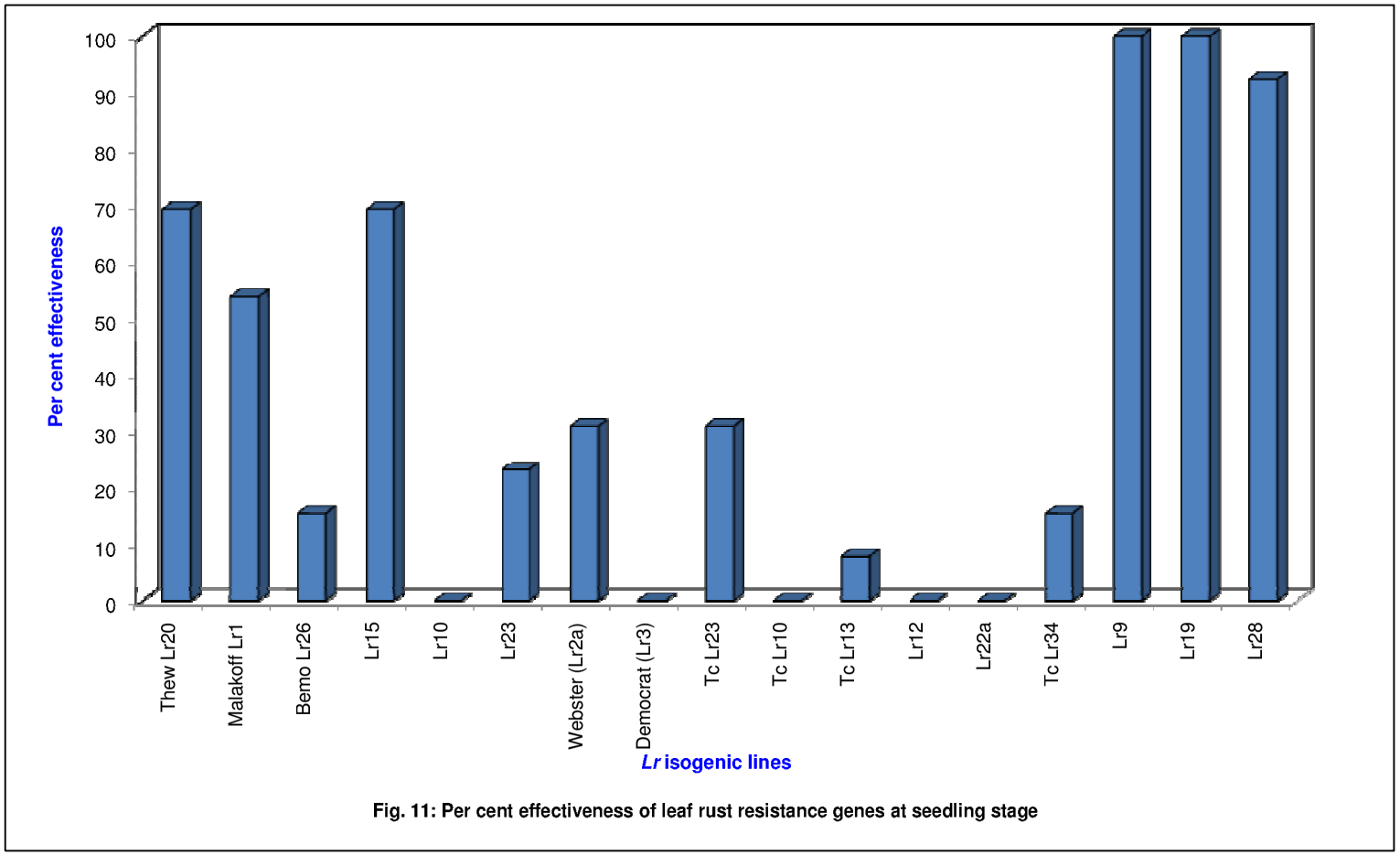


Fig. 11: Per cent effectiveness of leaf rust resistance genes at seedling stage

Fig 11: Per cent effectiveness of leaf rust resistance genes at seedling stage

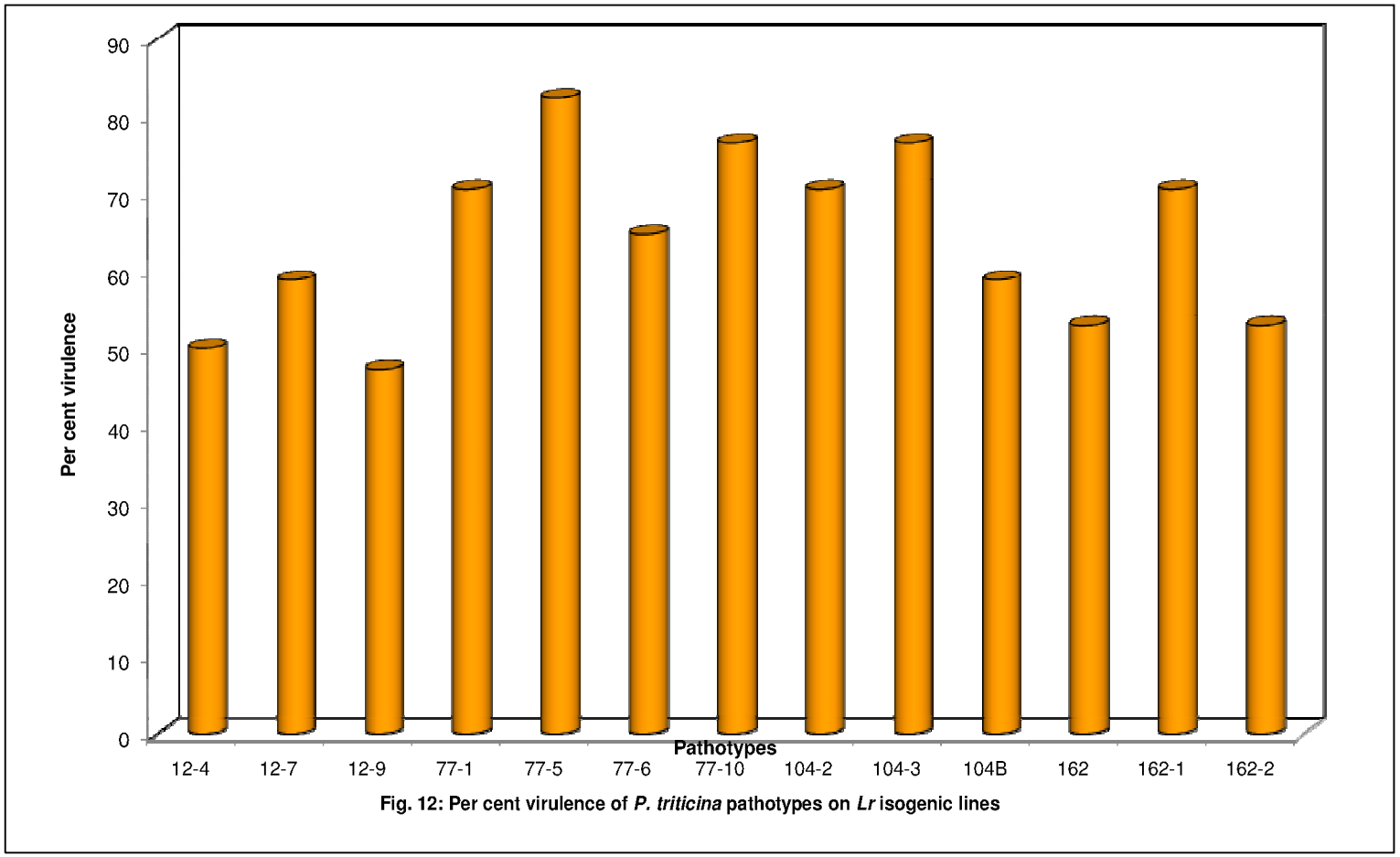


Fig. 12: Per cent virulence of *P. triticina* pathotypes on *Lr* isogenic lines

Fig 12: Per cent virulence of *P. triticina* pathotypes on *Lr* isogenic lines

Inoculation of individual leaf rust pathotypes on susceptible host

Single pustule culture on susceptible host

Plate 11: Mass multiplication and maintenance of individual leaf rust pathotypes at Shimla

LEGEND

Lane No.	Genotypes	Lane No.	Genotypes	Lane No.	Genotypes
M	Ladder size 25bp				
1	VL-943	22	VL-920	43	MACS-2496
2	VL-616	23	HI-977	44	Agra Local
3	VL-924	24	NIAW-917	45	HD-2864
4	GW-322	25	HS-533	46	HD-3091
5	HD-2932	26	DBW-17	47	MACS-3817
6	LOK-1	27	PBW-596	48	MACS-3828
7	Sonalika	28	HD-2733	49	HI-1563
8	UAS-326	29	PBW-343	50	HI-1544
9	RAJ-4083	30	DWR-162	51	HI-1584
10	HI-1500	31	PBW-590	52	HD-2189
11	HD--2888	32	HW-2004	53	GW-432
12	AKAW-4627	33	NW-4091	54	UP-2825
13	UAS-315	34	VL-829	55	HD-3093
14	NIAW-1415	35	HS-240	56	HD-3098
15	PBW-1415	36	VL-907	57	RAJ-4229
16	MACS-6145	37	HS-420	58	RAJ-4240
17	C-306	38	KRL-210	59	RAJ-4245
18	NI5439	39	Lal bahadur	60	RAJ-4270
19	UAS-304	40	PBW-343	61	NI-1689
20	DBW-16	41	PAVAN-76	62	-ve
21	KRL-210	42	RL-6077		

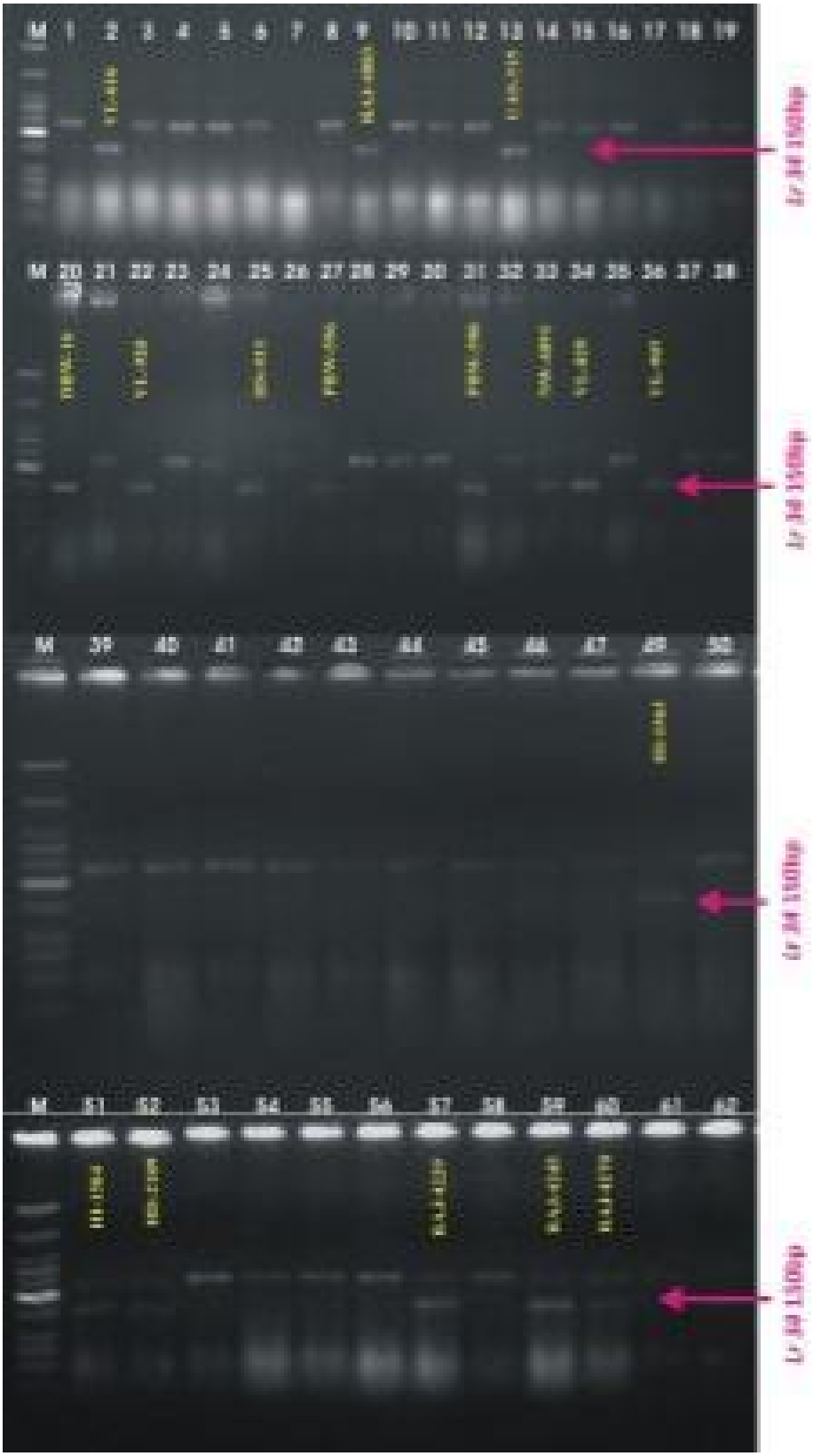


Plate 12: Bread wheat genotypes positive for slow leaf rust resistance gene *Lr34*

Table 21: List of genotypes positive for slow rust resistant genes at molecular level and their AUDPC values

Slow rusting resistance genes	Genotypes	AUDPC value
Lr34	RAJ-4083	4.38
	DBW-16	141.75
	VL-892	0.00
	HS-533	0.00
	PBW-596	0.00
	PBW-590	0.00
	VL-829	0.00
	VL-907	6.65
	HI-1563	0.00
	HI-1584	0.00
	RAJ-4229	0.00
	RAJ-4245	0.00
Lr46	GW-322	56.35
	HD-2932	268.80
	Sonalika	430.50
	HS-240	596.75
	HS-420	157.59
	HD-2864	0.00
	HD-3093	0.00
Lr67	RL-6077	112.88
Lr34+Lr46	VL-616	162.05
	HD-2189	196.52
	UAS-315	254.54
	NW-4091	0.00
	RAJ-4270	0.00

LEGEND

Lane No.	Genotypes	Lane No.	Genotypes	Lane No.	Genotypes
M	Ladder size : Lane 1, 2 100bp and Lane 3, 4 25bp				
1	VL-943	22	VL-920	43	-ve
2	VL-616	23	HI-977	44	MACS-2496
3	VL-924	24	NIAW-917	45	Agra Local
4	GW-322	25	HS-533	46	HD-2864
5	HD-2932	26	DBW-17	47	HD-3091
6	LOK-1	27	PBW-596	48	MACS-3817
7	Sonalika	28	HD-2733	49	MACS-3828
8	UAS-326	29	PBW-343	50	HI-1563
9	RAJ-4083	30	DWR-162	51	HI-1544
10	HI-1500	31	PBW-590	52	HI-1584
11	HD--2888	32	HW-2004	53	HD-2189
12	AKAW-4627	33	NW-4091	54	GW-432
13	UAS-315	34	VL-829	55	UP-2825
14	NIAW-1415	35	HS-240	56	HD-3093
15	PBW-1415	36	VL-907	57	HD-3098
16	MACS-6145	37	HS-420	58	RAJ-4229
17	C-306	38	KRL-210	59	RAJ-4240
18	NI5439	39	Lal bahadur	60	RAJ-4245
19	UAS-304	40	PBW-343	61	RAJ-4270
20	DBW-16	41	PAVAN-76	62	NI-1689
21	KRL-210	42	RL-6077	+ve	Pavon-76

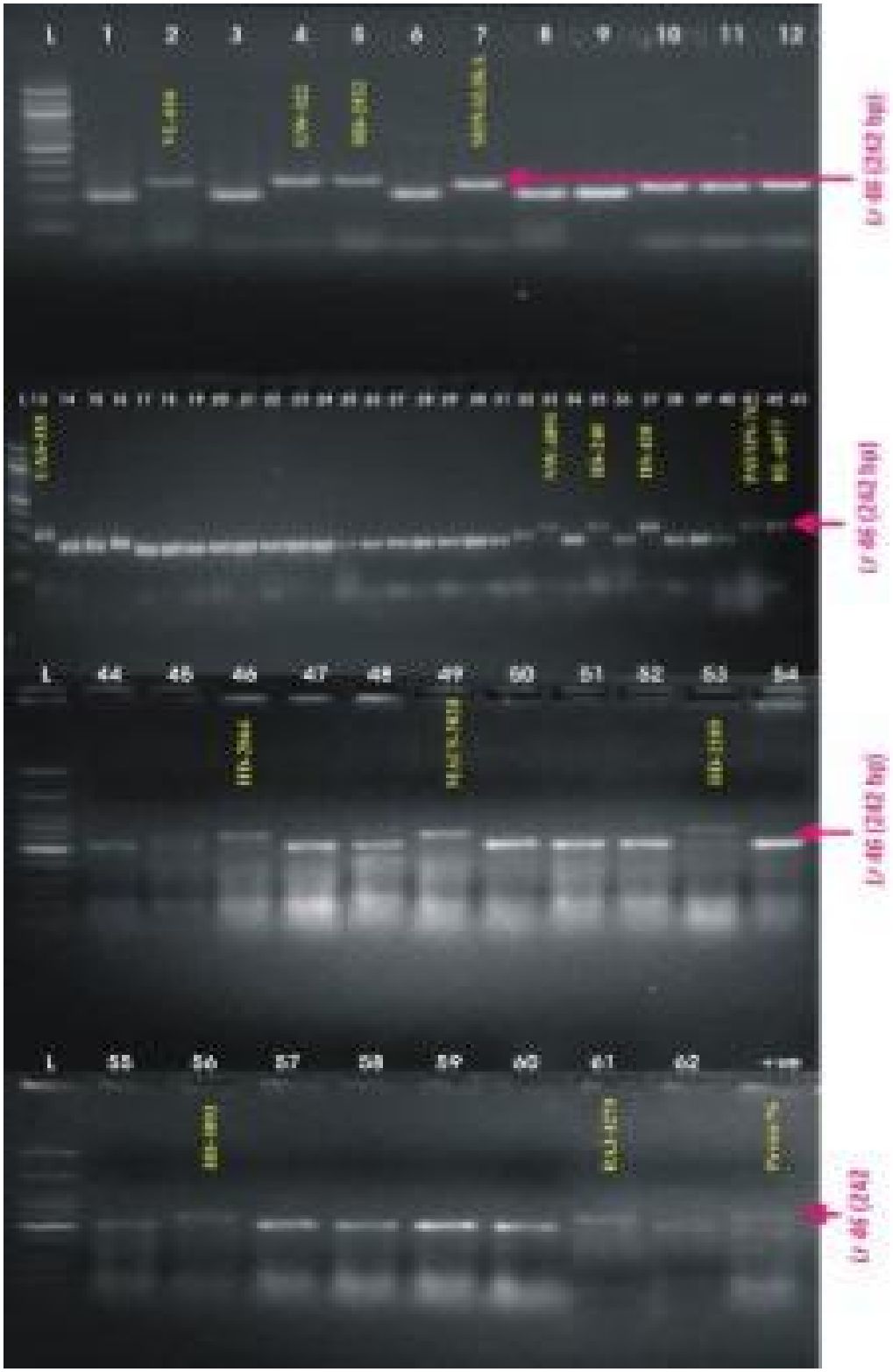


Plate 13: Bread wheat genotypes positive for slow leaf rust resistance gene *Lr46*

LEGEND

Lane No.	Genotypes	Lane No.	Genotypes	Lane No.	Genotypes
M	Ladder size : Lane 1, 2 100bp and Lane 3, 4 25bp				
1	VL-943	22	VL-920	43	RL-6077
2	VL-616	23	HI-977	44	MACS-2496
3	VL-924	24	NIAW-917	45	Agra Local
4	GW-322	25	HS-533	46	HD-2864
5	HD-2932	26	DBW-17	47	HD-3091
6	LOK-1	27	PBW-596	48	MACS-3817
7	Sonalika	28	HD-2733	49	MACS-3828
8	UAS-326	29	PBW-343	50	HI-1563
9	RAJ-4083	30	DWR-162	51	HI-1544
10	HI-1500	31	PBW-590	52	HI-1584
11	HD--2888	32	HW-2004	53	HD-2189
12	AKAW-4627	33	NW-4091	54	GW-432
13	UAS-315	34	VL-829	55	UP-2825
14	NIAW-1415	35	HS-240	56	HD-3093
15	PBW-1415	36	VL-907	57	HD-3098
16	MACS-6145	37	HS-420	58	RAJ-4229
17	C-306	38	KRL-210	59	RAJ-4240
18	NI5439	39	Lal bahadur	60	RAJ-4245
19	UAS-304	40	PBW-343	61	RAJ-4270
20	DBW-16	41	PAVAN-76	62	NI-1689
21	KRL-210	42	-ve	+ve	RL-6077

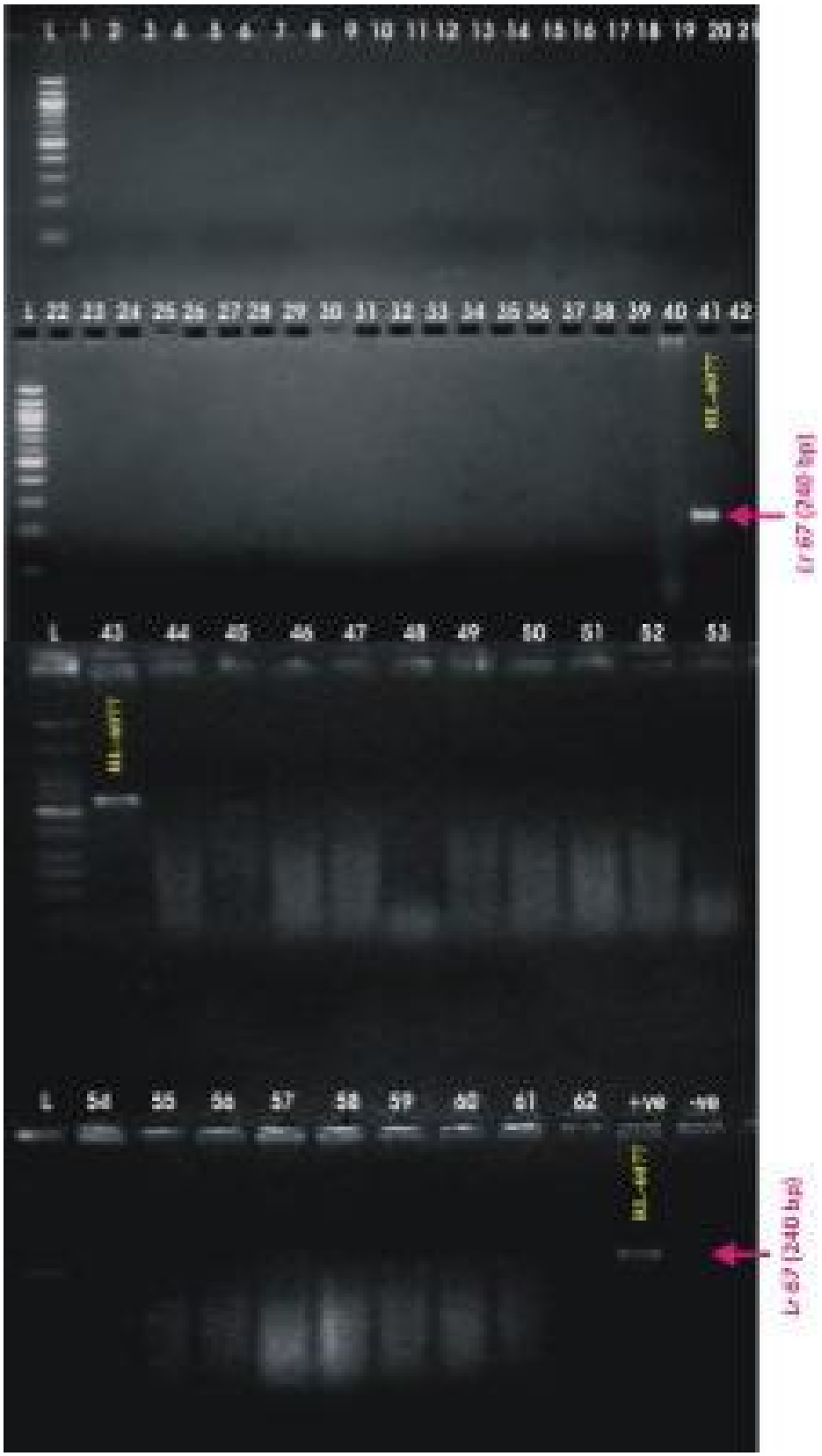
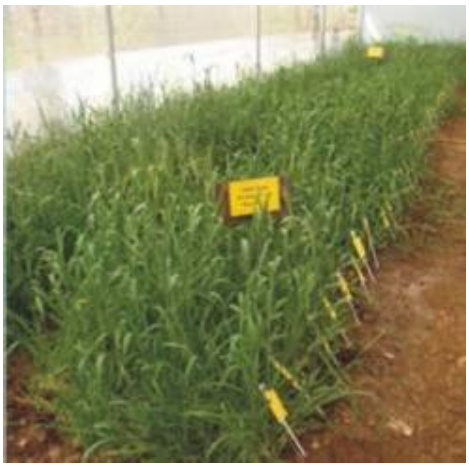
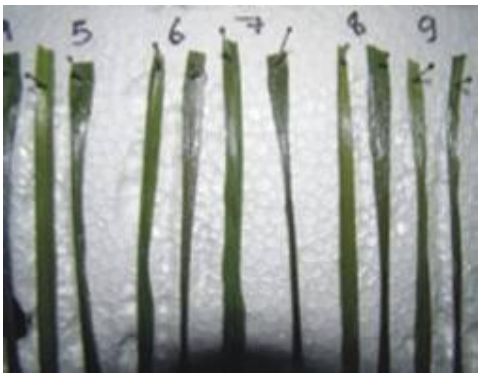


Plate 14: Bread wheat genotypes positive for slow leaf rust resistance gene *Lr67*

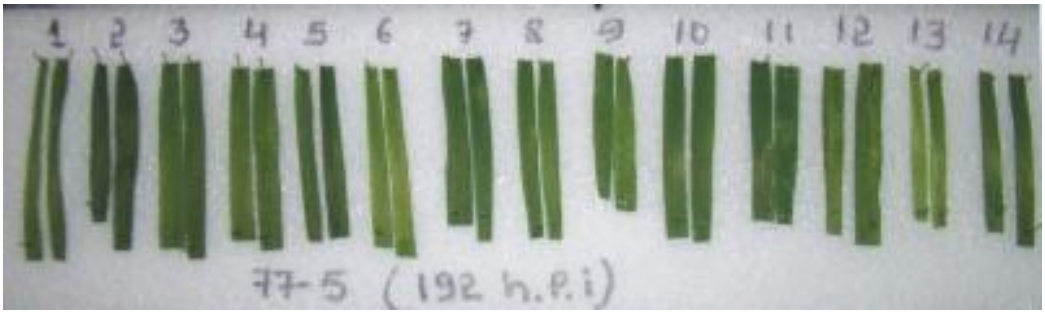


Two sets of bread whest genotypes grows under polyhouse for race reaction

Inoculation at adult plant stage



Smearing of xylene solution on leaf surface for histology study

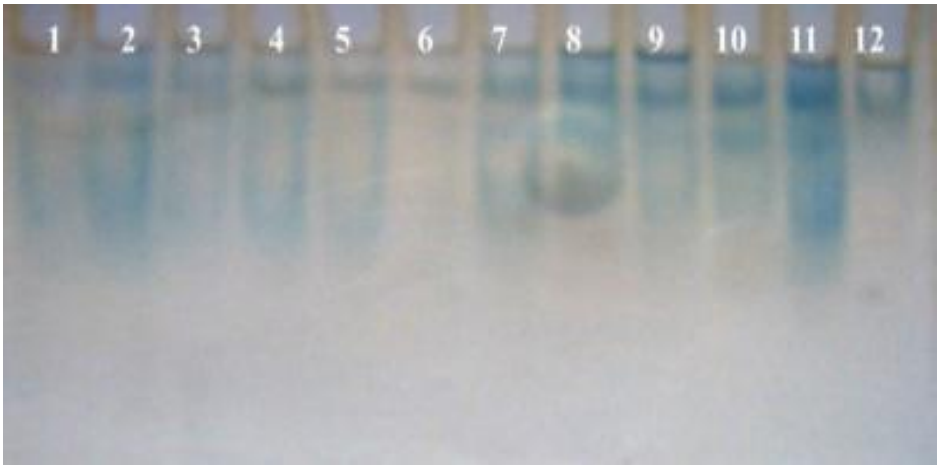


Sample mounting on thermocol sheet for histology study

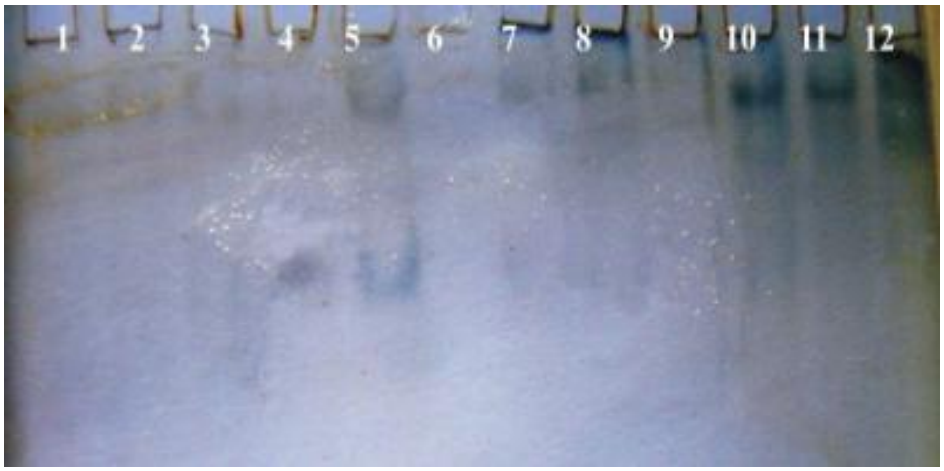
Plate 15: Adult plant stage of wheat genotypes for isozymes and histology study of two predominant pathotypes of *Puccinia triticina*

LEGEND

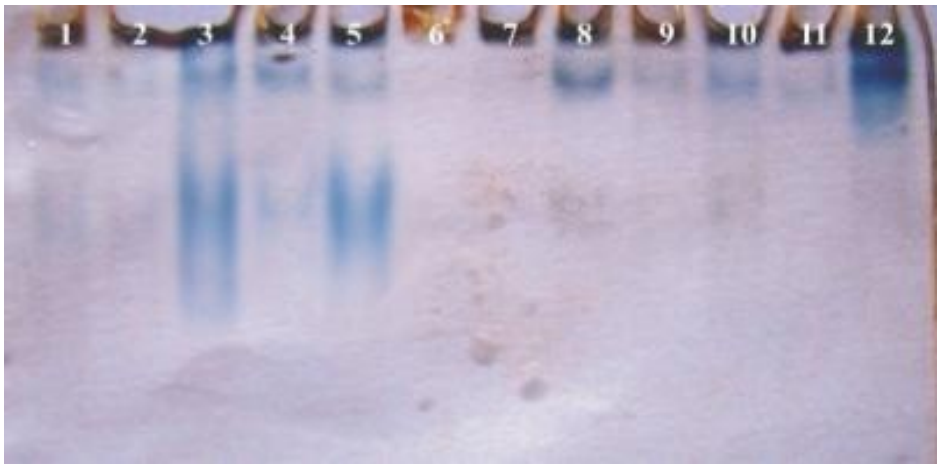
Lane No.	Details
1	Healthy at seedling stage
2	Seedling stage at 5 days after inoculation for race 77-5
3	Seedling stage at 10 days after inoculation for race 77-5
4	Seedling Stage at 5 Days after inoculation for race104-2
5	Seedling Stage at 10 Days after inoculation for race104-2
6	Tracking dye
7	Healthy at adult plant stage
8	Adult plant stage 5 days after inoculation race 77-5
9	Adult plant stage 10 days after inoculation for race 77-5
10	Adult plant stage: 5 days after inoculation of race 104-2
11	Adult plant stage 10 days after inoculation for race 104-2
12	Adult plant stage (field) at 15 days after inoculation



NIAW 917 (Resistant)



HD 2189 (Slow leaf ruster)

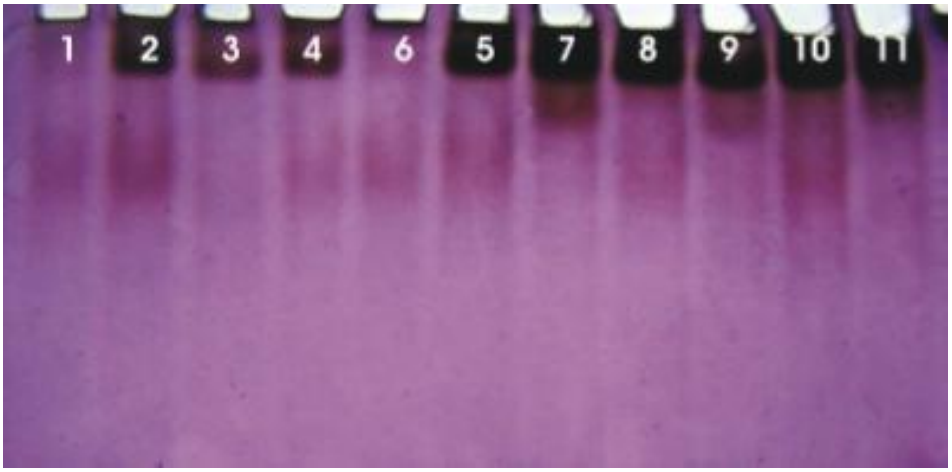


Agra Local (Susceptible)

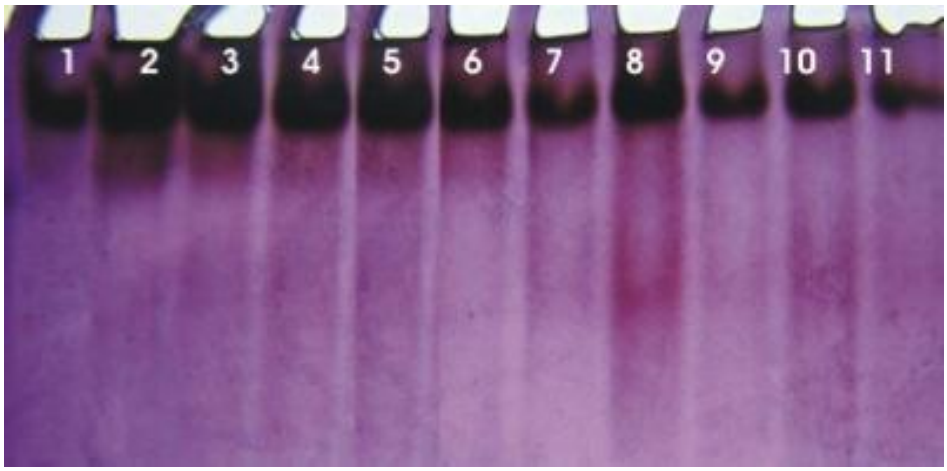
Plate 16: Peroxidase banding pattern of three bread wheat genotypes

LEGEND

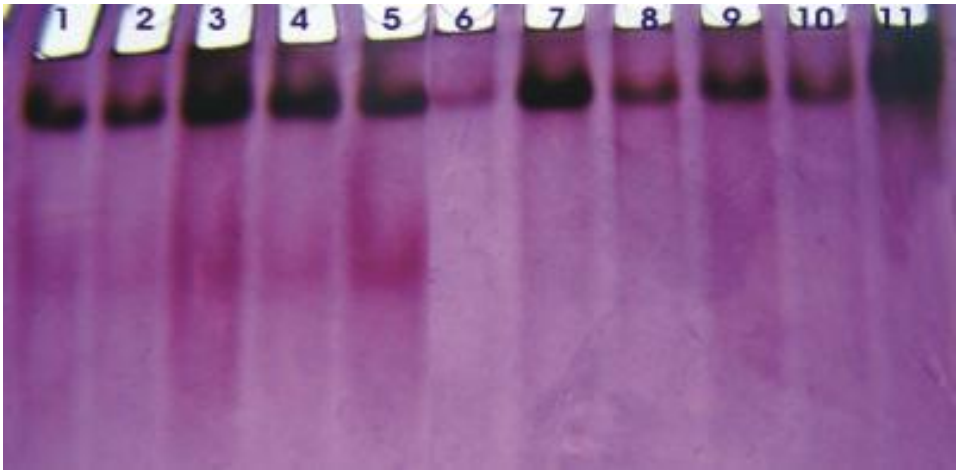
Lane No.	Details
1	Healthy at seedling stage
2	Seedling stage at 5 days after inoculation for race 77-5
3	Seedling stage at 10 days after inoculation for race 77-5
4	Seedling stage at 5 days after inoculation for race104-2
5	Seedling stage at 10 days after inoculation for race104-2
6	Healthy at adult plant stage
7	Adult plant stage 5 days after inoculation race 77-5
8	Adult plant stage 10 days after inoculation for race 77-5
9	Adult plant Stage: 5 days after inoculation of race 104-2
10	Adult plant stage 10 days after inoculation for race 104-2
11	Adult plant stage (field) at 15 Days after inoculation



NIAW 917 (Resistant)

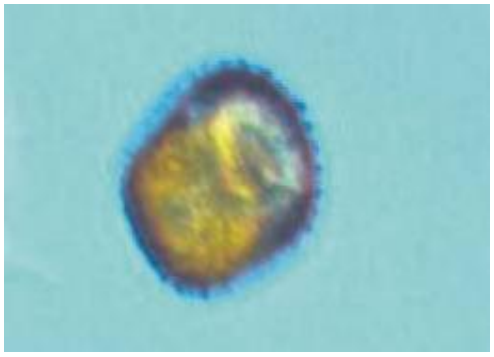


HD 2189 (Slow leaf ruster

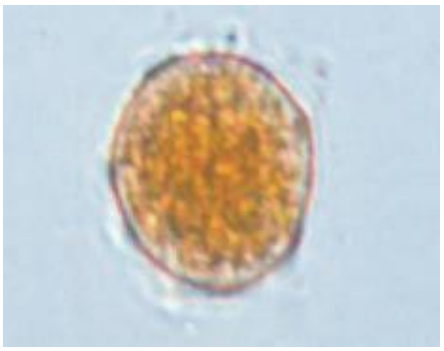


Agra Local (Susceptible)

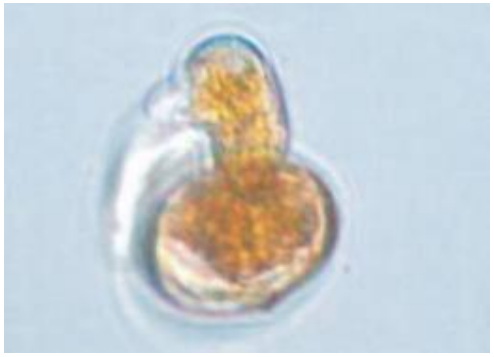
Plate 17: Polyphenol banding pattern of three bread wheat genotypes



Normal uredospore

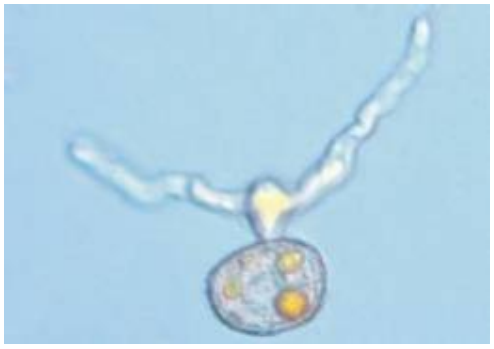


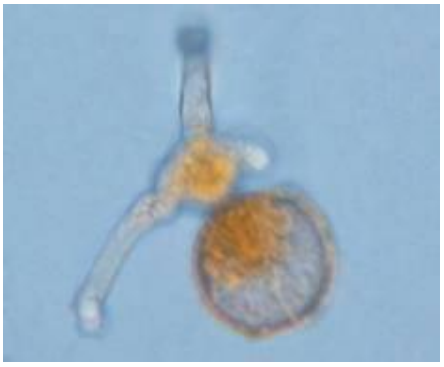
Uredospore after incubation



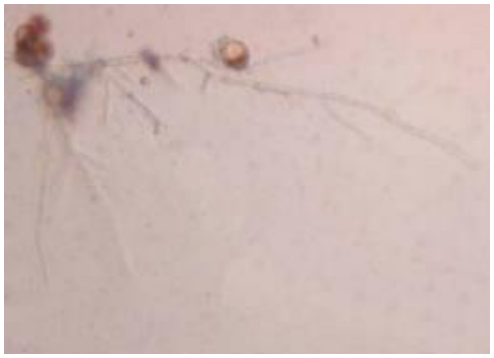
Germination of uredospore

Grem tube formation





Further development of germ tube



Mycelial formation

Teliospore

Plate 18: Uredospore germination process under sterile distilled water and teliospo

In the universal susceptible genotype, Agra Local documented absence of peroxidase at uninoculated conditions of both the stages. Whereas, expression of peroxidase were observed under inoculated conditions at seedling stage for both 77-5 and 104-2 races. At adult plant stage higher expression was observed in 5th day after inoculation for race 77-5 as that of race 104-2 at same interval, whereas very slight/ nil expression was observed at 10th day after inoculation for both the races. The prominent expression of peroxidase was observed at adult plant stage of field condition for mixture of races at 15th day after inoculation.

In comparison of all the three genotypes, it was found that no differences in the expression of peroxidase at all the stages and both the races in the resistant genotype, NIAW-917. Whereas, differences were observed among slow rusting and susceptible genotypes. In case of slow rusting genotype, HD-2189 differences for both stages as well as both races were noticed but in case of susceptible genotype difference observed only to the stage but not to the races was documented (Plate 16).

4.3.10.2 Polyphenol oxidases (PPO)

In the resistant genotype, NIAW-917 showed prominent expression of PPO irrespective of the growth stages for both inoculated *P. triticina* races (77-5 and 104-2) as well as mixture of races under field condition except 10th day after inoculation for race 77-5 and 5th day after inoculation for race 104-2 at seedling stage it was showed less expression as compared to other conditions (Lane 3 & 4) (Plate 17). Whereas, uninoculated conditions did not showed any expression of PPO at both the growth stage (Lane 1 & 5).

In slow rusting genotype, HD-2189 showed expression of PPO at all the stages irrespective of races inoculated as well as uninoculated conditions. But, expression level varies between the stages and also between inoculated and uninoculated conditions. The expression of PPO was less in uninoculated condition of both stages (Lane 1 & 6). At seedling stage expression was similar for both the races and interval, whereas, expression of PPO at adult plant stage differs between two intervals it stated that, expression of PPO was less in 5th day after inoculation for both races (Lane 7 & 9) as compare to the 10th day after inoculation (Lane 8 & 10). Under field condition for mixture of races at 15th day after inoculation showed less expression as compare to all other stages (Lane 11) (Plate 17).

In the universal susceptible genotype, Agra Local documented expression of PPO at all the stages irrespective of races inoculated as well as uninoculated conditions. But, expression level varies between the stages and also between inoculated and uninoculated conditions as that of slow rusting genotype. The expression of PPO was less in uninoculated condition of both stages (Lane 1 & 7). At seedling stage expression was similar for both the races and interval except 10th day after inoculation for race 77-5 (Lane 3) as it was showed higher expression, whereas, expression of PPO at adult plant stage differs between two intervals it stated that, expression of PPO was more in 5th day after inoculation for both races (Lane 8 & 10) as compare to the 10th day after inoculation (Lane 9 & 11) Under field condition for mixture of races at 15th day after inoculation showed more expression as compare to all other stages (Lane 12). This result is exactly opposite to the results obtained by slow rusting genotype, HD-2189 (Plate 17).

Overall results on expression of isozymes revealed that, among resistant, slow rusting and susceptible genotypes, higher variation between peroxidases and PPO expression at different stages of growth under inoculated and uninoculated conditions.

4.3.11 Histology of slow leaf rusters

Development of *P. triticina* uredospores on 14 bread wheat genotypes was studied histologically to determine differences among resistant (3), slow rusting (4) and susceptible (6) genotypes grown under polyhouse condition (Plate 15). Five sequential samples of flag leaves (24-192 hr after inoculation by two races, 77-5 and 140-2) were smeared with xylene solution and observed with a Differential Interference Contrast (DIC) microscope. Detailed procedure for sample collection and preparation was already explained in 'Material and Methods'. Uredospore germination and appressorial formation was also observed when incubated at different hour's interval in distilled water (Plate 18).

4.3.11.1 Spore germination

The spore germination was not observed at 24 h after inoculation in both races. It reveals that, upto 24 h no spore germination among all the genotypes tested. The spore germination started

between 24 and 48 h interval, whereas, average maximum per cent spore germination was observed between 72 and 96 h after inoculation for both the races (Table 22 & Fig. 13).

4.3.11.1.1 Race 77-5

The results of spore germination (%) is presented in Table 22 and Fig. 13 revealed that, genotype HS-240 (75 %) showed significantly highest per cent germination at 48 h after inoculation followed by Lal Bahadur (62.50 %). The lowest per cent germination was observed in UAS-304 (25.0 %) and NI-5439 (25.0 %). At 72 h after inoculation genotype, Sonalika and Agra Local showed significantly higher (75.0) per cent of germination and lowest (37.50 %) was found in NI-5439 and HD-2189. At 96 h after inoculation Sonalika showed higher (87.50) per cent germination followed by UAS-304, HS-420, C-306 and Agra Local showed 75.00 per cent germination. The average per cent germination was higher in Sonalika (70.84 %) followed by Agra Local (66.67 %) and lowest was obtained by NI-5439 (37.50) and HD-2189 (44.0). From the overall results, for race 77-5 shows that, per cent spore germination was observed significant differences between genotypes irrespective of the resistant, slow rusters and susceptible genotypes at different interval. Interestingly, 100 per cent germination was not observed in any of the genotypes tested.

4.3.11.1.2 Race 104-2

The results by inoculation of race 104-2 revealed that, genotype UAS-304 (75 %) showed significantly highest per cent germination at 48 h after inoculation followed by Sonalika, Lal Bahadur and Agra Local showed 62.50 per cent spore germination. The lowest per cent germination was observed in NI-5439 and Pavon-76 (25.0 % each). At 72 h after inoculation genotype, HD-2189, Sonalika and Agra Local showed significantly higher (75.0) per cent of germination and lowest (37.50 %) was found in NI-5439 and NIAW-917. At 96 h after inoculation Agra Local showed higher (87.50) per cent germination followed by NIAW-917, HS-420, Pavon-76, C-306, DWR-162 and Lal Bahadur showed 75.00 per cent germination. The least per cent germination was obtained by UAS-304 (50.0%). The average per cent germination was higher in Agra Local (75.00 %) followed by Lal Bahadur (70.83 %) and Sonalika (66.67 %) and lowest was obtained by NI-5439 (41.67). From the overall result for race 104-2 shows that, significant differences between genotypes were observed but not between the three group of genotypes *viz*., resistant, slow rusters and susceptible genotypes in the per cent germination of spore at different interval.

The significant increase in the average per cent germination of three groups of genotypes as increase in the interval among both the races was found. The spore germination was little higher in race 104-2 than race 77-5 was also observed (Fig. 13).

4.3.11.2 Appressoria formation

The appressorial formation (%) was not observed upto 48 h after inoculation in both races. The appressorial formation started between 48 and 72 h interval, whereas, average maximum per cent appressorial formation was observed between 96 and 192 h after inoculation for race 77-5 and for race 104-2 it was observed between 72 and 96 h after inoculation among all the genotypes tested (Table 23 & Fig. 14).

4.3.11.2.1 Race 77-5

The results of appressoria formation is presented in Table 23 and Fig. 14 revealed that, only few genotypes *viz*., NI-5439, HS-240 and Agra Local (12.5 %) started appressoria formation at 48 h after inoculation. Remaining genotypes were not formed appressoria at 48 h after inoculation. At 72 h after inoculation genotype, Agra Local showed significantly higher (cent per cent) appressoria formation followed by NI-5439 and UAS-304 (50.0%). Whereas, GW-322, HS-420, HD-2189 and C-306 were not formed appressoria at 72 h after inoculation. At 96 h after inoculation Agra Local and C-306 showed higher (75.50) per cent germination followed by UAS-304, NIAW-917 and Sonalika (62.5 %). The least was observed by NI-5439, HD-2189, Pavon-76 and DWR-162 showed 25.0 per cent formation of appressoria. At 192 h after inoculation higher (75.0%) per cent of appressoria formation was found in NI-5439 and DWR-162 followed by Lal Bahadur (62.5 %). The lowest was observed in GW-322 (25.0%). The average per cent appressoria formation was higher in Agra Local (62.50 %) followed by UAS-304 and NI-5439 (40.63 %) and lowest was obtained by GW-322 and HD-2189 (15.63%).

The significant increase in the average per cent appressoria formation of resistant group of genotypes was observed upto 96 h after inoculation and later it decreased at 192 h after inoculation. Whereas, slow ruster genotypes showed significant increase in the average per cent of appressorial

formation in each increase in the interval. But, susceptible genotypes were displayed significant increase in the average per cent of appressorial formation upto 96 h after inoculation and later it becomes constant at 192 h after inoculation.

4.3.11.2.2 Race 104-2

The results by inoculation of race 104-2 revealed that, genotype Sonalika (50.0 %) showed significantly highest per cent appressoria formation at 48 h after inoculation followed by C-306 and Agra Local showed 25.0 per cent appressoria formation. Remaining genotypes were not formed appressoria at 48 h after inoculation. At 72 h after inoculation genotype, Agra Local showed significantly higher (75.0%) appressoria formation. Whereas, HS-420 was not formed appressoria at 72 h after inoculation. Among remaining genotypes, eight genotypes showed 50.0 per cent appressoria formation. At 96 h after inoculation HS-420, Lal Bahadur and Agra Local showed higher (87.50) per cent appressoria formation. The least was observed by GW-322, UAS-304 and HS-240 showed 25.0 per cent formation of appressoria. At 192 h after inoculation higher (75.0%) per cent of appressoria formation was found in UAS-304, NIAW-917, NI-5439 and Agra Local followed by Sonalika (62.5 %). The lowest was observed in HS-420, Pavon-76 and DWR-162 (25.0%). The average per cent appressoria formation was higher in Agra Local (65.63 %) followed by C-306 (50.0%), Sonalika, NIAW-917 and Lal Bahadur (46.88%) and lowest was obtained by GW-322 and HS-420 (28.13%).

From the overall result for race 104-2 shows that, significant differences between genotypes were observed but not between the three group of genotypes *viz*., resistant, slow rusters and susceptible genotypes in the per cent appressoria formation at different interval.

The significant increase in the average per cent appressoria formation of resistant group of genotypes was observed from zero to 45.83 per cent and later it decreased to 41.67 per cent at 96 h after inoculation then it rises to 62.5 per cent at 192 h after inoculation. Whereas, slow ruster genotypes showed significant increase in the average per cent of appressorial formation upto 96 h after inoculation then it was decreased at 192 h after of inoculation. But, susceptible genotypes were displayed significant increase in the average per cent of appressorial formation upto 96 h after inoculation and later it decreases at 192 h after inoculation.

The overall pre penetration process on host was delayed largely as it was showed spore germination between 24 and 48 h after inoculation and appressorial formation between 48 and 72 h after inoculation. Whereas, it was observed that, spore germination within 4 h of incubation and appressorial formation within 8 h of incubation when incubated at room temperature in distilled water on cavity slide. The spore germination, germ tube elongation and appressorial formation on bread wheat host were documented by using Differential Interference Contrast microscope (400X) and given in Plate 19.

4.4 Evaluation of identified slow leaf rusters for quality traits

4.4.1 Grain Protein Content (GPC)

The mean GPC of resistant, slow rusting and susceptible genotypes were showed non significant differences under protected plots (13.99, 14.04 and 14.0% respectively) compared to the unprotected plots (14.03, 13.51 and 13.38% respectively).

The loss of GPC was either increased or decreased or no significant changes obtained when compared with protected and unprotected conditions among all the three group of genotypes. The average mean losses of GPC showed gain of GPC in unprotected condition for resistance genotype (-3.29 %). For slow rusting 3.53 per cent losses and susceptible genotypes showed 4.28 per cent losses (Table 24). In the individual analyses for each genotype, the GPC for majority of resistant genotypes were resulted with decrease as well as increase trend was observed. Whereas, NW-4091 showed no change in GPC losses.

Eight slow-ruster genotypes had GPC losses varied from 0.75 to 10.26 per cent. Remaining 3 slow ruster genotypes showed gain in the GPC under unprotected condition. Of the 10 susceptible genotypes, DWR-162, Lal Bahadur and Agra Local were showed gain in GPC under unprotected condition. Whereas, remaining susceptible genotypes were displayed losses varied from 1.11 to 13.93 per cent (Table 24).

Table 22: Percent germination of uredospores at different intervals on selected bread wheat genotypes with two *P.triticina* races

Races	Hours after inoculation							
	77-5				104-2			
Genotypes	**48**	**72**	**96**	**Mean**	**48**	**72**	**96**	**Mean**
Resistant								
GW-322	50.00	62.50	50.00	54.17	37.50	62.50	62.50	54.17
	(7.14)	(7.97)	(7.14)	(7.43)	(6.20)	(7.97)	(7.97)	(7.43)
UAS-304	25.00	50.00	75.00	50.00	75.00	62.50	50.00	62.50
	(5.10)	(7.14)	(8.72)	(7.14)	(8.72)	(7.97)	(7.14)	(7.97)
NIAW-917	50.00	50.00	62.50	54.17	37.50	37.50	75.00	50.00
	(7.14)	(7.14)	(7.97)	(7.43)	(6.20)	(6.20)	(8.72)	(7.14)
Mean	**41.67**	**54.17**	**62.50**	**52.78**	**50.00**	**54.17**	**62.50**	**55.56**
	(6.53)	**(7.43)**	**(7.97)**	**(7.33)**	**(7.14)**	**(7.43)**	**(7.97)**	**(7.52)**
Slow rusters								
NI-5439	25.00	37.50	50.00	37.50	25.00	37.50	62.50	41.67
	(5.10)	(6.20)	(7.14)	(6.20)	(5.10)	(6.20)	(7.97)	(6.53)
HS-420	37.50	50.00	75.00	54.17	50.00	50.00	75.00	58.33
	(6.20)	(7.14)	(8.72)	(7.43)	(7.14)	(7.14)	(8.72)	(7.70)
HD-2189	37.50	37.50	57.00	44.00	37.50	75.00	62.50	58.33
	(6.20)	(6.20)	(7.62)	(6.71)	(6.20)	(8.72)	(7.97)	(7.70)
Pavon-76	37.50	62.50	50.00	50.00	25.00	50.00	75.00	50.00
	(6.20)	(7.97)	(7.14)	(7.14)	(5.10)	(7.14)	(8.72)	(7.14)
Mean	**34.38**	**46.88**	**58.00**	**46.42**	**34.38**	**53.13**	**68.75**	**52.08**
	(5.95)	**(6.92)**	**(7.68)**	**(6.89)**	**(5.95)**	**(7.36)**	**(8.35)**	**(7.29)**
Susceptible								
Sonalika	50.00	75.00	87.50	70.83	62.50	75.00	62.50	66.67
	(7.14)	(8.72)	(9.41)	(8.48)	(7.97)	(8.72)	(7.97)	(8.23)
C-306	50.00	62.50	75.00	62.50	50.00	50.00	75.00	58.33
	(7.14)	(7.97)	(8.72)	(7.97)	(7.14)	(7.14)	(8.72)	(7.70)
Agra Local	50.00	75.00	75.00	66.67	62.50	75.00	87.50	75.00
	(7.14)	(8.72)	(8.72)	(8.23)	(7.97)	(8.72)	(9.41)	(8.72)
DWR-162	50.00	50.00	37.50	45.83	37.50	50.00	75.00	54.17
	(7.14)	(7.14)	(6.20)	(6.84)	(6.20)	(7.14)	(8.72)	(7.43)
HS-240	75.00	50.00	50.00	58.33	37.50	62.50	50.00	50.00
	(8.72)	(7.14)	(7.14)	(7.70)	(6.20)	(7.97)	(7.14)	(7.14)
Lal bahadur	62.50	62.50	50.00	58.33	62.50	75.00	75.00	70.83
	(7.97)	(7.97)	(7.14)	(7.70)	(7.97)	(8.72)	(8.72)	(8.48)
Mean	**56.25**	**62.50**	**62.50**	**60.42**	**52.08**	**64.58**	**70.83**	**62.50**
	(7.57)	**(7.97)**	**(7.97)**	**(7.84)**	**(7.29)**	**(8.10)**	**(8.48)**	**(7.97)**
Grand Mean	**46.15**	**55.77**	**61.12**	**54.35**	**46.15**	**58.65**	**68.27**	**57.69**
	(6.87)	**(7.53)**	**(7.88)**	**(7.44)**	**(6.87)**	**(7.72)**	**(8.32)**	**(7.66)**
SEm±	0.17	0.26	0.22		0.24	0.14	0.38	
CD (P=0.01)	0.72	1.12	0.96		1.04	0.62	1.62	

Data in parenthesis are square root transformed values ($\sqrt{X+1}$)

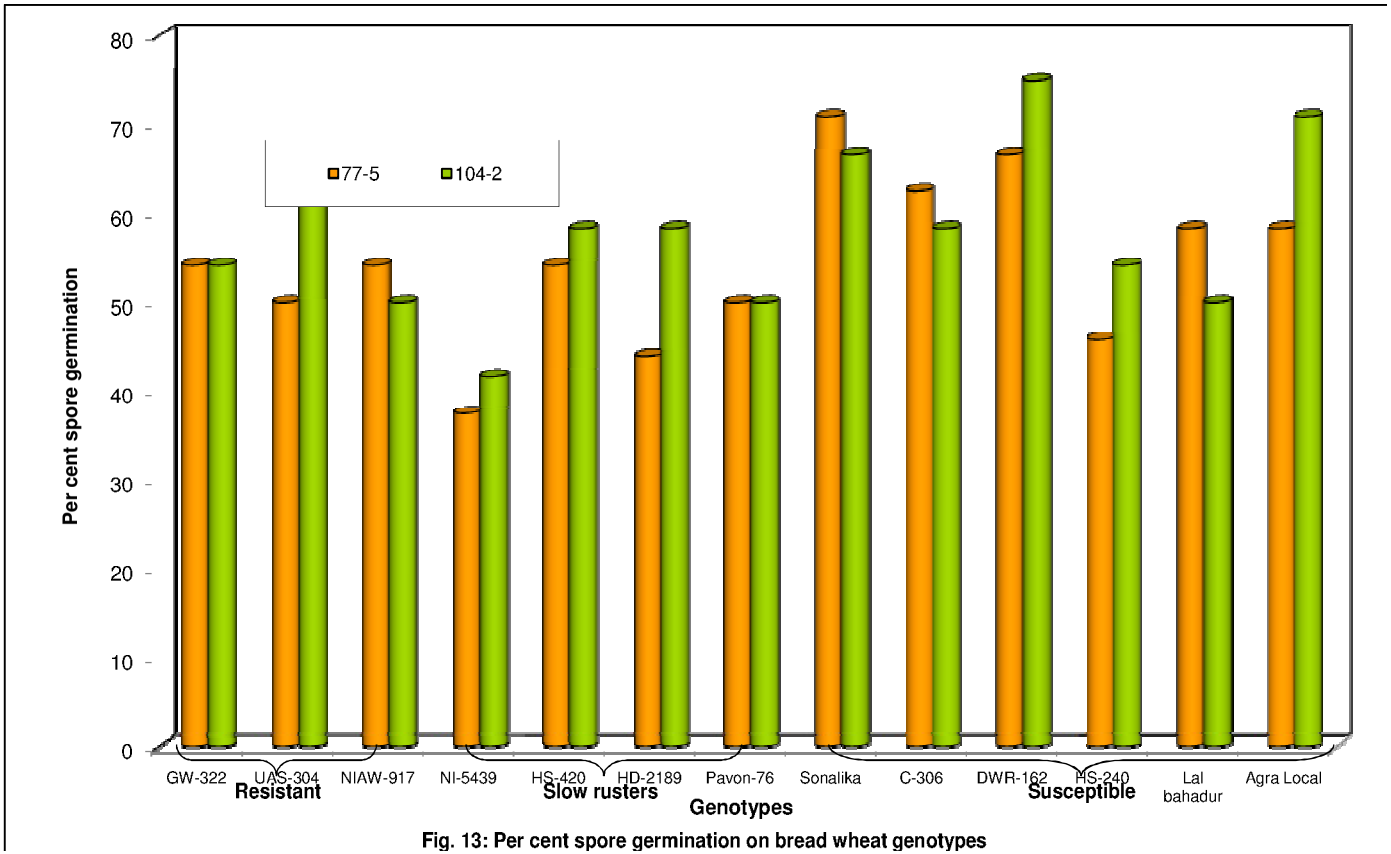


Fig. 13: Per cent spore germination on bread wheat genotypes

Fig 13: Per cent spore germination on bread wheat genotypes

Table 23: Percent appressorial formation at different intervals on selected bread wheat genotypes with two *P. triticina* races

Races	Hours after inoculation									
	77-5					104-2				
Genotypes	48	72	96	192	Mean	48	72	96	192	Mean
Resistant										
GW-322	0.00	0.00	37.50	25.00	15.63	0.00	50.00	25.00	37.50	28.13
	(1.00)	(1.00)	(6.20)	(5.10)	(4.08)	(1.00)	(7.14)	(5.10)	(6.20)	(5.40)
UAS-304	0.00	50.00	62.50	50.00	40.63	0.00	50.00	25.00	75.00	37.50
	(1.00)	(7.14)	(7.97)	(7.14)	(6.45)	(1.00)	(7.14)	(5.10)	(8.72)	(6.20)
NIAW-917	0.00	25.00	62.50	50.00	34.38	0.00	37.50	75.00	75.00	46.88
	(1.00)	(5.10)	(7.97)	(7.14)	(5.95)	(1.00)	(6.20)	(8.72)	(8.72)	(6.92)
Mean	**0.00**	**25.00**	**54.17**	**41.67**	**30.21**	**0.00**	**45.83**	**41.67**	**62.50**	**37.50**
	(0.00)	**(5.10)**	**(7.43)**	**(6.53)**	**(5.59)**	**(1.00)**	**(6.84)**	**(6.53)**	**(7.97)**	**(6.20)**
Slow Rusters										
NI-5439	12.50	50.00	25.00	75.00	40.63	0.00	25.00	75.00	75.00	43.75
	(3.67)	(7.14)	(5.10)	(8.72)	(6.45)	(1.00)	(5.10)	(8.72)	(8.72)	(6.69)
HS-420	0.00	0.00	50.00	50.00	25.00	0.00	0.00	87.50	25.00	28.13
	(1.00)	(1.00)	(7.14)	(7.14)	(5.10)	(1.00)	(1.00)	(9.41)	(5.10)	(5.40)
HD-2189	0.00	0.00	25.00	37.50	15.63	0.00	50.00	75.00	50.00	43.75
	(1.00)	(1.00)	(5.10)	(6.20)	(4.08)	(1.00)	(7.14)	(8.72)	(7.14)	(6.69)
Pavon-76	0.00	25.00	25.00	50.00	25.00	0.00	50.00	50.00	25.00	31.25
	(1.00)	(5.10)	(5.10)	(7.14)	(5.10)	(1.00)	(7.14)	(7.14)	(5.10)	(5.68)
Mean	**3.13**	**18.75**	**31.25**	**53.13**	**26.56**	**0.00**	**31.25**	**71.88**	**43.75**	**36.72**
	(2.03)	**(4.44)**	**(5.68)**	**(7.36)**	**(5.25)**	**(1.00)**	**(5.68)**	**(8.54)**	**(6.69)**	**(6.14)**
Susceptible										
Sonalika	0.00	37.50	62.50	50.00	37.50	50.00	25.00	50.00	62.50	46.88
	(1.00)	(6.20)	(7.97)	(7.14)	(6.20)	(7.14)	(5.10)	(7.14)	(7.97)	(6.92)
C-306	0.00	0.00	75.00	50.00	31.25	25.00	50.00	75.00	50.00	50.00
	(1.00)	(1.00)	(8.72)	(7.14)	(5.68)	(5.10)	(7.14)	(8.72)	(7.14)	(7.14)
DWR-162	0.00	25.00	25.00	75.00	31.25	0.00	50.00	75.00	25.00	37.50
	(1.00)	(5.10)	(5.10)	(8.72)	(5.68)	(1.00)	(7.14)	(8.72)	(5.10)	(6.20)
HS-240	12.50	25.00	50.00	50.00	34.38	0.00	50.00	25.00	50.00	31.25
	(3.67)	(5.10)	(7.14)	(7.14)	(5.95)	(1.00)	(7.14)	(5.10)	(7.14)	(5.68)
Lal bahadur	0.00	25.00	50.00	62.50	34.38	0.00	50.00	87.50	50.00	46.88
	(1.00)	(5.10)	(7.14)	(7.97)	(5.95)	(1.00)	(7.14)	(9.41)	(7.14)	(6.92)
Agra Local	25.00	100.00	75.00	50.00	62.50	25.00	75.00	87.50	75.00	65.63
	(5.10)	(10.05)	(8.72)	(7.14)	(7.97)	(5.10)	(8.72)	(9.41)	(8.72)	(8.16)
Mean	**8.65**	**35.42**	**56.25**	**56.25**	**38.54**	**16.67**	**50.00**	**66.67**	**52.08**	**46.35**
	(3.11)	**(6.03)**	**(7.57)**	**(7.57)**	**(6.29)**	**(4.20)**	**(7.14)**	**(8.23)**	**(7.29)**	**(6.88)**
Grand Mean	**8.65**	**23.08**	**48.08**	**51.92**	**32.93**	**7.69**	**43.27**	**62.50**	**51.92**	**41.35**
	(3.11)	**(4.91)**	**(7.01)**	**(7.27)**	**(5.83)**	**(2.95)**	**(6.65)**	**(7.97)**	**(7.27)**	**(6.51)**
SEm±	0.10	0.14	0.32	0.35		0.08	0.28	0.29	0.34	
CD (p=0.01)	0.43	0.60	1.39	1.50		0.36	1.22	1.26	1.48	

Data in parenthesis are square root transformed values ($\sqrt{X+1}$)

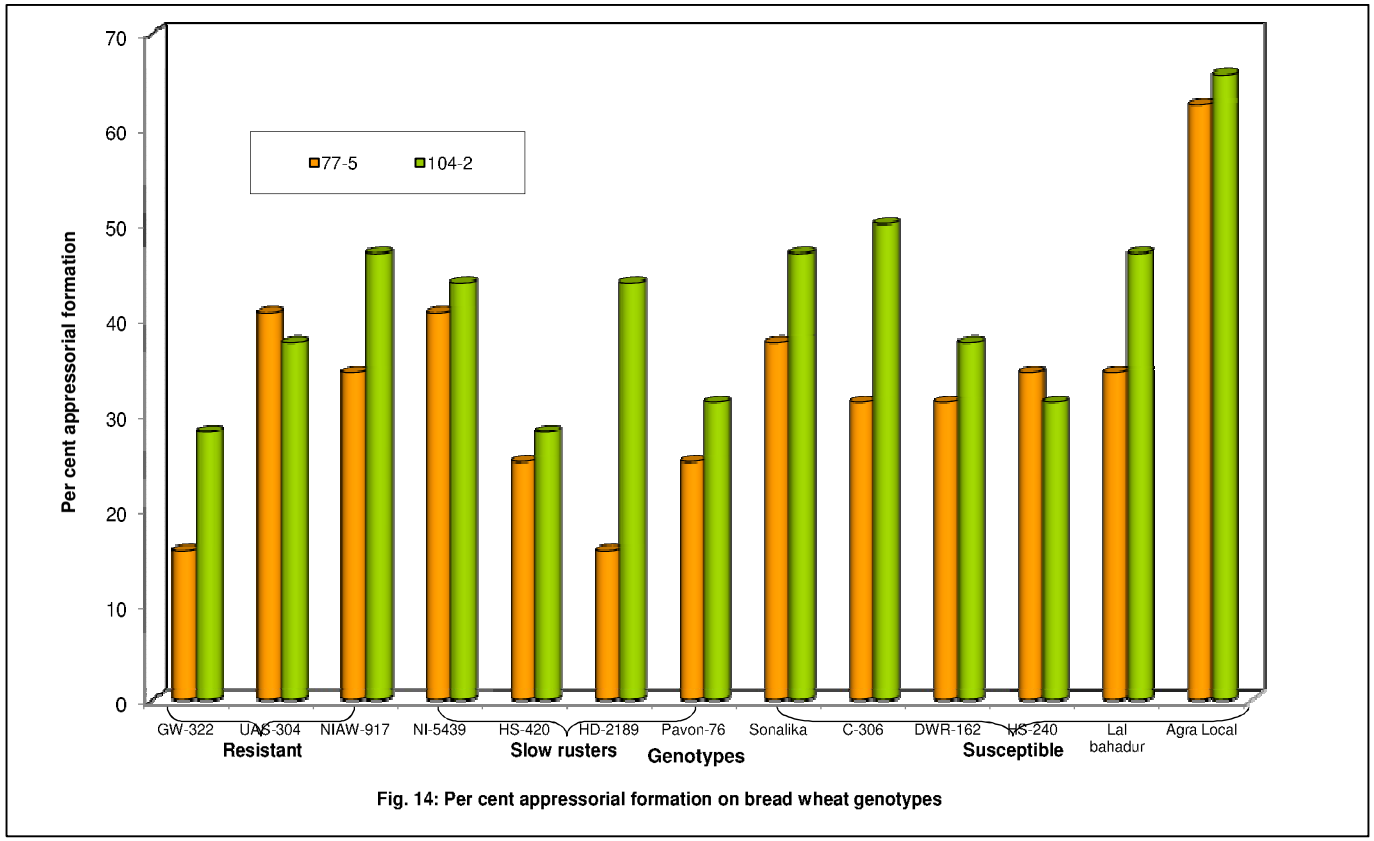
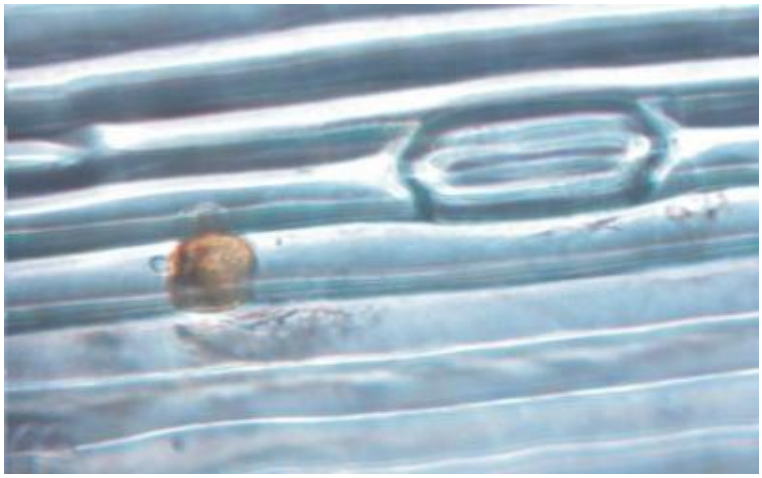
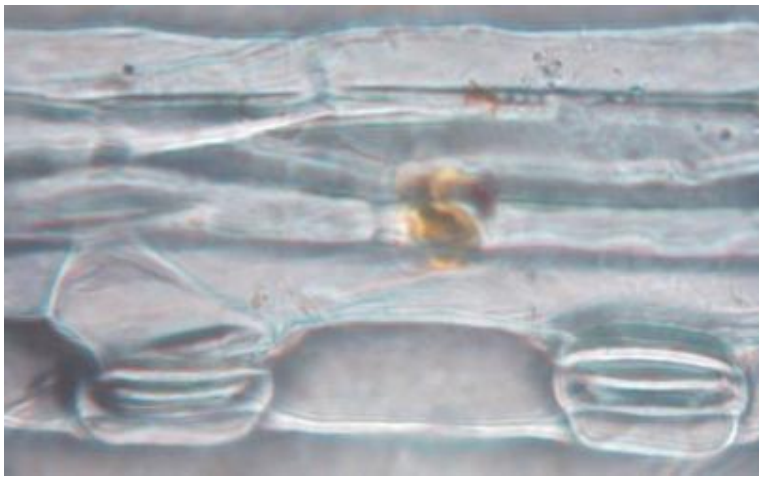


Fig. 14: Per cent appressorial formation on bread wheat genotypes

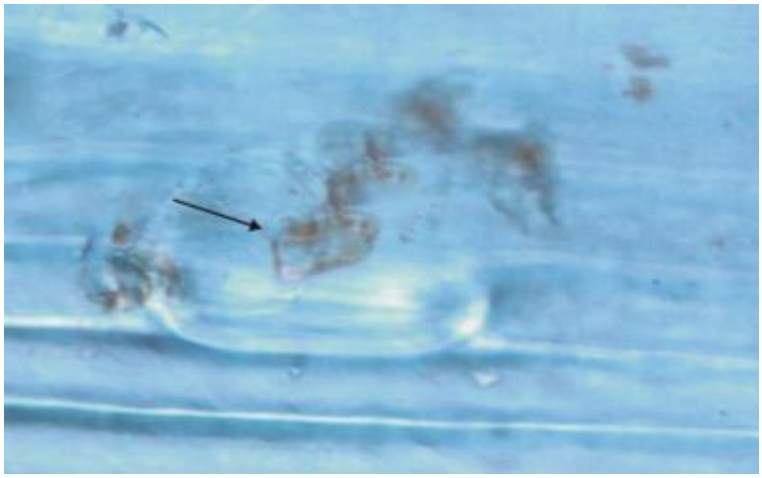
Fig 14: Per cent appressorial formation on bread wheat genotypes



Germination of uredospore near by stomata



Elongation of germ tube near by stomata



Appressorial formation on host

Plate 19: Uredospore germination and appressorial formation on bread wheat host at adult plant stage (400X)

Table 24: Effect of leaf rust on yield and quality traits of bread wheat genotypes during *rabi* 2012-13

Genotypes group	AUDPC	ACI	Yield (q/ha)			TGW (g)			Grain Protein Content (%)			Sedimentation value (ml)		
			Prot[b]	Un prot[b]	Loss[a] (%)	Prot[b]	Un prot[b]	Loss[a] (%)	Prot[b]	Unprot[b]	Loss[a] (%)	Prot[b]	Un prot[b]	Loss[a] (%)
Resistant														
AKAW-4627	0.00	0.00	40.33	40.83	-1.24	42.12	42.94	-1.95	13.10	13.55	-3.44	45.00	45.00	0.00
GW-322	56.35	1.62	46.92	48.96	-4.35	37.81	35.29	6.68	10.90	11.30	-3.67	34.00	35.00	-2.94
HD-2888	0.00	0.00	38.33	38.38	-0.11	37.26	34.88	6.39	12.40	14.65	-18.15	38.00	37.00	2.63
HI-1500	0.00	0.00	19.58	19.46	0.63	36.65	35.28	3.72	13.00	13.65	-5.00	43.00	43.50	-1.16
HS-533	0.00	0.00	27.67	27.71	-0.15	33.59	33.15	1.30	16.40	15.60	4.88	46.00	39.50	14.13
HW-2004	0.00	0.00	25.00	24.00	4.00	35.33	35.63	-0.85	14.40	13.30	7.6	47.00	44.00	6.38
MACS-6145	3.50	0.04	17.50	16.58	5.24	37.36	33.93	9.19	14.00	13.35	4.64	45.00	40.50	10.00
NIAW-1415	0.00	0.00	33.00	33.42	-1.26	35.23	34.08	3.25	12.80	13.80	-7.81	42.00	42.00	0.00
NIAW-917	0.00	0.00	48.67	48.88	-0.43	35.17	34.90	0.76	15.00	13.70	8.67	52.00	49.00	5.77
NW-4091	0.00	0.00	34.17	33.75	1.22	34.66	35.24	-1.69	13.70	13.70	0.00	38.00	34.50	9.21
PBW-590	0.00	0.00	34.00	34.38	-1.10	36.39	36.42	-0.09	15.50	14.85	4.19	46.00	43.50	5.43
PBW-596	0.00	0.00	40.92	39.08	4.48	45.36	38.25	15.68	15.30	15.40	-0.65	47.00	46.75	0.53
RAJ-4083	4.38	0.05	43.42	43.29	0.29	45.24	43.20	4.51	13.10	13.15	-0.38	46.00	42.50	7.61
RAJ-4270	0.00	0.00	45.17	44.38	1.76	46.71	45.47	2.67	13.90	14.00	-0.72	45.00	49.00	-8.89
UAS-304	93.22	2.94	39.92	35.75	10.44	34.07	31.70	6.96	13.70	13.35	2.55	45.50	44.50	2.20
VL-829	0.00	0.00	16.67	15.67	6.00	31.90	24.04	24.64	12.90	13.00	-0.78	42.50	41.50	2.35
VL-892	0.00	0.00	37.17	36.58	1.57	30.24	28.43	6.00	14.10	14.55	-3.19	48.00	45.00	6.25
VL-907	6.65	0.29	33.08	33.75	-2.02	29.63	29.64	-0.03	16.00	15.75	1.56	48.00	48.50	-1.04
VL-943	14.88	0.34	26.17	24.88	4.94	34.04	34.24	-0.59	15.60	15.85	-1.60	38.50	37.00	3.90
Mean	**9.42**	**0.28**	**34.09**	**33.67**	**1.57**	**36.78**	**35.09**	**4.55**	**13.99**	**14.03**	**-3.29**	**44.03**	**42.54**	**3.28**
Slow-ruster														
DBW-16	141.75	3.76	32.42	28.17	13.11	28.97	28.46	1.77	13.80	14.00	-1.45	40.00	36.00	10.00
DBW-17	299.25	7.62	43.67	37.71	13.65	31.86	28.97	9.08	13.70	13.55	1.09	38.00	41.75	-9.87
HD-2189	196.52	5.15	27.83	24.96	10.32	32.51	29.97	7.83	14.10	13.30	5.67	34.00	36.00	-5.88
HI-977	206.33	4.73	44.08	42.04	4.63	36.07	34.61	4.05	14.20	13.55	4.58	47.00	47.00	0.00
HS-420	157.59	3.84	52.00	46.04	11.46	35.09	33.75	3.81	14.3	13.55	5.24	49.5	42.25	14.65

Contd…

Genotypes group	AUDPC	ACI	Yield (q/ha)			TGW (g)			Grain Protein Content (%)			Sedimentation value (ml)		
			Prot[b]	Un prot[b]	Loss[a] (%)	Prot[b]	Un prot[b]	Loss[a] (%)	Prot[b]	Unprot[b]	Loss[a] (%)	Prot[b]	Un prot[b]	Loss[a] (%)
KRL-210	191.80	6.06	42.50	36.83	13.33	39.86	37.06	7.03	15.20	13.80	9.21	40.00	41.00	-2.50
NI-5439	168.53	5.08	40.42	34.63	14.33	33.29	28.98	12.93	13.60	13.80	-1.47	35.50	37.50	-5.63
UAS-315	254.54	6.82	36.17	31.13	13.94	32.41	30.05	7.28	12.30	12.45	-1.22	40.00	43.50	-8.75
UAS-326	171.50	3.37	33.92	29.58	12.78	34.77	33.15	4.65	13.40	13.30	0.75	46.00	51.50	-11.96
VL-616	162.05	5.41	16.33	15.92	2.55	32.91	28.06	14.73	15.10	13.55	10.26	32.00	37.50	-17.19
VL-924	207.20	6.95	40.75	38.00	6.75	41.58	41.12	1.11	14.70	13.80	6.12	38.00	44.50	-17.11
Mean	**196.10**	**5.34**	**37.28**	**33.18**	**10.62**	**34.48**	**32.20**	**6.75**	**14.04**	**13.51**	**3.53**	**40.00**	**41.68**	**-4.93**
Susceptible														
AGRA LOCAL	2222.50	68.00	30.00	9.33	68.89	31.09	25.21	18.89	14.30	14.50	-1.40	51.50	54.00	-4.85
C-306	923.30	24.30	45.58	35.71	21.66	34.78	29.22	15.97	13.50	13.35	1.11	35.00	41.00	-17.14
DWR-162	317.45	7.89	33.17	30.21	8.92	36.98	31.27	15.43	13.80	13.90	-0.72	44.00	46.50	-5.68
HD-2733	275.45	7.55	40.08	34.50	13.93	35.44	31.90	9.99	14.30	12.85	10.14	43.00	40.00	6.98
HD-2932	268.80	6.13	44.50	40.13	9.83	43.32	43.16	0.37	14.40	12.90	10.42	44.00	52.50	-19.32
HS-240	596.75	17.68	30.17	22.71	24.72	27.29	22.23	18.56	14.60	14.10	3.42	43.50	44.50	-2.30
LAL BAHADUR	2012.50	64.20	38.42	12.71	66.92	39.82	33.55	15.75	12.40	13.50	-8.87	35.00	37.00	-5.71
LOK-1	873.25	28.58	28.67	24.08	15.99	45.72	40.02	12.45	14.00	12.05	13.93	42.00	38.50	8.33
PBW-343	542.24	13.06	43.58	33.71	22.66	37.25	34.10	8.45	14.70	13.95	5.10	39	36.5	6.41
SONALIKA	430.50	13.28	33.75	32.25	4.44	33.06	32.93	0.39	14.00	12.65	9.64	47.00	54.00	-14.89
Mean	**846.27**	**25.07**	**36.79**	**27.53**	**25.80**	**36.47**	**32.36**	**11.62**	**14.00**	**13.38**	**4.28**	**42.40**	**44.45**	**-4.82**
Grand Mean	**269.97**	**7.87**	**35.64**	**32.00**	**10.12**	**36.07**	**33.61**	**6.93**	**14.01**	**13.72**	**1.76**	**42.51**	**42.78**	**-1.00**
SEm±			1.63			1.43			0.34			1.11		
CD at 5%			4.67			4.10			0.98			3.17		
CD at 1%			6.25			5.49			1.31			4.24		

[a] Loss (%) = (protected yield – nonprotected yield) × 100/protected yield; * and ** = significant at *P* = 0.05 and 0.01, respectively.
[b] Prot and Unprot=Protected and Unprotected respectively.

When leaf rust was the only evidently severe disease, an increased trend of grain protein content due to leaf rust was obtained in grains of the susceptible varieties *viz*., DWR-162, Lal Bahadur and Agra Local.

4.4.2 Sedimentation value (SDS)

In SDS also varied as it was observed in GPC but slightly varies with genotype to genotype. The mean SDS of resistant, slow rusting and susceptible genotypes were showed non significant differences under protected plots (44.03, 40.0 and 42.40 ml respectively) compared to the unprotected plots (42.54 and 41.68 and 44.45 ml respectively). Thirteen resistant genotypes observed losses in SDS varied from 0.53 to 10.0 per cent. Among 6 remaining genotypes, 4 genotypes had negligible gain in the SDS under unprotected condition and 2 genotypes were showed no loss in SDS. Of the 11 slow-rusting genotype, 2 genotypes had losses in SDS but remaining genotypes showed gain in the SDS value ranged from -17.19 to -2.50 in SDS under unprotected condition except HI-977 showed no change. Among 10 susceptible genotypes, 7 genotypes including Lal Bahadur and Agra Local showed gain in the SDS under diseased condition whereas other three genotypes showed losses in SDS (Table 24 & Plate 20).

4.4.3 Genotypic correlations

In general, the genotypic correlations among grain yield, yield losses, AUDPC, ACI, TGW, GPC and SDS traits in the unprotected treatment were higher compared with the correlations in the protected treatment. The AUDPC and ACI of both the years were negatively correlated with grain yield, TGW, GPC and positively correlated with yield losses and SDS. In the unprotected treatments, grain yield was positively correlated with TGW and SDS. Whereas negatively correlated with GPC similar trend was also followed in the protected yield. Yield losses also were negatively correlated to TGW, PC and SDS. The GPC was positively non significantly correlated with SDS (Table 25).

4.4.4 Damaged starch

The results of damaged starch in the wheat flour measured by AACC (American Association of Cereal Chemists) units of slow rusters in comparison with resistant and susceptible genotypes were presented in Table 26 and documented in Plate 20. The results revealed that, all the slow ruster and susceptible genotypes under unprotected condition had higher damaged starch in the flour. Whereas, resistant genotypes displayed significantly lower starch damage under unprotected condition except NW-4091 showed difference in the damaged starch of both conditions. Results of damaged starch under protected condition showed higher damage in case of resistant group except NW-4091 and lower starch damage in slow rusters as well as susceptible group of genotypes.

Among the resistant genotypes under protected condition highest damaged starch was noticed in HI-1500 (6.22) and lowest was observed in RAJ-4270 (5.51), whereas under unprotected condition highest was in RAJ-4240 (6.10) and lower in HD-2888 (5.08). Among slow ruster genotypes, UAS-326 had highest and VL-924 had lowest (5.41) damaged starch under protected condition, whereas under unprotected condition UAS-326 was found higher (6.53) damaged starch and lowest was in VL-616 (5.84). Among susceptible genotypes, highest was found in C-306 and lowest was observed in Agra Local (5.53) under protected condition, whereas, under unprotected condition DWR-162 (6.42) had highest and Agra Local (6.00) had lowest damaged starch.

The loss (%) of damaged starch when compared both protected and unprotected condition showed increase in the loss in case of protected condition of resistant genotypes. Whereas loss was decreased under protected conditions of slow rusters as well as susceptible genotypes. This has been clearly says that, there was a differences observed in the loss of damaged starch between three group of genotypes.

The mean damaged starch of resistant and slow rusting genotypes was showed non significant between unprotected and protected condition. Whereas, susceptible genotypes were showed significant differences under unprotected and protected conditions.

The mean loss of damaged starch was higher in resistant genotypes (2.75 %). But, in case of susceptible mean loss was decreased to 6.50 per cent under protected condition and in case of slow rusters it decreased to 3.84 per cent. It clearly shows that, higher starch damage was observed in susceptible genotypes than slow ruster genotypes.

SD matic machine for estimation of damaged starch content in wheat flour

Dry gluten of 20 bread wheat genotypes

Diacid digestion of wheat flour for estimation of mineral nutrients

Sedimentation study of bread wheat flour

Plate 20: Evaluation of bread wheat genotypes for quality traits

Table 25: Genotypic correlations of yield and quality traits of bread wheat genotypes during *rabi* 2012-13

Variable	Treatment	TGW	Protein	SDS	AUDPC [a]	ACI [b]	Yield loss
Protein	Unprot[c]	-0.09					
	Prot[c]	-0.08					
SDS	Unprot[c]	0.15	0.044				
	Prot[c]	-0.006	0.42**				
AUDPC	Unprot[c]	-0.24	-0.071	0.048			
ACI	Unprot[c]	-0.25	-0.073	0.052	0.99**		
Yield loss	Unprot[c]	-0.45**	-0.112	-0.019	0.94**	0.95**	
Yield	Unprot[c]	0.48**	-0.0827	0.183	-0.53**	-0.52**	-0.67**
	Prot[c]	0.38**	-0.155	0.086			

[a] AUDPC = Area Under Disease Progress Curve of 2012.
[b] ACI=Average co-efficient of infection of 2012.
[c] Prot and Unprot = Protected and Unprotected respectively

Table 26: Effect of leaf rust on starch damage, wet gluten content, dry gluten content and gluten index of selected bread wheat genotypes

	Damaged Starch (AACC units)			Wet Gluten Content (%)			Dry Gluten Content (%)			Gluten Index (%)		
	Prot	Unprot	Difference[a]	Prot	Unprot	Loss[b] (%)	Prot	Unprot	Loss[b] (%)	Prot	Unprot	Loss (%)
Resistant												
HD-2888	5.60	5.08	0.52	33.59	41.18	-22.58	11.55	14.85	-28.53	78.63	93.21	-18.54
HI-1500	6.22	6.04	0.18	36.98	40.36	-9.14	14.21	14.23	-0.14	95.40	82.88	13.12
NW-4091	5.82	5.83	-0.01	34.95	31.75	9.16	11.71	11.45	2.22	95.77	96.74	-1.02
RAJ-4240	6.19	6.10	0.09	40.84	37.89	7.24	12.85	13.17	2.43	96.44	92.78	3.79
RAJ-4270	5.51	5.49	0.02	40.35	36.21	10.26	14.85	13.99	11.77	93.16	96.73	-3.83
Mean	**5.87**	**5.71**	**0.16**	**37.34**	**37.48**	**-1.01**	**13.30**	**13.37**	**-2.45**	**91.88**	**92.47**	**-1.30**
Slow Rusters												
HD-2189	6.20	6.33	-0.13	40.83	38.91	4.71	14.04	13.63	2.96	92.04	85.90	6.67
HD-2932	5.82	6.27	-0.45	41.18	35.02	14.97	16.33	12.85	21.34	96.16	94.34	1.89
HS-420	5.89	5.97	-0.08	37.34	35.23	5.66	15.80	14.70	6.93	97.20	97.02	0.18
NI-5439	6.15	6.20	-0.05	34.05	32.85	3.52	13.37	11.30	15.52	97.06	85.00	12.43
UAS-315	5.89	6.13	-0.24	34.47	32.70	5.13	12.47	11.70	6.21	93.29	90.95	2.50
UAS-326	6.32	6.53	-0.21	35.23	31.50	10.60	13.01	12.10	6.96	96.41	96.82	-0.43
VL-616	5.68	5.84	-0.16	46.27	35.21	23.91	18.22	13.79	24.31	90.86	96.09	-5.75
VL-924	5.41	5.87	-0.46	41.41	36.19	12.61	13.68	13.39	2.12	88.77	95.94	-8.07
Mean	**5.92**	**6.14**	**-0.22**	**38.85**	**34.70**	**10.14**	**14.61**	**12.93**	**10.79**	**93.97**	**92.76**	**1.18**
Susceptible												
Agra Local	5.53	6.00	-0.47	34.99	34.35	1.84	13.94	13.02	6.66	96.46	96.93	-0.48
C-306	6.28	6.36	-0.08	34.00	32.31	4.96	10.80	12.36	12.59	94.62	86.87	8.19
DWR-162	6.24	6.42	-0.18	35.58	34.81	2.16	12.00	13.74	12.66	97.12	97.19	-0.07
HS-240	5.77	6.08	-0.31	31.68	30.51	3.71	11.13	11.07	0.54	96.46	96.72	-0.26
Lal Bahadur	5.70	6.22	-0.52	34.31	33.045	3.69	12.02	11.37	5.45	96.00	95.59	0.43
Local red[+]	5.56	6.14	-0.58	49.04	33.99	30.69	19.05	14.29	25.01	91.79	89.13	2.90
Sonalika	5.60	6.07	-0.47	36.45	34.42	5.57	15.96	14.15	11.37	97.22	96.35	0.90
Mean	**5.81**	**6.18**	**-0.37**	**36.58**	**33.35**	**7.52**	**14.03**	**12.38**	**10.60**	**95.67**	**94.11**	**1.66**
Grand Mean	**5.87**	**6.05**	**-0.18**	**37.68**	**34.92**	**6.43**	**13.85**	**13.06**	**7.42**	**94.04**	**93.16**	**0.73**
SEm±	0.06			0.59			0.32			1.07		
CD (P=0.01)	0.25			2.40			1.31			4.35		

Unprot: Unprotected, Prot: Protected, [a] := (Protected – Unprotected), AACC: American Association of Cereal Chemists,
[b] : (Protected – Unprotected) x (100/Protected) , +: Durum Susceptible Check.

4.4.5 Wet gluten content (WGC)

The interaction of gliadin and glutenin protein types when mixed with water forms gluten. Water retention capacity, loaf volume and dough strength of bread are functions of gluten. Any change in gluten content due to disease, thus affect either of these baking quality parameters.

In the present investigation, the studies on effect of wet gluten content (%) was affected by leaf rust (Table 26). The average WGC under protected condition of slow rusters (38.85) was higher than resistant (37.34) and susceptible (36.58) genotypes. Whereas, the average WGC under unprotected condition was significantly higher in resistant (37.48) genotypes followed by slow rusters (34.70) and lowest in susceptible (33.35) genotypes.

Among the resistant genotypes, two genotypes (HI-1500 and HD-2888) showed decrease in the WGC under protected condition, whereas, remaining three genotypes showed increase in the WGC. However, the mean loss (%) of wet gluten content showed slightly decreased among resistant genotypes shows there was little negative effect in the spray of fungicide. All the slow ruster genotypes showed less WGC under diseased condition as compare to protected condition and the higher reduction was observed in VL-616 (35.21 to 46.27 %) whereas, lower reduction was found in NI-5439 (32.85 to 34.05%). The mean loss (%) WGC of slow rusters (10.14 %) showed higher as compare to susceptible (7.52 %) as well as resistant (-1.01 %) genotypes. In case of susceptible genotypes all the genotypes revealed reduction in the WGC under diseased condition. The higher reduction was observed in the Local red (Durrum susceptible check) followed by Sonalika and least reduction was observed in Agra Local.

4.4.6 Dry Gluten Content (DGC)

Results of DGC (%) showed similar trend as in the WGC (Table 26). The two resistant genotypes (HI-1500 and HD-2888) showed decrease in the DGC under protected condition, whereas, remaining three genotypes showed increase in the DGC. However, the mean loss (%) of dry gluten content showed negative effect in protected condition among resistant genotypes shows there was little effect in the spray of fungicide. All the slow ruster genotypes showed less DGC under diseased condition as compare to protected condition and the higher reduction was observed in VL-616 (13.79 to 18.22 %) whereas, lower reduction was found in VL-924 (13.68 to 13.39 %). The mean loss (%) DGC of slow rusters (10.79 %) showed higher as compare to resistant (-2.45 %) and susceptible (10.60 %) genotypes. In case of susceptible genotypes, all the genotypes revealed reduction in the DGC under diseased condition. The higher reduction was observed in the Local red (Durrum susceptible check) followed by DWR-162 and C-306 (Plate 20). The least reduction was observed in HS-240 and showed non significant.

4.4.7 Gluten Index (GI)

The results of GI showed very negligible gain and loss among majority of genotypes selected (Table 26). However, it was observed differences in the mean loss (%) among three groups of genotypes that, resistant genotypes showed slight increase (-1.30 %) in the GI of unprotected condition, whereas, among slow rusters and susceptible genotypes showed reduction in the GI under unprotected condition.

4.4.8 Alveoconsistograph

Three selected bread wheat genotypes including resistant (NIAW 917), slow leaf ruster (HD 2189) and susceptible (Agra local) seeds were obtained from both protected and unprotected conditions as explained in 'material and methods' were evaluated for alveograph parameters. The samples were divided into five subsamples gives five curves for each samples. The evaluation involves obtaining several indexes representing the average of all the recorded curves.

The principal indexes obtained from this average alveograph were maximum overpressure (P), average abscissa at rupture (L), swelling index (G), deformation energy (W), configuration ratio (P/L) and elasticity index (I_e). These indexes and theire relevance in assessing the rheological quality of the test dough as well as theire significance in predicting the baking quality of the flour used were given hereunder.

4.4.8.1 Maximum overpressure (P)

The maximum overpressure was highest in HD-2189 under unprotected condition (161mm) followed by protected condition (119mm) (Appendix VI & V). Whereas, least was observed in

unprotected condition of Agra local (83 mm) as shown in Appendix VIII and followed by protected condition of NIAW 917 (104mm) and protected condition of Agra local (106 mm) (Appendix III & VII).

4.4.8.2 Average abscissa at rupture (L)

The maximum average abscissa at rupture was highest in Agra local under unprotected condition (117 mm) followed by protected condition (107mm) (Appendix VIII & VII). Whereas, least was observed in unprotected condition of HD-2189 (13mm) and followed by protected condition (Appendix VI & V).

4.4.8.3 Swelling index (G)

Similar trend as shown in the average abscissa at rupture was observed in the swelling index of the selected genotypes under protected and unprotected condition.

4.4.8.4 Deformation energy (W)

It represents the energy necessary to inflate the bubble until it ruptures. The maximum deformation energy was recorded in NIAW 917 under unprotected condition (137) followed by unprotected condition (101) of the HD-2189 as shown in Appendix IV & VI. Whereas, zero deformation energy was recorded in unprotected condition of Agra local as shown in Appendix 8 and followed by protected condition of same genotype (6.47) (Appendix VII).

4.4.8.5 Configuration ratio (P/L)

The highest configuration ratio was observed in HD-2189 under unprotected condition (12.4) followed by protected condition (8.5) of the same genotype (Appendix VI & V) whereas, least was observed in unprotected condition of Agra local (0.71) as shown in App. 8 and followed by protected condition of the same genotype (0.99) (Appendix VII).

4.4.8.6 Elasticity index (I_e)

The elasticity index was shown zero in NIAW 917 and HD-2189 under both protected and unprotected condition whereas, highest was observed in Agra local under protected condition followed by unprotected condition of same genotype (Appendix VII & VIII).

The overall alveograph results on the rheological quality of the test dough indicated that highly susceptible genotype Agra local under diseased condition showed very poor rheological quality whereas, slow ruster did not affected much of the rheological quality.

4.4.9 Micronutrients

The selected bread wheat genotypes from the identification of slow ruster experiment (during 2011-12) were categorized based on AUDPC values (Table 27) into three different groups *viz*., resistant (33), slow rusters (3) and susceptible (4) genotypes were used to study the micro-nutrients by using Atomic Absorption Spectrophotometer (AAS) standard instrument for micronutrients estimation. The obtained parts per million (ppm) values were converted to milligrams per Kg of grains and the results were presented in Table 27 and diacid digestion of samples were documented in Plate 20.

4.4.9.1 Zinc

The average zinc content was found significantly highest in slow rusters (33.73 mg/Kg) as compared to the resistant (29.52) and susceptible (30.65) genotypes. Among all the genotypes, highest zinc content was observed in UAS-315 (40.05) and least was found in Lok-1 (12.65). The overall grand mean of zinc content was 30.08 mg/Kg of grains.

4.4.9.2 Iron

The average iron content was observed highest in slow rusters (42.04 mg/Kg) as compare to the resistant (41.99) and susceptible (40.72) genotypes and found on par with each other. Among the slow rusters VL-616 (44.40) was found highest followed by HD-2189 (42.05). Among resistant genotypes, highest iron content was observed in HD-2888 (48.15) and least was found in MACS-6145 (39.95). Among susceptible group of genotypes, Lal Bahadur (41.05) was found highest iron content and remaining three genotypes showed similar (40.60) content of iron except C-306. The overall grand mean of iron content was 41.84 mg/Kg of grains.

Table 27: Effect of leaf rust on micronutrients of slow rusters in comparison with resistant and susceptible selected bread wheat genotypes during *rabi* 2011-12

Sl. No.	Genotypes	AUDPC	ACI	Zn (mg/kg)	Fe (mg/kg)	Mn (mg/kg)	Mg (mg/kg)	Cu (mg/kg)
	Resistant							
1	AKAW-4627	0.00	0.00	33.35	42.10	34.05	994.80	10.06
2	DBW-16	0.00	0.00	30.90	41.00	24.20	994.85	9.94
3	DBW-17	0.00	0.00	27.80	41.05	33.00	993.10	9.56
4	GW-322	29.05	0.79	25.10	42.75	33.15	988.70	10.28
5	HD-2733	82.60	2.65	24.95	40.75	28.00	1002.20	9.55
6	HD--2888	0.00	0.00	36.15	48.15	31.65	988.50	10.01
7	HD-2932	4.20	0.10	28.35	41.85	36.30	994.35	10.21
8	HI-1500	0.00	0.00	34.45	40.95	29.10	1001.15	10.06
9	HS-240	84.18	2.33	28.30	41.95	27.00	991.90	9.42
10	HS-420	0.00	0.00	25.45	42.60	28.00	997.65	9.23
11	HS-533	0.00	0.00	27.65	41.55	36.45	994.75	9.64
12	HW-2004	0.00	0.00	28.50	43.15	35.55	1015.90	9.39
13	KRL-210	71.75	2.09	28.05	42.05	37.85	994.85	9.31
14	LOK-1	70.70	1.85	12.65	40.40	22.30	937.50	9.86
15	MACS-6145	0.00	0.00	36.60	39.95	34.30	1004.25	9.92
16	NIAW-1415	0.00	0.00	39.50	40.35	38.15	994.80	10.06
17	NIAW-917	0.00	0.00	29.90	42.80	36.50	988.60	9.79
18	NW-4091	10.06	0.22	24.95	41.35	31.20	997.65	9.34
19	PBW-343	15.66	0.25	31.05	41.65	32.90	984.75	9.50
20	PBW-590	0.00	0.00	24.20	43.20	32.60	993.15	9.39
21	PBW-596	0.00	0.00	30.65	41.10	33.50	994.90	9.66
22	RAJ-4083	3.50	0.07	29.30	41.60	30.90	994.80	10.04
23	RAJ-4270	0.00	0.00	29.10	42.70	35.60	1000.55	9.56
24	UAS-304	0.00	0.00	30.70	41.10	32.60	994.75	9.91
25	UAS-315	49.88	1.01	40.05	40.75	38.50	999.50	10.05
26	UAS-326	0.00	0.00	30.65	42.45	35.50	1010.65	10.22
27	VL-829	0.00	0.00	33.30	42.40	38.00	999.65	9.40
28	VL-892	0.00	0.00	25.20	41.25	31.40	997.15	9.86
29	VL-907	0.00	0.00	25.75	40.70	22.30	1009.05	9.29
30	VL-924	0.00	0.00	35.15	43.75	36.80	1000.50	10.86
31	VL-943	0.00	0.00	27.50	44.30	32.20	981.00	10.34
	Mean	**13.60**	**0.37**	**29.52**	**41.99**	**32.57**	**994.71**	**9.80**
	Slow Rusters							
32	HD-2189	132.82	3.92	35.05	42.05	36.55	989.00	10.06
33	NI-5439	120.37	2.53	32.90	40.55	34.95	986.85	9.92
34	Sonalika	154.70	3.09	38.75	41.15	22.30	995.10	10.20
35	VL-616	168.44	4.55	28.20	44.40	28.25	927.35	10.18
	Mean	**144.08**	**3.52**	**33.73**	**42.04**	**30.51**	**974.58**	**10.09**
	Susceptible							
36	Agra Local	2275.00	81.20	29.60	40.60	32.50	987.60	9.23
37	C-306	436.68	10.01	36.15	40.75	35.65	995.20	9.94
38	DWR-162	296.36	9.59	30.60	40.60	32.10	993.00	9.55
39	HI-977	239.31	5.10	28.25	40.60	26.55	988.55	9.79
40	Lal Bahadur	2135.00	78.10	28.65	41.05	33.00	994.75	9.20
	Mean	**1076.47**	**36.80**	**30.65**	**40.72**	**31.96**	**991.82**	**9.54**
	Grand Mean	**159.51**	**5.24**	**30.08**	**41.84**	**32.29**	**992.33**	**9.79**
	SEm±			0.77	0.54	0.67	9.23	0.33
	CD at 1%			2.95	2.08	2.58	35.35	NS

4.4.9.3 Manganese

Manganese showed the grand mean of 32.29 mg per kg with a range from 22.30 to 38.50 mg per kg. The average manganese content of slow ruster genotypes had lowest (30.51) and highest in resistant (32.57) genotypes in-between was observed in susceptible genotypes (31.96). The manganese content of resistant and susceptible genotypes found statistically on par with each other.

4.4.9.4 Magnesium

The average magnesium content was observed lowest in slow rusters (974.58 mg/Kg) as compare to the resistant (994.71) and susceptible (991.82) genotypes and found on par with each other. Among the slow rusters Sonalika (995.10) was found highest followed by HD-2189 (989.0). Among resistant genotypes, highest magnesium content was observed in HW-2004 (1015.90) and least was found in VL-943 (981.0). Among susceptible group of genotypes, C-306 (995.20) was found highest and Agra Local was found with lowest (987.60) magnesium content. The overall grand mean of magnesium content was 992.33 mg/Kg of grains.

4.4.9.5 Copper

The overall copper content was found non significant among the genotypes. However, slow ruster genotypes had highest (10.09 mg/Kg) copper content followed by resistant genotypes (9.80) and susceptible genotypes had lowest (9.54) copper. The grand mean of copper content among all the genotypes was 9.79 mg/Kg and it ranges from 9.20 to 10.86 mg/ Kg of grains.

4.4.10 Correlation coefficient of evaluated micronutrients

The genotypic correlations between AUDPC, ACI and micronutrients were performed, the results indicated that, AUDPC and ACI were negatively correlated with all the analyzed micronutrients except manganese with ACI value. The copper element was significantly negatively correlated with AUDPC and ACI values and also with zinc element (@ P=0.05 level). The manganese was highly significantly positively correlated with zinc (Table 28).

4.4.11 Yield and thousand grain weight assessment

4.4.11.1 Disease development and expression of resistance

Leaf rust severity was high and uniform throughout the experiment during *rabi* 2012-13. The fungicide-protected plots remained free from leaf rust during the entire crop season. The 11 slow-rusting genotypes and 10 susceptible genotypes showed compatible interaction with leaf rust pathogen. Among 19 resistant genotypes, 13 genotypes were displayed with race-specific resistance were either immune or near immune (i.e., showed hypersensitive flecking) remaining 6 genotypes displayed resistant to moderately resistant reaction, noticed as small uredinia surrounded by chlorosis.

The two susceptible checks Agra local and Lal Bahadur had highest AUDPC and ACI. The mean AUDPC and ACI for resistant, slow-rusting and susceptible genotypes were 9.42 & 0.28, 196.10 & 5.34 and 846.27 & 25.07 respectively (Table 24).

In individual analysis, 13 genotypes with resistance were found immune and had an AUDPC and ACI of 0 per cent. Remaining, 6 genotypes showed increase in AUDPC and ACI. The slow-rusting genotypes showed large variations in AUDPC and ACI values, indicating phenotypic diversity for slow-rusting resistance. A majority of the slow-rusting genotypes had lower AUDPC and ACI values *rabi* 2012-13. Among 10 susceptible genotypes, Agra Local and Lal Bahadur were showed higher AUDPC and ACI values as shown in the Table 24.

4.4.11.2 Yield loss assessment

The mean grain yield of resistant genotypes were showed there was no significant changes in protected plots (34.09 q/ha) compared to the unprotected plots (33.67 q/ha). Results indicated that there was a little differences in mean yield under protected and unprotected condition for slow ruster (37.28 and 33.18 q/ha respectively) genotypes. Whereas, susceptible genotypes showed significant reduction in yield under unprotected condition as compare to protected condition (27.53 and 36.79 q/ha respectively) (Table 24 & Fig. 15).

The average yield of protected plots were recorded higher in slow ruster (37.28 q/ha) as compare to resistant (34.09) and susceptible (36.79) genotypes. It indicates higher yield potentiality of slow ruster genotypes. Under unprotected condition average yield was significantly higher in resistant

(33.67) followed by slow ruster genotypes (33.18) which were on par with each other. Whereas, lowest was observed in susceptible (25.80) genotypes.

The average yield loss for resistance genotype was very slight 1.57 per cent, whereas, for slow ruster was 10.62 per cent acceptable and susceptible genotypes showed very high (25.80 %) (Table 24). In the individual analyses for each genotype, the yield losses were negligible for most of the resistant genotypes whereas, MACS-6145, VL-943 and UAS-304 resistant genotypes had moderate yield losses. This was not surprising because they were the only genotypes with low to intermediate leaf rust responses when the rest of the resistant genotypes displayed immune responses. In contrast, in the resistant genotype VL-829 even though it was showed immune response, it had a yield loss. In this experiment, yield reductions in the unprotected compared with the protected treatment were negligible for most of resistant bread wheat genotypes. However, higher yield loss was observed in VL-829 bread wheat resistant genotypes that were immune to the *P. triticina* populations.

All slow-rusting genotypes had tolerable yield losses varied from 2.55 to 14.33 per cent. The slow-rusting genotypes HD-2189, HI-977, VL-616 and VL-924 had lower losses than the resistant UAS-304 (10.44 %). Of the 10 susceptible genotypes, the yield loss for Lal Bahadur (66.92 %) and Agra Local (68.89 %) was very high as compare to all other susceptible genotypes (Table 24 & Fig. 15).

The slow-rusting bread wheat genotypes lost less than 14.33 per cent. All the slow-rusting bread wheat genotypes had the lowest AUDPC and ACI values (range of 60.67 to 199.15 and 3.25 to 11.0 respectively) compared with susceptible genotypes.

4.4.11.3 Thousand grain weight loss assessment

The average thousand grain weight (TGW) of protected plots were recorded higher in resistant (36.78 g) as compare to slow ruster (34.48) and susceptible (36.47) genotypes. Whereas, under unprotected condition average TGW was higher in resistant (35.09) followed by susceptible genotypes (32.36). Whereas, lowest was observed in slow ruster (32.20) genotypes.

The mean TGW of resistant and slow rusting genotypes were reduced non significantly, in protected plots (36.78 and 34.48 g respectively) compared to the unprotected plots (35.09 and 32.20 g respectively). However, there was a significant difference in mean TGW under protected and unprotected condition for susceptible genotypes (36.47 and 32.36 g respectively) (Table 24).

The average mean loss of TGW was showed for resistance genotype (4.55 %), slow rusting (6.75 %) and susceptible genotypes (11.62 %) (Table 2). In the individual analyses for each genotype, the TGW losses were negligible for most of the resistant genotypes, whereas, HD-2888, VL-892, PBW-596, VL-829, MACS-6145, RAJ-4083, GW-322 and UAS-304 resistant genotypes had moderate level of TGW losses. Slow-rusting genotypes had TGW losses varied from 1.11 to 14.73 per cent. Of the 10 susceptible genotypes, the TGW loss for Lal Bahadur (15.75 %) and Agra Local (18.89 %) was very high as compare to all other susceptible genotypes (Table 24).

4.5 Management of leaf rust through chemicals

In the present investigation, treatments were composed based on best results obtained in previous year separate experiments on pest and disease management of wheat under the concept of Integrated Pest Management (IPM). Totally 11 treatments including single and combi-products of different pesticides and fungicides were composed to manage both shoot fly and leaf rust. Data on the effect of chemicals in the management of leaf rust has been given more importance and are presented in the Table 29.

4.5.1 Average coefficient of infection at 45 DAS

After the first spurt fortnight, RIL 071/F1 (20%FS) @ 1.5ml/kg seed (Seed treatment alone) and T_1 + Propiconazole @0.1 % two spray (45 & 60 DAS) was most effective among all treatments which acquired only 5 ACI value as compared to T9 and T10 showed 26.67 and 23.33 ACI respectively.

4.5.2 Average coefficient of infection at 60 DAS

After first spray, T4, T5 and T10 showed utmost control of leaf rust with 4.67, 4.33 and 4.67 ACI respectively. These three treatments were statistically on par with each other. The next best treatments are T6 (5.33 ACI) followed by T8 (10 ACI) as given in the Table 29.

Table 28: Genotypic correlations between micronutrients and disease observations

	AUDPC [a]	ACI [b]	Zn (mg/kg)	Fe (mg/kg)	Mn (mg/kg)	Mg (mg/kg)	Cu (mg/kg)
AUDPC [a]	1.0000						
ACI [b]	0.9978**	1.0000					
Zn (mg/kg)	-0.0149	-0.0288	1.0000				
Fe (mg/kg)	-0.1987	-0.1852	0.0306	1.0000			
Mn (mg/kg)	-0.0005	0.0088	0.4185**	0.1367	1.0000		
Mg (mg/kg)	-0.0660	-0.0530	0.3725*	-0.1601	0.3096*	1.0000	
Cu (mg/kg)	-0.3430*	-0.3530*	0.3720*	0.2382	0.1126	-0.1798	1.0000

[a] AUDPC = Area Under Disease Progress Curve of 2011.
[b] ACI=Average co-efficient of infection of 2011.

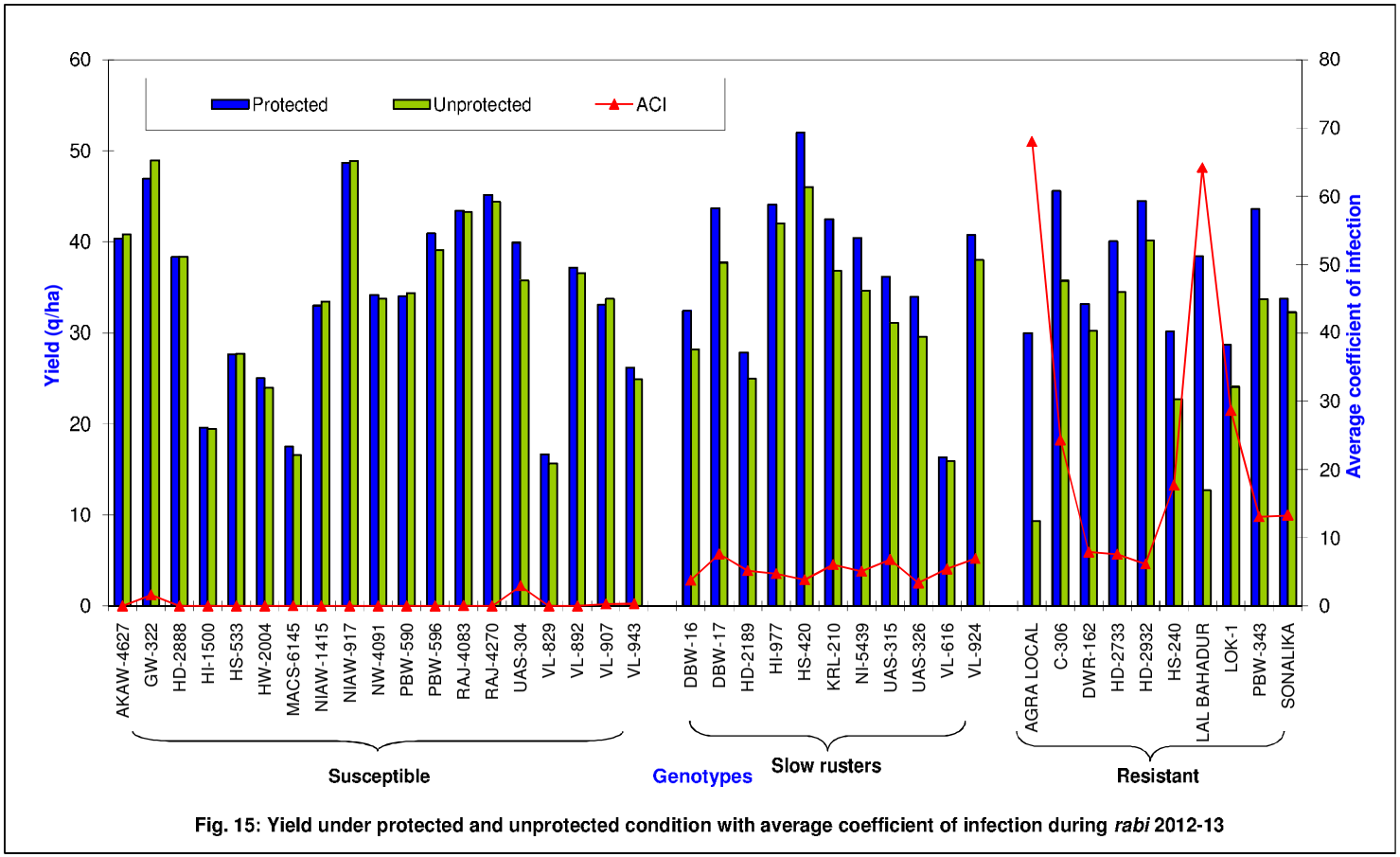


Fig. 15: Yield under protected and unprotected condition with average coefficient of infection during *rabi* 2012-13

Fig 15: Yield under protected and unprotected condition with average coefficient of infection during *rabi* 2012-13

Table 29: Effect of chemical treatments on leaf rust severity, thousand grain weight and yield during *rabi* 2012-13

Trt. No.	Treatment Details	Average co-efficient of infection				Yield (q/ha)	TGW (g)
		45 DAS	60 DAS	75 DAS	Mean		
1	RIL 071/F1 (20%FS) @ 1.5ml/kg seed (Seed treatment alone)	5.00 (2.45)	36.67 (6.14)	43.33 (6.66)	28.33 (5.42)	10.76	20.84
2	RIL 071/F1 (20%FS) @ 2ml/kg seed (Seed treatment alone)	6.67 (2.77)	40.00 (6.40)	50.00 (7.14)	32.22 (5.76)	13.37	21.38
3	Imadachloprid 600FS @ 0.77ml/kg seed (Seed treatment alone)	6.67 (2.77)	46.67 (6.90)	56.67 (7.59)	36.67 (6.14)	14.61	22.18
4	T_1 + Propiconazole @0.1 % two spray (45 & 60 DAS)	5.00 (2.45)	4.67 (2.38)	4.67 (2.38)	4.78 (2.40)	31.20	24.35
5	T_2 + Propiconazole @0.1 % two spray (45 & 60 DAS)	6.67 (2.77)	4.33 (2.31)	4.00 (2.24)	5.00 (2.45)	26.61	24.06
6	T_3 + Propiconazole @0.1 % two spray (45 & 60 DAS)	13.33 (3.79)	5.33 (2.52)	5.67 (2.58)	8.11 (3.02)	22.48	22.72
7	Pyraclostrobin 13.3% + Epoxiconazole 5% (Opera 18.3% SE) @ 0.1 % two spray (45 & 60 DAS)	16.67 (4.20)	10.00 (3.32)	9.33 (3.21)	12.00 (3.61)	28.56	26.70
8	T_3 + T_7	6.67 (2.77)	10.00 (3.32)	8.67 (3.11)	8.85 (3.07)	32.85	27.31
9	Chloropyriphos 20%EC @ 3ml/kg seed (Seed treatment alone)	26.67 (5.26)	46.67 (6.90)	73.33 (8.62)	48.89 (7.06)	9.91	21.65
10	Propiconazole @0.1 % two spray (45 & 60 DAS)	16.67 (4.20)	4.67 (2.38)	4.67 (2.38)	8.67 (3.11)	24.00	23.49
11	Control	23.33 (4.93)	46.67 (6.90)	100.00 (10.05)	56.67 (7.59)	9.30	22.79
	Mean	12.12 (3.62)	23.24 (4.92)	32.76 (5.81)	22.71 (4.87)	20.33	23.41
	SEm±	0.35	0.27	0.23		1.65	0.83
	CD at 5%	1.04	0.80	0.67		4.86	2.43

DAS: Days after sowing, TGW: Thousand grain weight,
Data in the parenthesis are square root transformed values ($\sqrt{X+1}$)

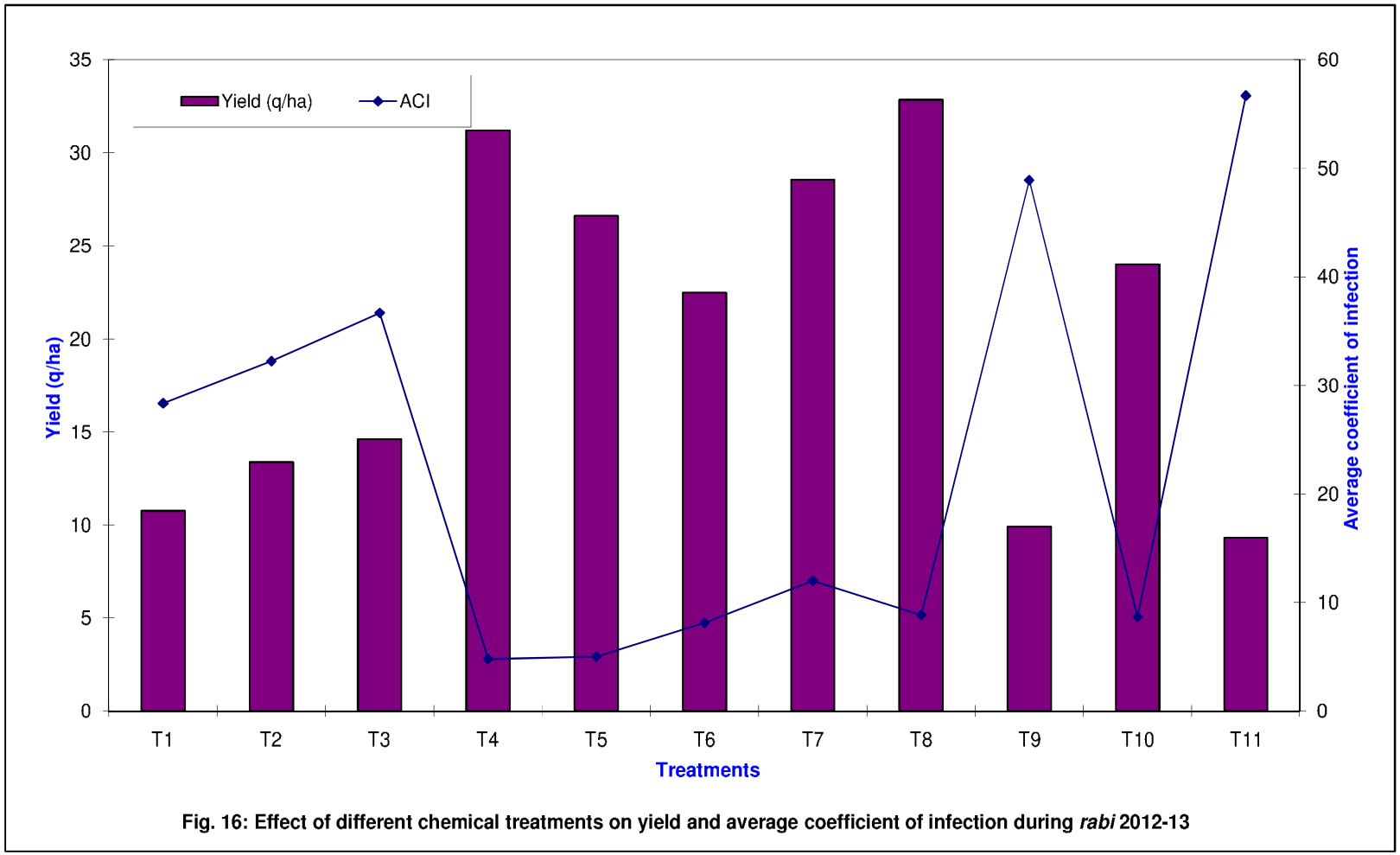


Fig. 16: Effect of different chemical treatments on yield and average coefficient of infection during *rabi* 2012-13

Fig 16: Effect of different chemical treatments on yield and average coefficient of infection during *rabi* 2012-13

4.5.3 Average coefficient of infection at 75 DAS

After the second spray, T5, T4 and T10, showed least leaf rust (4.00, 4.67 and 4.67 ACI respectively) which was followed by T6 (5.67 ACI) and T8 (8.67 ACI). The highest ACI was recorded in the control treatment (100.0) which was followed by T9 (73.33). However, on an average, in different category of chemical treatments, ACI was least in the T4 (4.78) followed by T5 (5.00) and T6 (8.11). The highest average ACI was observed in T11 (control treatment) of 56.67 (ACI) followed by T9 (48.89).

4.5.4 Yield and thousand grain weight

The data on effect of leaf rust and different treatments on yield and 1000 grain weight are presented in the Table 29 and the graphical representation of yield and ACI were given in Fig. 16.

Among all the treatments T8 (Seed treatment with Imadachloprid 600FS @ 0.77ml/kg of seed followed by Pyraclostrobin 13.3% + Epoxiconazole 5% (Opera 18.3% SE) @ 0.1 % two spray between 45 & 60 DAS) was obtained with highest yield (32.85 q/ha) followed by T4 (seed treatment with RIL 071/F1 (20%FS) @ 1.5ml/kg of seed followed by Propiconazole @ 0.1 % two spray between 45 & 60 DAS) with yield of 31.20 q/ ha and T7 (Pyraclostrobin 13.3% + Epoxiconazole 5% (Opera 18.3% SE) @ 0.1 % two spray between 45 & 60 DAS) given 28.56 q/ha which were on par with each other. The lowest yield was recorded in control (9.30 q/ha) followed by T9 (seed treatment of Chloropyriphos 20%EC @ 3ml/kg seed) given 9.91 (q/ha) and T1 (seed treatment with RIL 071/F1 (20%FS) @ 1.5ml/kg seed) obtained 10.76 (q/ha). The next least yield was obtained by T2 (seed treatment with RIL 071/F1 (20%FS) @ 2ml/kg seed) of 13.37 (q/ha). The treatments *viz*., T9, T2 and T1 are on par with control.

Maximum thousand grain weight was recorded in the T8 (27.31 g) followed by T7 (26.70 g) which were statistically on par with each other. The next highest was in T4 (24.35 g) which showed statistically on par with T7 and T5 (24.06 g). The lowest thousand grain weight was recorded in T1 showed 20.84 g followed by T2 (21.38 g).

In the present study the best alternative to the propiconazole in the management of leaf rust was identified based on observing lower disease score (ACI), highest yield and highest thousand grain weight. The fungicide combination Pyraclostrobin 13.3% + Epoxiconazole 5% (Opera 18.3% SE) @ 0.1 % two spray between 45 and 60 DAS is the best alternative to the propiconazole.

DISCUSSION

Wheat commonly known as "World's Hunger Eradication Active Tool" is been accorded a premier place among the cereals. Bread wheat is an important cereal food crop in the world. This cereal as the axiom comes true that "necessity is the mother of invention" in the way that this cereal converted the ancient food gatherers and hunters into the modern, well organized and civilized human beings in the course of evolution. The cultivation of wheat dates back to farther antique era in the history of civilization and evolution. The wheat crop as a second most liked and pin food of the human beings was attacked by a number of innate pathogens since time eternal which, dates back to epoch of Babylonians, Egyptians, Greece and Roman Emperors. Among the most important pathogens of wheat three rusts *viz.,* stem rust, leaf rust and stripe rust caused cruel losses in the production of this cereal in the history. Among these three rusts the leaf rust is of primary in importance and reign right through the world.

India is the second largest producer of wheat next to China. India, owing to its diverse climatic conditions prevailing throughout the country, suffers more or less of all the three rusts. The entire country offers the good climatic conditions for the occurrence and spread of the leaf rust while the Nilgiri and Palani hills in the south acts as the stockpile for inoculated of stem and leaf rust throughout the country as well as the primary source of inocula for conception of epiphytotic conditions. Whereas, in northern India, the foothills of Himalaya conserve the infective primary spores of brown and stripe rust to serve in the northern parts of the country. In south, due to its high prevailing temperature and humid conditions, favors the high temperature requiring innate pathogens of the wheat crop such as stem and leaf rust while, North owing to its cooler environmental conditions and fluctuating temperature during the month of January-March let the stripe rust and leaf rust of wheat to cause disease in the susceptible wheat crop.

5.1 Survey, surveillance and race identification of leaf rust in wheat growing region of Karnataka

The rusts are responsible for the considerable damage to the wheat crop. The losses caused due to rusts vary from region to region. In the present investigation survey and surveillance for leaf rust incidence was carried out in off-seasons and also in regular seasons of 2010-11, 2011-12 and 2012-13.

The study on the variations and identification of physiologic races of leaf rust was carried out with a view of assisting plant breeders in development of rust resistant wheat varieties. This information will help the plant breeders to develop resistant varieties against prevailing physiologic races of leaf rust and also the resistant genetic stock could be maintained for further use in the breeding programme.

Kulkarni (1984), Navi (1986) and Hegde (1991) reported off-season wheat of Chikmagalur and Chitradurga districts of Karnataka are important source of infection. Since, this area serves as a secondary focus of infection of rusts in addition to primary foci such as Nilagiri and Palni hills of Tamil Nadu. Kulkarni (1984) recommended to ban the cultivation of wheat in these areas during off-season. In contrast to above findings, the off-season (*kharif* 2010-11 and 2011-12) survey revealed that, there was no wheat crop in the surveyed area because with the introduction of irrigations, farmers were growing commercial crops *viz.,* chilli and onion etc., the practice of growing wheat in off-season was stopped. In the interview and thorough discussion with farmers, fertilizer dealers, private company field assistants (Bayer crop science India Ltd., MCF Ltd. and Syngenta India Ltd.) and concerned Govt. department officials, it came to know that, 20 years before farmers were growing off-season wheat on a small scale. But later it was discontinued because Government of Karnataka has banned cultivation of off-season wheat in the area. This result is on par with study conducted by Hasbanis (1998), who reported that, the practice of growing the wheat in off-season was discontinued to some extent since it is being banned for cultivation by the Govt. of Karnataka.

The normal season survey revealed that, leaf rust was comparatively more severe during *rabi* 2012-13. The mean higher temperature of *rabi* 2010-11 had influence on less incidence of leaf rust on rainfed and comparatively lower severity on irrigated wheat too and adopted to many unrecognized components in a particular environment. The component of parasitic population may be genetically competent however their reproductive potential will also be determined by temperature, humidity and other factors (Bahadur, 1986). During all the years severity was different from one location to other but marked variation were noticed among the host varieties. The recently released durum wheat

variety *viz.,* UAS-415 also showed leaf rust disease severity of 5S-10S. The variation in severity of infection was due to change in weather parameters in different locations. Nagarajan and Joshi (1985) also concluded that once the leaf rust of wheat appears, subsequent development is dependent on prevailing local weather conditions *viz.,* temperature and relative humidity.

Mehta (1941) initiated the research on cereal rusts and systematic virulence analysis in India during 1923 and 1931 respectively. In the present investigation, when virulences were detected by following new system proposed by Nagarajan *et al*. (1983) revealed that, the group 77 was dominant over the surveyed area. Kulkarni (1978, 1979a) reported the high frequency of race group 77 and 162 from Karnataka State. The pathotype 121R63-1 (77-5) had expressed maximum mean frequency of 41.12 per cent over the three seasons. This clearly indicated that still farmers are growing DWR-162 extensively in Karnataka may be because of quality food product prepared out of DWR-162 was excellent. The pathotype 121R63-1 (77-5) is a matching virulence of combined genes *Lr* 23 + *Lr* 26 (Nagarajan and Murlidharan, 1995). Hasabnis (1998) surveyed different districts in Karnataka and Maharastra states during 1996-97 and 1997-98 for virulence monitoring. He reported that, pathotypes from group 77 were widely distributed in surveyed areas. The extensively cultivated wheat variety DWR 162 has *Lr* 23 + *Lr* 26 gene combination conditioning vertical resistance. This is in confirmation with the host-pathogen interaction concept proposed by Van der Plank (1968). Similarly virulence of *P. recondita* f. sp. *tritici* and cultivar relationship in Texas from 1985 to 1987 has been explained by Marshall (1989). Existence of variability in rust pathogens makes it necessary to have an effective system of virulence analysis. It provides an important clue for the management of rust resistance genes by way of their efficient deployment. Leaf rust virulence survey since many decades reveals that, the population is highly variable leading to the evolution of new pathotypes through mutation and rarely through somatic hybridization (Bhardwaj *et al*., 2005).

With the introduction of more and more rust resistant varieties, the quality and productivity of wheat is often reduced because of leaf rust caused by *Puccinia recondita* f. sp. *tritici*. The extent of yield losses caused by leaf rust depends on the nature and number of leaf rust resistance genes in the cultivars and the composition of virulence in the respective geographical region (McIntosh *et al.,* 1995).

5.2 Study of genetic diversity in *Puccinia triticina* population of Karnataka through molecular techniques

All the isolates of *P. triticina* that were tested produced a unique pattern of EST-SSR alleles confirming the high genetic diversity within populations of the leaf rust fungus. This was in agreement with the statement of Bhardwaj *et al*. (2006 & 2011), who opined that, leaf rust of wheat is the most widely distributed and normally causes more losses than any other rust of wheat in India. It is observed in all the wheat growing areas. Because of its widespread occurrence in India and it is probably the most variable pathogen in India. Kolmer (2013) reviewed that cereal rust fungi are highly variable for virulence and molecular polymorphism. Leaf rust is the most common rust of wheat on a worldwide basis. Many different races of *P. triticina* that vary for virulence to leaf rust resistance genes in wheat differential lines are found annually in the US. Molecular markers have been used to characterize rust populations in the US and worldwide.

The molecular diversity studies on *P. triticina* are limited in India. It may be because of inability to culture *P. triticina in vitro* and the relatively large genome size estimated to be 100–124 Mbp (Eilam *et al*., 1994).

In the present study three phenotypically known races (12-4, 77-5 and 77-6) were grouped under single cluster. It reveals that, even they were phenotypically identified as dissimilar but, showed genetic similarity when checked by using EST-SSR molecular markers. This was in agreement with following studies. Molecular markers such as RAPDs and AFLPs have been used to characterize variation in *P. triticina* populations. Different groups of *P. triticina* isolates in Canada and from international collections could be distinguished with RAPD and AFLP markers that also correlated with grouping based on avirulence/virulence to single gene differential lines (Kolmer, 2001; Kolmer and Liu, 2000). In Europe, multiple isolates of the same race from different countries had identical RAPD banding patterns (Park *et al*., 2000). Wang *et al*. (2010) developed gene-associated simple sequence repeat (SSR) markers for *P. triticina* through the data mining of existing EST libraries and they analysed of 7134 expressed sequence tags (ESTs) from cDNA libraries of *P. triticina* detected 204 EST-SSRs with a minimum of 12 repeating nucleotides. These EST-SSRs were evaluated on 35 *P.*

triticina isolates collected in Canada and 21 EST-SSRs were polymorphic and informative in determining intraspecific genetic diversity.

In the similarity co-efficient study, stated that, high degree of similarity between the isolates which were collected from different wheat growing areas shows common distribution of similar pathotypes in those areas. This was in agreement with studies conducted by Ordonez and Kolmer (2007), observed high degree of virulence and simple sequence repeat (SSR) genotypic similarity between *P. triticina* isolates from durum wheat in Europe, South America, Mexico and California suggested that *P. triticina* populations on durum wheat in these regions originated from a single original founder population.

The high degree of similarity for SSR genotype of isolates from both South America and North America suggested a common European origin of *P. triticina* that was introduced to both continents. The emergence of the same *P. triticina* virulence phenotypes with highly related SSR genotypes in the United States in 1996 and in Uruguay in 1999 indicated the likely intercontinental migration of these genotypes from Mexico to both South America and North America (Ordonez *et al*., 2010).

In the present investigation EST-SSR markers were used to study the genetic dissimilarity as they identified as co-dominant and can distinguish between heterozygote and homozygote genotypes, in contrast to RAPD and AFLP markers that are dominant. Similar opinions were given by following researchers working on diversity study of *P. triticina* across the world. Recently, locus-specific microsatellite or SSR markers have been developed for *P. triticina* (Duan *et al*., 2003; Szabo and Kolmer, 2007). SSR markers are co-dominant and can distinguish between heterozygote and homozygote genotypes, in contrast to RAPD and AFLP markers that are dominant. *P. triticina* populations studied with SSR markers have several attributes in common, including higher levels of heterozygosity than expected compared with populations in Hardy–Weinberg equilibrium and high levels of linkage disequilibrium, and they are genetically differentiated by continental region (Kolmer and Ordonez, 2007) or due to selective effects of resistance genes in wheat cultivars (Goyeau *et al*., 2007). All populations of *P. triticina* that have been examined with SSR markers (Goyeau *et al*., 2007; Kolmer and Ordonez, 2007) have genetic characteristics typical of clonal diploid or dikaryotic populations in which high levels of heterozygosity are maintained by sequential mutation in the absence of recombination (Balloux *et al*., 2003; Halkett *et al*., 2005). Somatic recombination has been reported in *P. triticina* (Park *et al*., 1999), although the rates of such variation remain unknown.

Singh *et al*. (2011) reported the abundance and inherent potential for extensive allelic variations in simple sequence repeats (SSRs) or microsatellites resulted in valuable source for genetic markers in eukaryotes. They analyzed and compared the abundance and organisation of SSR in the genome of two important fungal pathogens of wheat leaf rust (*P. triticina*) and stem rust (*P. graminis* f. sp. *tritici*). *P. triticina* genome with two fold genome size as compared to *P. graminis* f. sp *tritici* has lower relative abundance and SSR density.

5.3 Identification of slow leaf rusters

Leaf rust caused by *P. triticina* appropriate to its surviving nature in ample range of climatic conditions appears to be the most important pathogen in the country as well as in the world. Pathologists, breeders and scientists, apprehensive with the management of this pathogen were attentive of the fact that due to its wide spreading nature it is cumbersome and very tricky to manage the disease and avoid epiphytotic conditions in the country by the use of expensive and perilous chemicals which, are the most important deterrent measure in the management strategy. The trend to look for resistant sources against leaf rust running up to the current epoch and will be continued as the increase in the world population and diversity of the pathogen to overcome the resistant genes. The current strategy to combat this ever evolving pathogen is by breeding for durable leaf rust resistance and needs constant efforts to identify new sources of durable rust resistance genes as well as genotypes.

The search and use of genotypes with less terminal leaf rust severity is an important practice to manage the obligate parasites. The slow rusters which are characterized by longer latent period, slow development of leaf rust, lesser number of uredia per unit area of leaf, lesser size of uredia and lesser value of AUDPC which, ultimately results in low terminal disease severity. The low terminal disease severity append in the yield of slow rusters because more leaf area is available for photosynthesis during the peak growth period.

The experiment was conducted to identify slow rusters among selected genotypes of bread wheat during *rabi* 2011-12 and 2012-13. The results revealed that Agra Local, Lal Bahadur, Lok-1,

Sonalika, C-306, DWR-162, PBW-343, DBW-17 and HS-240 were identified as fast rusters, whereas, 34 genotypes were displayed immune to resistant reaction to the prevailing pathotypes of *P. triticina* during the course of investigation. Ultimately by considering two year data and all the studied slow rusting components, it could be possible to identify genotypes, UAS-326, UAS-315, VL-616, VL-924, HD-2189, HD-2932, HD-3091, NI-5439, HI-977, HS-420, DBW-16, KRL-210, Pavon-76, RL-6077 and Parula as slow leaf rusters with low terminal disease severity, less rate of disease development, minimum values of AUDPC, longer latent period, lower pustule density and smaller size of uridinium (Table 14, 15, 16 and 17). These genotypes were categorized based on highest scores of slow rusting components obtained during both the years of investigation. Results of slow rusting components were discussed hereunder.

During *rabi* 2011-12, among 40 bread wheat genotypes, Agra Local (29.00 ACI) and Lal Bahadur (27.00 ACI) were first to show high leaf rust incidence and later it reached to maximum of 100 and 98.00 respectively. The fifty per cent of (20) genotypes showed zero ACI. Ten genotypes showed medium range of ACI between 0.25 to 22.36. The genotype HD-2189 showed 6.3 ACI at the end. During the *rabi* 2012-13 the overall disease development was higher as compared to previous year and it is indicated in the Table 15. This may be due to the weather which is strongly influencing the disease development as it is one of the disease triangle components (Stevens, 1960). Among 57 bread wheat genotypes sown, the first observation at 54 days after sowing on two susceptible checks, Agra Local (27 ACI) and Lal Bahadur (16.00 ACI) showed very high leaf rust incidence followed by Lok-1(10.40), Sonalika (7.6), HS-240 (7.10) and Pavon-76 (5.92). Agra Local and Lal Bahadur reached highest final disease score as 100 and 99 ACI respectively. Totally, 21 genotypes showed zero ACI with immune to resistant reaction (Table 15).

The lowest ACI was observed at 82 days after sowing in RAJ-4083 (0.25) and VL-907 (0.38) followed by VL-943 (0.85) and GW-322 (2.54) which were on par with RAJ-4083 and VL-907. The next lowest ACI at last disease score was recorded by HI-977, UAS-315, KRL-210, NI-5439, DBW-16, HD-2733, HS-420, MACS-2496, HD-3091, Pavon-76, RL-6077 and Parula which were statistically on par with earlier known slow rusting genotype, HD-2189 (9.48 ACI). Similar tread was observed in the AUDPC value as it was calculated by using ACI values shown in table 16 and 17 for both the years (during 2011-12 and 2012-13 respectively). This result reveals two possibilities and is different in slow and fast rusters. With the advancement of leaf rust, availability of green tissue for further disease development went on diminishing in fast rusters. In case of slow rusters, inbuilt defense mechanism was activated with the advance of infection and restricted the further spread of the pathogen in host tissues.

The results are in agreement with the concepts proposed by Wilcoxson *et al*. (1975) for values of AUDPC. Meenakumari *et al*. (1994) reported high values of AUDPC and no logarithmic leaf rust development in Kalyanasona, Lal Bahadur and WL-711. Navi (1986) found that, HD-2189 and Kiran (DWR-137) as slow rusters. Hasabnis *et al.* (2002) and Thombare (1981) reported similar results regarding ACI and AUDPC values. Similar results revealed by Nargund (1989) identified DWR-39, HD-2189, HI-977, Keerti, Sonalika, WH-147 and WH-416 as slow leaf rusters on the basis of values of AUDPC. Navi (1986) identified C-464, DWR-39, HD-2189 and DWR-16 as slow rusters. Hasabnis and Srikant Kulkarni (2002) identified HD-2189 as slow ruster based on AUDPC values. Sabharwal (1986) identified Lal Bahadur and WH-711 as fast rusters and Arjun as slow ruster based on AUDPC and 'r' values. Prabhu *et al.* (1993) reported that among the wheat varieties S-57, S-69 and HB-208 expressed stable slow rusting resistance, as measured by AUDPC. Ten wheat cultivars, namely B. Yellow, C 306, GW 173, HD 2189, HD 2501, HD 2687, K 8962, PBW 396, Sujata and WH 542, had AUDPC values ranging from 10.00 to 77.50 and rate of infection from 0.00 to 0.12 units per day. Considering lower terminal disease score, smaller values of AUDPC and slow rate of infection as good donors of desirable and durable leaf rust resistance (Hasabnis *et al.,* 2003; Patil *et al.,* 2005). Susceptible genotypes had higher AUDPC range which were being cultivated by many years and hence, the resistance has broken by ever evolving pathotypes of *P. triticina* and therefore they depicted higher AUDPC values. Similar results were obtained by Priyamvada *et al*. (2009)

Patidar *et al.* (2007) characterized that genotypes HD-2189, HW-2021 showed the typical characteristics of slow rusters. These genotypes recorded low ACI, low AUDPC, considerable more latent period, medium pustule size, pustule density and lesser rate of infection with good quantity of yield per hectare. However, genotypes DWR-195, DWR-162 and MACS-2496 recorded maximum ACI and relevant adverse values compared to slow leaf rusting genotypes and identified as fast rusters.

In general, the genotypes with a stumpy initial leaf rust severity or delayed onset of the leaf rust invariably wrecked up with a low terminal disease severity and *vice-versa*. The terminal ACI was

lesser in the genotype which showed later appearance of the disease and also depended on the presence and type of resistant genes.

The maximum 'r' value were noticed in DWR-162 (0.17), Agra Local (0.12) and Lal Bahadur (0.12 units per day) between first two observations of the disease development. The HD-2189 showed 0.08 'r' value in first two interval but shown 0.09 and 0.04 units per day at second and third interval subsequently it was reduced to 0.02 unit per day. More or less similar trend was observed by HI-977, NI-5439, GW-322, UAS-315, VL-616, and HS-240 during 2011-12 (Table 16). The peak average 'r' value was observed in the genotype DWR-162 (0.14) followed by HI-977, LOK-1 and Sonalika with 'r' values 0.12, 0.10 and 0.08 respectively. The least average 'r' value was noticed in the genotype RAJ-4083 (0.01) followed by NI-5439 (0.02) and KRL-210 (0.02). During 2012-13 maximum 'r' value were noticed in KRL-210 (0.32), C-306 (0.27) and PBW-343 (0.18 units per day) between first two observations of the disease development. After r1 there was steady increase in 'r' value observed in HD-2189. Whereas, decrease in 'r' value was also observed in VL-924, C-306, NI-5439, DBW-17, MACS-2496, RL-6077 and Parula.

The rate of disease development 'r' is calculated by using formula given by Van der Plank (1963). The genotypes not show any relation between 'r' value and slow rusting nature. This results were supported by Wilcoxson *et al*. (1975), who pointed out that, 'r' values are not useful, as AUDPC values in studying the disease development. And also similar results were obtained by Nargund (1989). Patidar *et al.* (2007) showed that, lowest initial rate of infection was recorded in the genotype HW-2021 (0.04 units per day) followed by HD-2189 (0.058 units per day) between 40 and 47 DAS. Whereas, mean rate of infection was lowest in the genotype DWR-195 (0.035 units per day) followed by HW-2021 (0.040 units per day) and GW-344 (0.042 units per day). Similar results were obtained by Statler *et al.* (1977b) and Gupta and Singh (1982).

Mackenzie (1976) proposed application of slow rate of increase in stem rust of Bonaza 55 to the identification of slow rusting mechanism. The cultivars with lower infection rate, fewer pustule number, pustule area and lower number of uredospore per unit area are slow rusters (Sharma and Gupta*.,* 1986). Hasabnis *et al.* (2002) reported cultivars CPAN 4011, K 9324, UP 2358, CPAN 4059, YCBE-13 and K 9305 showed low 'r' and AUDPC values.

The results revealed that the slow rusting genotypes showed longer latent period, produced less number of uredia per square centimeter of leaf with a lesser pustule size and lesser terminal leaf severity. Longer latent period of slow rusting cultivars may be effective in reducing the rate of rust development (Johnson, 1980). The longest latent period recorded in the genotype GW-322 in both the years with pustule size of 4.95 x10 μm^2 coupled with less pustule density (0.05 per cm^2). Latent period was always found to be longer for all the cultivars in adult plant stage than in seedling stage (Kapoor, 1979) whereas, among slow rusting ones recorded a latency period of 27.00 to 29.63 days with a pustule density of 0.01 and 1.54 pustules per square centimeter. Cultivars with longer latent period, less production of uredospores per unit area, pustule size rusted slowly (Sokhi and Singh, 1984).

Kapoor and Joshi (1981) reported that Sonalika, produced comparatively fewer flecks and pustule number per centimeter of leaf area than Agra Local. The latent period for Sonalika was longer by one to two days than for Kharchia and Agra Local. Leaf rust developed more slowly on cultivar that showed longer latent period, smaller and fewer uredia (Shaner and Finney, 1980; Sareen *et al*., 2012).

Lehman and Shanner (1996) revealed that in simulated epidemics, isolates with short latent period caused two to two and half times more disease and overcame 13 to 35 per cent of the resistance of four partially resistant cultivars. Cultivar 'Kundan' produced lower pustule number, uredial size and longer latency period. These measures can be used as direct parameters for identifying slow rusting genotypes (Ahmad and Singh., 2003). Marina and Putnik-Deliac (2009) observed that correlation coefficient between latent period, reaction types, infection frequency and AUDPC values, was 0.828.

The results regarding yield and thousand grain weight revealed a complex picture about the slow rust resistance cultivars. It is generally expected that the decrease in disease there is significant increase in the yield of the genotype but data revealed that there is no relationship due to varietal characters and specific weather requirement as one of the Mexican genotype, RL-6077 even though displayed slow rusting mechanisms but it did not given any yield. Yield data showed reductions in yield to a greater extent, in fast rusters than the slow rusters. The increased thousand grain weight

was correlated with less intensity of the disease among the genotype evaluated (Table 15). However, the yield and thousand grain weight differed irrespective of genotypes.

It was noticed that dwindle in the average coefficient of infection (ACI) was optimistically correlated with a swell in the thousand grain weight. The yields of different genotypes differ with each other appreciably.

These results were in agreement with many earlier workers on slow rusting resistant bread wheat genotypes and their yield potentiality (Navi, 1986; Nargund, 1989; Hasabnis, 1998; Patidar *et al*., 2007).

Khan *et al.* (1997) revealed that the cultivars with moderately slow rusting nature losses the yield ranged from 8 to 19 per cent irrespective of cultivars. Although cultivars showing fast rusting responded as rust tolerant with respect to yield loss. AUDPC was highly correlated with the yield (Buchenau, 1975).

5.3.1 Seedling reaction test and gene postulation

The identification of resistance genes and their relationship with known genes has great relevance to plant breeders their endeavor to breed the varieties for greater stability. The infection type conditioned by the gene to particular pathotype is useful in identifying resistant genes. Nagarajan *et al*. (1984) identified the various types of genes by selecting appropriate pathotypes by matching techniques in large number of wheat genotypes. McIntosh *et al*. (1995) catalogued resistance genes to three rusts of wheat. Nayar *et al*. (1988) postulated *Lr14a* in HUW 12 and *Lr10* in WH 322 varieties. Total nine known and one unknown gene was postulated singly or in combinations from CIMMYT germplasm by Singh and Gupta (1991). Twenty commercially grown varieties of wheat under study possessed *Lr13*, *Lr23*, *Lr24*, *Lr26* and *Lr34* genes singly or in combinations. The leaf rust resistance genes *Lr26* as singly as in combinations with *Lr10*, *Lr13* and *Lr23* were detected at higher frequency from 36 wheat lines developed at Dharwad (Hasabnis, 1998).

In the present investigation with the inoculation of different pathotypes and by matching the infection types of *Lr* isogenic lines, *Lr* genes were postulated from 41 bread wheat genotypes collected across India. The genes *viz*., *Lr1*, *Lr10*, *Lr13*, *Lr23* and *Lr26* in various combinations were identified (Table 18). Among the single gene or in combinations with *Lr26* was observed in highest frequency among Indian bread wheat genotypes. High frequency of presence of *Lr26* in Indian bread wheat has been reported by Nayar *et al*. (1991), Bahadur *et al*. (1995) and Bharadwaj *et al*. (1996).

Kiyar (2002) postulated *Lr* genes from the varieties *viz*., DDK1001, DDK1019 and DDK1022 were found to carry gene combination of *Lr23*+*Lr26*, *Lr23*+*Lr26,* and *Lr10*+*Lr26* respectively with an unknown additional gene. Whereas, DWR 225 carries *Lr26* with an unknown additional gene.

In the most of the gene postulated, the *Lr* genes were found in two or three gene combinations. This is in agreement with the statement of Samborski and Dyck (1982), who reported several cases in which pairs of genes for leaf rust resistance in Thatcher background had cumulative effect. They emphasized the value of combining genes in the disease resistance wheat breeding programme. Thus, the present study have *Lr* genes *viz*., *Lr1, Lr10, Lr13, Lr23, Lr24, Lr26* and *Lr28.* The frequency of *Lr26* was found to be considerably at higher level.

Nagarajan and Muralidharan (1995) cited Indian experience on the host induced evolution of pathotypes of *P. recondita* f. sp. *tritici* over last two decades. At least six pathotypes from group 77 have been intercepted by gaining or losing the virulence for *Lr20*, *Lr23* and *Lr26*. Thus, it is necessary to trap the new sources of diversified resistance against evolving complex pathotypes. Mebrate *et al*. (2008) stated that gene postulation helps to undertake a quick identification of the probable leaf rust resistance genes (*Lr* genes) present in a large number of wheat cultivars at a time. To identify the race-specific *Lr*-genes present in 36 wheat cultivars from Ethiopia and Germany. Seventy-six wheat genotypes, including 40 near-isogenic lines (NILs), were tested against 31 isolates of *P. triticina* isolates collected from both countries. *Lr*-genes *Lr1*, *2c*, *3*, *3ka*, *9*, *10*, *14a*, *14b*, *13*, *16*, *18*, *21*, *23*, *27+31*, *30*, *37*, and *44* were postulated to be present in the Ethiopian wheat cultivars. *Lr* genes *Lr9*, *20*, and *21* were present in the German wheat cultivars. The *Lr*-genes present in some wheat cultivars could not be postulated because of non-matching virulence combinations with any of the NILs. .

Li *et al*. (2010) documented fourteen leaf rust resistance genes *Lr1, Lr2a, Lr3bg, Lr3ka, Lr14a, Lr16, Lr17a, Lr18, Lr20, Lr23, Lr24, Lr26, Lr34,* and *LrZH84* either singly or in combinations

from 65 genotypes and also resistant gene *Lr26* was present in 44 accessions of Chinese bread wheat.

In the present investigation, seventeen *Lr* isogenic lines were tested against 13 pathotypes of leaf rust at seedling stage. At seedling stage *Lr9* and *Lr19* were not matched by any of the pathotypes tested in the study. The group 77 had higher frequency for virulence, of which 77-5 had maximum of 82.4 per cent. The minimum was noticed in 12-9 as of 47.1 per cent. The *Lr9* and *Lr19* have been identified as resistant sources universally (Reddy, 1974; Kulkarni, 1979; Shinde, 1987; Singh and Rajaram, 1991 and Hasabnis, 1998). Saini *et al*. (1993) concluded that, *Lr1*, *Lr10*, *Lr13*, *Lr14a*, *Lr17*, *Lr23* and *Lr26* genes were not useful singly or in combination against pathotypes *viz*., 77A, 77-1 and 77-2. But *Lr3*, *Lr16* and *Lr34* genes confirmed resistance against leaf rust. Sawhney *et al*. (1992) studied genetic diversity to leaf rust in near isogenic lines and discussed the possible presence of *Lr34* gene in Indian bread wheat varieties and its role in durable resistance. Singh *et al.* (2004a) evaluated 44 cultivars and lines of wheat for resistance to leaf rust. They found that 14 wheat lines showed seedling resistance, while 30 cultivars/lines showed seedling susceptibility to race 77-5. The 14 wheat lines possessing seedling resistance. Cultivars with seedling resistance gene combinations including *Lr16* or single genes *Lr47* (detected with molecular marker), *Lr19* and *Lr41*, showed high levels of resistance against all 17 different pathotypes of leaf rust collected from Argentina (Vanzetti *et al*., 2011).

Abdelbacki *et al*. (2013) observed the most frequently occurring gene in ten Egyptian wheat cultivars was *Lr35* (70%), followed by *Lr22 (60%), Lr27 (40%), Lr34 (30%), Lr19 (30%), Lr18 (10%), Lr36* (10%) and *Lr46* (10%), eight out of sixteen *Lr* genes were not present in the tested cultivars. Four genes; *Lr28*, *Lr24*, *Lr34* and *Lr19* were confirmed using molecular marker. It is concluded that there was a good variation in *Lr* genes carried by wheat cultivars commercially grown in Egypt.

5.3.2 Genetics and molecular characterization of bread wheat varieties for durable/slow leaf rusting resistance

The adult plant resistance gene *Lr34* has been reported in many genotypes of Indian origin on the basis of leaf tip necrosis and AUDPC scores (Singh and Gupta, 1991; Saini *et al*., 1998; Bahadur, 1998).

In the present study, STS marker (*csLV34*) was used and has given positive for *Lr34* and which is highly linked marker given consistent results than other markers used. Earlier works in the 7DS chromosomal region demonstrated the close genetic linkage of *Lr34* with SSR (*XGwm130* & *XGwm295)* and EST *(cdo475, bf473324* & *be493812)* markers (Suenaga *et al.,* 2003; Schnurbush *et al.,* 2004; Spielmeyer *et al.,* 2005). Lagudah *et al*., 2006 got success in conversion of the RFLP to a co-dominant sequence tagged site (*csLV34*), revealing bi-allelic locus in which 79 bp insertion in an intron sequence was with cultivars that lacked *Lr34/Yr18*.

Upon comparing the AUDPC values, genotypes found positive for *Lr34* gene were towards lower AUDPC value, DBW-16 showed AUDPC between 100-200 as it showed slow rusting mechanisms in the field as well as presence of Lr34 gene at molecular level. Whereas, other 11 genotypes even though showed for the presence of *Lr34* gene they had very 0.0 to 6.65 AUDPC value, it may be because of known and unknown major genes which have proven resistance against leaf rust (Table 18). Genotypes falling in the range of 101-200 for AUDPC truly represent the slow rusters. These genotypes offer long lasting broad spectrum field resistance and must be preferred while breeding to develop durable rust resistant genotypes and also can be recommended for cultivation in farmers field helpful in getting economic yield. These results were in agreement with Priyamvada *et al*. (2009) they also opined similar statements by 82 bread wheat lines screened for *Lr34,* obtained 16 lines positive for *Lr34* and compared the AUDPC values. Similar results were obtained for *Lr46* gene (Singh *et al*., 2005) with *Xwmc44* (Lillemo *et al*., 2008) for other genotypes and hence, discussion is same as that of *Lr34.* Whereas, slow rusting gene *Lr67* showed only in RL-6077 Mexican genotype for the first time recently identified (Herrera-Foessel *et al*., 2011). Utilization of this particular genotype in Indian wheat breeding programme is limited so that, none of the genotypes showed presence for *Lr67* gene. In this study RL-6077 alone showed monomorphic amplification with *Xbarc288* SSR marker (Herrera-Foessel *et al*., 2011).

In the current study interesting results were obtained with presence of combination of two slow rust resistant genes (*Lr34* & *Lr46*) among five Indian bread wheat genotypes. It has been showed enhanced slow rusting mechanisms with lower AUDPC values and optimum yield reveals the effectiveness of combinations of two or more slow rusting resistance gene. Singh *et al*. (2000) opined

that, slow rusting resistance genes have small-to-intermediate effects when present alone, high levels of resistance have been achieved by combining 4-5 such slow rusting genes. The multiple or broad spectrum disease resistance conferred by slow rusting genes *Lr34* and *Lr46* implies an added value for wheat breeding (Herrera-Foessel *et al*., 2011 & 2012).

5.3.3 Studies on expression of oxidative enzymes (isozymes) in selected bread wheat under the pathogenesis of leaf rust races

The studies on peroxidases and polyphenol oxidases (PPO) were carried out. Two growth stages of host such as seedling and adult plant stages were selected. The expression of these two isozymes in resistant, slow rusting and susceptible genotypes was discussed hereunder.

Enhanced peroxidase activity during resistance has been reported in a number of wheat-rust fungus interactions (Fric and Fuchs, 1970; Johnson and Cunningham, 1972; Johnson and Lee, 1978; Moerschbacher *et al*., 1988). Similar result were obtained in this study that, resistant genotype, NAIW-917 showed expression of peroxidase irrespective of the growth stages for both inoculated *P. triticina* races (77-5 and 104-2) as well as mixture of races under field condition. Grisebach (1981) and Northcote (1985), who reported that, peroxidase is involved in many processes in plants including the final step in lignin synthesis and thereby expression of resistance to plant pathogens and also Southerton and Deveralli (1990) noticed that, peroxidase activity was increased more rapidly after 40 h of inoculation in the presence of avirulent strain in two rust resistant varieties.

The slow rusting genotype HD-2189 not showed expression of peroxidase for race 77-5 at both seedling and adult plant stage but it showed expression of peroxidase for race 104-2 this may be because of race 77-5 is highly virulent (82.4 %) as shown in Table 20. Due to higher virulence it may be having mechanisms to deactivate/inactivate produced peroxidase in the inoculated host. Whereas race 104-2 allowed the expression of peroxidase as it was less (70.6 %) virulent than race 77-5 similar results were obtained by Hasabnis, 1998, he reported that, when susceptible and resistant (HD-2189 considered as resistant) varieties were inoculated with highly virulent and less virulent pathotypes at seedling stage, they behaved distinctly for peroxidase banding pattern. Variation in peroxidase expression to both the races found in the susceptible genotype, Agra Local showed higher expression to the highly virulent race than less virulent race (at 5^{th} day after inoculation) this result also in agreement with Hasabnis (1998) stated that, when susceptible varieties inoculated with 77-5 isozyme activity was higher and bands were prominent. Results were also on par with work of Nagaveni (2005), reported that peroxidase activity was higher in the resistant barley genotype than in the susceptible genotype. It was noticed that at 30 and 60 DAS in susceptible varieties, the number of isozyme bands were comparatively less with low Rm value at 30 and 60 DAS compared to the resistant varieties. Mohammadi and Kazemi (2002), when carried out same studies between *Fusarium graminearum* and wheat host interaction obtained similar results.

Overall results of this study indicated activity of peroxidases higher in resistant genotype. This is in agreement with the findings of Polilova and Fomchenko (1993), who reported higher activity of peroxidase isozyme in highly effective resistance genes *viz*., *Lr 9*, *Lr 15*, *Lr19* and *Lr24*. Wand *et al*. (1994) observed peroxidase activities in wheat lines carrying different *Lr* genes when inoculated with two different leaf rust races.

Polyphenol oxidase is important in the expression of disease resistance mechanism. Kuc (1964) expressed the possibility that, precursors of phenolic compounds via shikimic acid pathway might have induced synthesis of polyphenol oxidase in infected tissues.

Mohan and Khanna (1988) studied polyphenol oxidase activities in 10 near isogenic lines carrying resistance genes to *P. recondita* and compared with two susceptible genes carrying lines. The polyphenol oxidase activities was increased initially, followed by decline and then another increase in response to infection of leaf rust pathogen.

In the present investigation, in comparison of resistant, slow rusting and susceptible genotypes, it was found that differences between seedling and adult plant stages in the expression of PPO in the resistant genotype, NIAW-917. Whereas, differences were not observed among slow rusting and susceptible genotypes at seedling stage for both the races. At adult plant stages these two genotypes revealed exactly reverse expression of PPO for both races and mixture of races in the field.

It was noticed that, the activity of polyphenol oxidase was not observed under uninoculated condition in resistant genotype, NIAW-917. Whereas it was present in both slow rusting and

susceptible genotypes at both the growth stages. It means both the genotypes had higher inbuilt activity of polyphenol oxidase. Under the set of inoculations, activity of polyphenol oxidase was noticed higher by expressing prominent isozyme bands as compared to respective uninoculated ones. This is in agreement with the results of Nargund (1989) and Hasabnis (1998), who reported higher acivity of PPO in different wheat varieties, when inoculated with leaf rust pathogen. Agra Local showed higher expression of PPO at 5th day after inoculation whereas higher expression in HD-2189 was in 10th day after inoculation for both races at adult plant stage. Thus, it can be concluded that even it is susceptible genotypes it can defend against pathotypes initially by expression of more PPO and later it may be decreased due to the virulence mechanisms of the pathogen similar results were also obtained by Hasabnis (1998). This was in agreement with Saini *et al*. (1993) inoculated, wheat cultivar WL 711 with two races of leaf rust pathogen and noticed high activity of polyphenol oxidase in susceptible interaction when inoculated with race 77.

Thus, it can be concluded that, the activity of peroxidase and polyphenol oxidase isozymes in wheat leaf rust interaction is need based. In response to stimuli of the fungal pathogen, activity showed increased trend. In slow rusting genotype, with less virulent pathotype activity of the isozymes was increased.

In the present investigation, activities of peroxidase and polyphenol oxidase were found to be enhanced in response to infection. Further, with increase in the expression of resistance. The role of peroxidase in removal of toxic hydrogen peroxide accumulated in tissues as a result of enhanced metabolic activity during srtessfull condition is well documented (Moerschbacher *et al*., 1988). Infection of tissue brings about lot of changes in the metabolic activity of the host tissue. Thus, these enzymes activities provided a tool to monitor durable leaf rust resistance in bread wheat genotypes.

5.3.4 Histology of slow leaf rusters

In the pre penetration interaction between host and pathogen, the results of spore germination and appressorial formation showed significant differences between selected genotypes irrespective of the three group of genotypes *viz*., slow rusters, resistant and susceptible group of genotypes. The present investigation was in agreement with study of Lee and Shaner (1984), stated that, there were no significant differences among slow rusters, intermediate slow rusters and susceptible cultivars in frequencies of spore germination, appressorium formation and substomatal vesicle formation. Similarly many other workers reported the same results (Clifford, 1972; Niks, 1981; Sztejnberg and Wahl, 1976)

Chang and Line (1983) reported that there are no significant differences in appresorium formation among susceptible genotypes and with partial resistance in wheat. The differences in percentage of appresorium formation between the apex and the central part of the leaf are relating all differences in environmental conditions (Broers and Jacobs, 1989). Hu and Rijkenberg (1998) studied the morphology of infection structure development of *P. recondita* f. sp. *tritici* on and in susceptible and resistant wheat lines inoculated with urediospores was examined by SEM and opined that, there were no significant differences between the infection processes on the three wheat lines examined.

In the present investigation slow rusters with *Lr34* and *Lr46* slow rust resistant gene selected these genes also not showed any differences in the two components of pre penetration studied. The results were in agreement with study conducted by Garcia-Lara *et al.* (2007), studied the infection process with partial resistance. The genes *Lr34*, *Lr46*, and one gene (Gene 1) not yet named confer to prehaustorial resistance, Jupateco 73R+*Lr34*, Avocet+*Lr34*, Lalbahadur+*Lr46*, and Lalbahadur+Gene 1, and in genotypes that do not contain the gene: Jupateco 73S-*Lr34*, Avocet-*Lr34*, and Lalbahadur. In the percentage of urediospore germination, there were no significant differences in multiple comparison of means ($p>0.05$; DMS). Whereas the contrasting results were also obtained in stripe rust and wheat host-pathogen interaction by Stubs and Plotnikova (1972) observed differences in spore germination and germ tube penetration of race 60 of *P. striiformis* on wheat varieties investigated *T. spelta* L. var. Album was the most resistant, strongly inhibiting spore germination and retarding germ tube penetration. Heath (1981) suggested that the mechanisms, presented before the formation of the first haustorium are those of the strongest impact. After the fungus invades the tissues of an incompatible leaf, some adverse effects on growth or the appearance of sub-stomal bladder and infection hypha become evident. Although this is more common in non-hosts, it is also reported for resistant cultivars (Leath and Rowell, 1966). Poyntz and Hyde (1987) reported differences among wheat varieties, resistant and susceptible to leaf rust in germination and germinative tube length; the lowest percentage in both types corresponded to the susceptible

varieties, and in the infection process the largest colonization was presented by the susceptible varieties.

In the present investigation delayed pre penetration process were observed as it was showed that, start of spore germination and appressorial formation on genotypes was very late as compare to the normal (before 4 h after inoculation). This may due to microclimate required for spore germination and appressorial formation which largely influenced as the plants grown under polyhouse condition. This is in agreement with the work of Broers and Jacobs (1989) showed the differences in percentage of spore germination and appresorium formation between the apex and the central part of the leaf are relating all differences in environmental conditions.

5.4 Evaluation of identified slow leaf rusters for quality traits

The effect of leaf rust on selected bread wheat genotypes were highly influenced by resistance level of the varieties used (Hailu and Fininsa, 2007). The loss of GPC was either increased or decreased or no significant changes obtained when compared with protected and unprotected conditions among all the three group of genotypes. When leaf rust was the only evidently severe disease, an increased trend of grain protein content due to leaf rust was obtained in grains of the susceptible varieties *viz*., DWR-162, Lal Bahadur and Agra Local. Similar results were obtained by Hailu and Fininsa (2007) with stripe rust and susceptible bread wheat genotypes at Agarfa and At Sinana where less stripe rust severity was recorded, the disease severities on flag and penultimate leaves were negatively and significantly (P<0.05) correlated with grain protein content of the susceptible variety with coefficients of correlation ranging from -0.530 to -0.716. The negative correlation coefficients indicate reduction in protein content due to leaf rust. There was no significant correlation between leaf rust and protein content for the selected bread wheat genotypes. The inconsistency of the effect of leaf rust on grain protein content among genotypes was found. Everts *et al*. (2001) also found that effect of wheat disease on protein was not consistent for varieties and environments. Dill-Macky *et al*. (1990 & 1991) reported that stem rust resulted in increased protein content of wheat but caused the opposite effect on barley.

However, leaf rust shows the effect on grain quality. When diseases such as leaf rust affect photosynthesis negatively, starch biosynthesis and its storage in seed are affected. Jiao *et al.* (1996) reported a reduction of starch biosynthesis of common bean (*Phaseolus vulgaris* L.) due to infection by common bacterial blight (caused by *Xanthomonas axonopodis* pv. *phaseoli*). An interruption of normal starch synthesis due to disease may result in nitrogen increase at the loss of starch. Leaf rust infection was so severe that it resulted in shrivelled kernels. Shriveling of kernels most often results in increased grain protein due to an increase in the ratio of protein to starch (Everts *et al*., 2001). Increased grain protein content in seeds harvested from severely rusted plots in this study seems to be due to reduced starch filling in the endosperms. Shriveling, in turn, causes proportionate losses in flour yield. In the resistant and slow-rusting genotypes, where there was no shriveling of kernels, leaf rust showed a tendency to decrease grain protein content.

Almost similar trend was followed in the SDS value of protected and unprotected conditions as shown in the GPC among selected bread wheat genotypes and it was studied previously by Dhaka *et al*. (2012) stated that protein content and SDS were significantly positively correlated. Similar results were also found in our study. Hence, discussion of protein content is similar to SDS of this study.

The higher starch damage was observed in case of resistant genotypes under protected condition. This may be due to the chemical spray as it has phytotonic effect of crop growth there by grain hardness increases ultimately, it reflects in the higher damage of starch in flour and the genotype does not showed any significant difference in starch damage it may be because of vary in the starch damage from genotype to genotype. This was in agreement with Kwasniewska-Karolak (2011) showed that the degree of starch damage shows a high positive correlation with grain hardness (r =0.7), which depends mostly on wheat genotype. Excessive starch damage, which results from milling of wheat with increased kernel hardness, can result in high farinograph absorptions (Preston *et al*., 1991). This, in turn, can lead to short mixing times and relatively large mixing tolerance (Dexter *et al*., 1985).

The slow rusters and susceptible genotypes showed higher damaged starch under unprotected condition this is because of infected condition by the leaf rust pathogen as it clearly showed reduced damage in the protected condition. Direct relevance to the effect of leaf rust on damaged starch in bread wheat flour was observed. These types of studies were limited to support

the present investigation. Hence, generally opined by Everts *et al*. (2001) that quality issues related to disease damage of wheat are most commonly milling related. Wheat diseases generally affect the yield of a wheat crop by causing shriveled kernels and reduced test weight, and these, in turn, tend to reduce flour yield. Experiments confirm that wheat flour for starch production should not contain damaged starch grains, and flour for baking, on the other hand, should contain a certain amount of damaged starch, since it affects volume increase in bakery products (Martin *et al.,* 2007; Miller *et al*., 2008). Baking quality parameters (including loaf volume and crumb and crust characteristics) deteriorate as a result of frost injury to wheat. This effect is due to both high levels of starch damage as well as to inferior gluten properties in flour produced from frost-damaged wheat. Additionally, poor crumb color is related to the high flour ash and color values (Preston *et al*., 1991 and Dexter *et al*., 1985) that are associated with milling of frost-damaged wheat.

There is an inverse relation between gluten content and leaf rust severity on the slow ruster and susceptible genotypers (Table 26). The effect of leaf rust on wet gluten content was similar to dry gluten content and also gluten index was showed similar trend as that of wet gluten content. Similar results were obtained by Hailu and Fininsa (2007) studied the effect of stripe rust on wet gluten content of susceptible varieties and stated inverse relationship between gluten content and stripe rust severity. There was also strong positive association between wet gluten and dry gluten content of the slow ruster and susceptible genotypes. This was in agreement with Dhaka *et al*. (2012) observed a significant positive correlation (r^2=0.948) between wet gluten and dry gluten content of bread wheat varieties. O'Brien *et al*. (1990) reported that flour from stripe rust affected grains resulted in weaker dough as measured by shorter dough development time. According to Czuchajowska and Pasczyñska (1996), bread volume increases by 46 to 65 cm^3 for each 1% increase of wet gluten content. Wet gluten ranked the second most desired test to assess field testing of wheat functional quality and end-use characteristics (Chinnaswamy *et al*., 2005).

In the study of alveograph parameters, unprotected condition of susceptible genotype Agra local showed poor rheological quality whereas, slow ruster (HD-2189) not affected much of the rheological quality. Similar results were obtained with following workers. Mobarak *et al*. (2007) studied the rheolegical tests showed increment of water absorption, development time dough stability, extensibility, resistance to extension and dough energy in infected wheat dough compared with protected wheat dough of the same wheat cultivars. Manthey *et al* (2006) evaluated the rheological and gelatinization properties of durum wheats grown in the USA with Mixolab (Alveoconsistograph), their results showed variability in terms of protein quality and starch pasting properties, which indicated that Mixolab could be used to determine durum wheat quality. Pena *et al* (2006) found that the Mixolab dough development time, stability and breakdown parameters showed high correlation with the Alveograph W value when testing the whole grain flour. However, the studies related to the utilization of Mixolab to evaluate the bread making quality of flours are limited. Overall results of the present study indicated that Mixolab can be used to predict the bread wheat quality and Mixolab can be used to differentiate wheat genotypes in terms of different quality characteristics.

Mineral elements play essential roles in the biochemical and physiological functions of any biological system. In plants, appropriate mineral availability is essential for almost every aspect of development, from seed germination and seedling development (Welch, 1999) to yield formation and mineral deposition in grains (Yilmaz *et al*., 1998; Welch, 1999). Mineral elements are also essential nutrients for animal and human well-being. It is estimated that over three billion people suffer from micronutrient malnutrition worldwide (Bouis, 2003; Welch and Graham, 2004; White and Broadley, 2009), resulting in overall poor health, anemia, increased morbidity and mortality rates, and low worker productivity (Holtz and Brown, 2004; Sanchez and Swaminathan, 2005; Cakmak, 2008). Currently, mineral malnutrition is considered to be among the most serious global challenges for humans (Copenhagen Consensus 2004; http:// www.copenhagenconsensus.com).

The availability of sufficient amounts of mineral nutrients in the human diet depends primarily on their composition in higher plants (Grusak and Cakmak, 2005; Cakmak *et al*., 2010; Sands *et al*., 2009), particularly on mineral nutrient concentration in staple food crops such as cereal grains. Therefore, enhancement of grain nutrients (biofortification), either agronomically (application of mineral fertilizers) or genetically (breeding) or protecting loss from biotic causes, is considered the most promising and cost-effective approach to alleviating malnutrition and related health problems (Welch and Graham, 2004; Bouis, 2003; Cakmak, 2008; Peleg *et al*., 2008). This solution, however, requires a comprehensive exploration of potential genetic resources and an in-depth understanding of the physiological and genetic basis of nutrient-accumulation processes in grains.

Disturbed mineral nutrition is one of the common effects of disease, and symptoms of infection. Diseases are frequently diagnosed as mineral deficiency or toxicity. This may result from reduced uptake, altered distribution, impaired utilization, or toxicity from excess accumulation around infection sites.

In the present investigation, 40 genotypes of wheat were evaluated (Three different groups *viz*., resistant (33), slow rusters (3) and susceptible (4) genotypes) for grain nutrient content to assess their genetic potential by studying grain yield and other quality characters. The results obtained from the present investigation are discussed hereunder.

In the present study, slow ruster genotypes showed highest content of three micro-nutrients *viz*., zinc, iron and copper. Whereas, lowest was observed in case of manganese and magnesium content as compared to resistant and susceptible genotypes. Even under diseased condition slow rusters genotypes showed highest content of three micro-nutrients, it may be because of genetic potentiality of the slow ruster genotypes or not allowed to lose their nutrient content even under pathogenesis of leaf rust or may be due to influence of pathogen to uptake more of these three nutrient during the slow phase of development or may not be utilized by the pathogen. And other two (manganese and magnesium) nutrients were showed less in slow rusters as compared to remaining two groups of genotypes. This may be because of strong requirement by the leaf rust pathogen for their growth and development since, we are allowing that pathogen to multiply slowly there by pathogen utilize those two nutrients without allowing to transformation from source to sink in the plant. AUDPC and ACI were negatively correlated with all the analyzed micronutrients except manganese with ACI value. The manganese was highly significantly positively correlated with zinc. These results were in agreement directly or indirectly with following researchers.

Utilizing leaf rust effective resistant genes in wheat durable resistant breeding programs and growing slow rust resistant cultivars on a large scale would most likely decrease leaf rust related yield and quality losses (Akin *et al*., 2008).

Ortiz-Monestrio *et al.* (2007) studied a wide range of germplasm lines at CIMMYT and reported the variability ranging from 28.8 to 56.5 mg per kg for iron and 25.2 to 53.3 mg per kg for Zn and also showed that among all wheat germplasm studied the species *T. dicoccum* had the highest concentration and they also noted the presence of positive correlation between Zn and Fe (Welch *et al.,* 2005; Liu *et al.,* 2006; Zhao *et al*., 2009).

Ozkan *et al.* (2005) accessed the variation for seed micronutrient content in 54 accessions of einkorn wheat (*T. monococcum*). The result showed the existence of large genotypic variation in content of micronutrients. The contents of Zn and Fe varied from 0.21 to 2.16 mg /seed for Zn with average of 1.19 mg/seed and from 0.54 to 3.09 mg/seed for Fe with average of 1.19 mg/seed and also showed the presence of positive relationship between Fe and Zn, the results of the four traits showed that a major QTL which is common to all four micronutrients explaining from 10 to 30 per cent observed on chromosome-5.

Chhuneja *et al.* (2006) analyzed *Aegilops kotschyi* and *A. tauschi* for Fe and Zn content in grains and showed that S and SD genome species accumulates significantly higher iron and zinc in the grains than that cultivated wheats observed that one of the CIMMYT synthetics also had significantly higher Fe and Zn in the grain as compared with the cultivated wheat. The study shows that *Aegilops kotschyi* as a promising source for Iron and Zinc.

Morgounov *et al.* (2007) selected sixty-six spring and winter common wheat genotypes from Central Asian breeding Programs and evaluated for grain concentrations of iron (Fe) and zinc (Zn). Iron showed large variation among genotypes, ranging from 25 mg kg^{-1} to 56 mg kg^{-1} (mean 38). Similarly, Zn concentration varied among genotypes, ranging between 20 mg kg^{-1} and 39 mg kg^{-1} (mean 28 mg kg^{-1}). Spring wheat cultivars possessed higher Fe-grain concentrations than winter wheats. Ficco *et al.* (2009) studied 84 Italian durum wheat cultivars of old and new germplasm in two locations and showed that content of Zn is ranged from 28.5 to 46.3 mg per kg with average of 37.4 mg per kg and Fe ranged from 33.6 to 65.6 mg per kg with average of 49.6 mg per kg grain, Phosphorus content was 0.46 to 0.76 mg per g showing positive correlation with all minerals except Cu and Zn also showed the significance G x E interaction.

The 265 genotypes displayed a large variation for all mineral elements investigated including Fe and Zn, ranging from 28.0 to 65.4 mg kg-1 and 21.4 to 58.2 mg kg-1 for Fe and Zn, with mean values of 39.2 and 32.3 mg kg-1, respectively. Jimai 26, Henong 326 and Jingdong 8 displayed high

Fe and Zn concentrations. Jimai 26 and Henong 326 also displayed high concentrations of Cu, Mg, K, P and protein content (Zhang *et al*., 2010).

Ferney *et al.* (2010) studied 19 wild emmer wheat genotypes and the largest variation was observed in Mn concentration (13-87 mg/kg). Accessions with higher nutrient concentration had also shown higher grain yield, analysis of variance showed that significant for environmental variation *i.e*. Up to 44 per cent but genotypic effect was also important for Mg, Zn, Mn and S.

The yield losses due to leaf rust among genotypes were showed negligible for most of the resistant genotypes whereas, MACS-6145, VL-943 and UAS-304 resistant genotypes had moderate yield losses. This was not surprising because they were the only genotypes with low to intermediate leaf rust responses when the rest of the resistant genotypes displayed immune responses. In contrast, in the resistant genotype VL-829 even though it was showed immune response, it had a yield loss. In this experiment, yield reductions in the unprotected compared with the protected treatment were negligible for most of resistant bread wheat genotypes. However, higher yield loss was observed in VL-829 bread wheat resistant genotypes that were immune to the *P. triticina* populations. The plants respond to inoculation with energy-demanding physiological processes, probably defense reactions, using stored host energy that otherwise would go to growth and seed production. In addition, a reduction in photosynthetic leaf area due to hypersensitive flecking also can cause yield reductions (Samborski and Peturson, 1960). The use of broad spectrum systemic fungicides often results in yield increases. Fungicide treatments with triazoles have been shown to have a beneficial effect on the plants by delaying senescence, thereby prolonging the duration of green leaf area and increasing yield (Bertelsen *et al*., 2001). Same results were obtained by Herrera-Foessel *et al*. (2006) among durum wheat genotypes. Sayre *et al*. (1998) found that leaf rust caused losses irrespective of the level of resistance possessed by the cultivars. Smedegaard-Petersen and Tolstrup (1985) observed that powdery mildew resistance in barley, highly resistant plants do not show any visible disease symptoms after inoculation does not mean that the plants are not affected.

All slow-rusting genotypes had tolerable yield losses. The slow-rusting genotypes HD-2189, HI-977, VL-616 and VL-924 had lower losses than the resistant UAS-304 (10.44 %). Of the 10 susceptible genotypes, the yield loss for Lal Bahadur and Agra Local was very high as compared to all other susceptible genotypes as they displayed very high disease severity. Slow-rusting genotypes displayed slow disease development with tolerance mechanisms and it is the ability of plants to maintain yield (or quality) performance in the presence of disease symptoms or when the plants appear susceptible to the disease (Caldwell *et al*., 1958; Parker *et al*., 2004). Individual components of yield are less likely to describe disease effects on yield than the yield measurement itself. Yield loss, rather than yield performance or disease development, should more accurately reflect the tolerance of a cultivar (Caldwell *et al*., 1958). At the same time, it is important to take into account yield performance per se because a high yielding line with high leaf rust tolerance will have a superior value for wheat breeders and producers.

The slow-rusting bread wheat genotypes lost less than 14.33 per cent. All the slow-rusting bread wheat genotypes had the lowest AUDPC and ACI values (range of 60.67 to 199.15 and 3.25 to 11.0 respectively) compared with susceptible genotypes. This indicates that slow-rusting genotypes were better option to tackle ever evolving leaf rust pathogen by use of these identified slow rusting genotypes with less yield loss could be possible to achieve targeted production (Bhardwaj *et al*. 2011; Kolmer, 2013).

In the present study, yield losses due to leaf rust were mostly attributed to a reduction in TGW (Fischer, 2001). Afzal *et al*. (2008) reported that a wheat yield loss in the northern Punjab and North West Frontier Province (NWFP) there was existence of direct linkage between the disease level of *P. striiformis* and weight loss of kernel in the most common wheat varieties sown in Pakistan. The kernel weight was significantly negatively correlated with the proportion of leaf area affected by stripe rust. The correlation coefficient (-0.9185) depicted highly significant effect of stripe rust on lowering 1,000 grain weight, ultimately the wheat yield.

As would be expected, the grain yield losses between the protected and unprotected plots generally were much lower and the yield losses were attributed mainly to changes in kernel weight. In a similar study by Sayre *et al*. (1998) observed that grain yield losses due to leaf rust in bread wheat were associated with reductions in kernel weight, kernels per square meter, spikes per square meter, and grain-filling rate. Singh and Huerta-Espino (1994) investigated the effect of the slow-rusting gene *Lr34* in bread wheat and found that yield losses due to leaf rust was associated with biomass,

kernel weight, kernels per spike, harvest index, and test weight. Similar results among durum wheat were also supporting above facts by Herrera-Foessel *et al*. (2006).

5.5 Management of leaf rust through chemicals

The management of leaf rust by using the genetic resistance is of utmost importance. But in the conditions of epiphytotic it is necessary to check the multiplication and spread of inoculum by using the chemicals which provide quick and easy management of deadly obligate pathogens. In the present study, in search of best alternative to the propiconazole was attemptented with eleven treatments including single and combi-products of different pesticides and fungicides.

The results of chemical management as a short term strategy showed T4 (seed treatment with RIL 071/F1 (20%FS) @ 1.5ml/kg of seed followed by Propiconazole @ 0.1% two spray between 45 & 60 DAS) best treatment in suppressing leaf rust pathogen followed by T5 (Seed treatment with RIL 071/F1 (20%FS) @ 2ml/kg seed followed by Propiconazole @ 0.1 % two spray between 45 & 60 DAS). This result was in agreement with the work of Patidar *et al*. (2007) obtained, by spray of 0.1 per cent Propiconazole produced maximum yield (43.45 q/ha) with an ACI of 3.33 per cent which was on par with triadimefon (43.06 q/ha) and hexaconazole (38.41 q/ha) with 5.33 and 8.00 ACI. Nargund (1989) reported that maximum 1000 grain weight was noticed in propiconazole treatment (25.04 g) followed by diclobutrazol (23.20 g) and triadimefon (23.20 g) also obtained similar trend in grain yield (Patidar, 2006). Kalappanavar and Patil (1998) reported that propiconazole, tridimefon and hexaconazole were the most effective fungicides for management of leaf rust of wheat.

Khan and Ilyas (1996) reported that a single application of either Tilt or Folicur at growth stage 10 resulted in significant reduction in the rate of leaf rust and spot blotch development and lower AUDPC at FSD-85. Brahma *et al.* (1991) revealed that the fungicide Propiconazole was found effective against all rusts. Barros *et al. (*1982) reported that triadimefon and mancozeb were the most effective fungicides against stem and leaf rust of wheat.

Kalappanavar *et al*. (2008) reported that among the 13 treatments imposed fungicide propiconazole performed best followed by triadimefon and hexaconazole. However, in different categories, neem leaf extract, *Trichoderma harzianum* and Panchgavya were the succeeding treatments effective against leaf rust of wheat. The yield of propiconazole, triadimefon and hexaconazole sprayed plots were significantly superior over control indicating marked influence of the leaf rust on yield. The 1000-grain weight was also significant in the above said treated plots compared to other treatments.

In the present study the best alternative to the propiconazole in the management of leaf rust was identified based on observing lower disease score (ACI), highest yield and highest thousand grain weight. The fungicide combination Pyraclostrobin 13.3% + Epoxiconazole 5% (Opera 18.3% SE) @ 0.1 % two spray at 45 and 60 DAS is the best alternative to the propiconazole. These results are in agreement with following workers. Chen *et al*. (2012) reported the integrated use of pyraclostrobin + epoxiconazole applied at 150 + 150 g a.i./ha provided over 85% efficacy in management of fusarium head blight (FHB) of wheat. Pyraclostrobin and epoxiconazole should be good alternatives to benzimadazole fungicide for the control of FHB, and integrated use of these two fungicides might achieve greater efficacy. Bertelsen *et al*. (2001) showed that the effects of the fungicides azoxystrobin (a strobilurin) and epoxiconazole (a sterol biosynthesis inhibitor) on phyllosphere fungi, senescence and yield were studied in winter wheat in field trials. Inoculation in a glasshouse experiment with the saprophytic fungi *Alternaria alternata* and *Cladosporium macrocarpum* accelerated wheat senescence. Both fungicides reduced *A. alternata* induced papilla formation in wheat leaves, with epoxiconazole being more effective. Inoculation with either of the two saprophytes did not significantly increase wheat leaf respiration, in contrast to inoculation with the nonhost pathogen *Erysiphe graminis* f. sp. *hordei*. It is proposed that the greater inhibition of infection attempts from *Mycosphaerella spp*. by azoxystrobin, compared with epoxiconazole, may account for the greater yield given by azoxystrobin in field plots.

Future line of work

1. There is a needs to relook on presently believed *Puccinia* path in India to unravel the existence of unknown *Puccinia* path, since, there is a change in the movement of uredospore as well as climate and the current study on survey and surveillance supplying/supporting necessary information to identify existence of new *Puccinia* path in India.

2. Survival mechanisms and presence of alternative host for leaf rust pathogen need to be identified in the absence of secondary foci of infection in the state.
3. It is necessary to develop race specific molecular markers for leaf rust pathogen races develop our own race analysis method at molecular level is felt essential.
4. In-depth analysis of known/unknown slow rust resistant genes at molecular level needs to be studied.
5. The effectiveness of newly identified slow rust resistance genes (*Lr67* & *Lr68*) need to be studied in different bread wheat genotypes having varied genetic background.
6. Identified slow rusters should be utilized for durable rust resistance breeding programme to manage ever evolving leaf rust pathogen.
7. More number of genotypes should be screened for presence of identified slow rust resistant genes.

SUMMARY AND CONCLUSIONS

The investigations on leaf rust of wheat caused by *Puccinia triticina* Eriks. include prime aspects *viz*., Survey, Surveillance and race analysis, genetic diversity of *P. triticina*, identification of slow rusters, yield loss and quality assessment. The experiments were undertaken during *rabi* 2011-12 and 2012-13 at Dr Sanjay Rajaram wheat laboratory, All India coordinated wheat improvement project. Main Agricultural Research Station (MARS), University of Agricultural Sciences Dharwad and Directorate of Wheat Research (DWR), Regional Station, Flowerdale, Shimla, Himachal Pradesh, India.

Two years (2010-11 and 2011-12) survey from off-season wheat growing area of Chikmagalur and Chitradurga districts of Karnataka revealed that farmers have stopped growing wheat during off-season. The cultivation is discontinued as per the scientific and technological reports submitted to the concerned authorities of Government of Karnataka. Earlier farmers were growing wheat, but Government has banned growing of wheat, since it serves as a secondary focus of infection of rusts in addition to primary foci as Niligiri and Palni hills of Tamil Nadu. During normal wheat growing season of 2010-11, 2011-12 and 2012-13, disease appeared comparatively in more severe form during 2012-13 than the previous two years in the surveyed areas. The marked differences in the leaf rust severity were more related to the type of variety grown in different localities. The wheat varieties *viz.,* DWR 162 having vertical resistance was severely affected. The pathotype 121R63-1 (77-5) was dominant in population such as 35.71 and 58.90 per cent during *rabi* 2010-11 and 2011-12, respectively, during 2012-13 pathotype 121R60-1 (77-9) was dominant with 31.00 per cent.

All the isolates of *P. triticina* that were tested produced a unique pattern of EST-SSR alleles confirming the high genetic diversity within populations of the leaf rust fungus. In the similarity co-efficient study, high degree of similarity between the isolates which were collected from different wheat growing areas had common distribution of similar pathotypes in those areas.

In the present study, identification of slow leaf rusters in two cropping seasons (during *rabi* 2011-12 and 2012-13), revealed that Agra Local, Lal Bahadur, Lok-1, Sonalika, C-306, DWR-162, PBW-343, DBW-17 and HS-240 were identified as fast rusters, whereas, 34 genotypes showed immune to resistant reaction to the prevailing pathotypes of *P. triticina* during the course of investigation. Ultimately by considering two year data and all the studied slow rusting components, it could be possible to identify genotypes, UAS-326, UAS-315, VL-616, VL-924, HD-2189, HD-2932, HD-3091, NI-5439, HI-977, HS-420, DBW-16, KRL-210, Pavon-76, RL-6077 and Parula as slow leaf rusters with low terminal disease severity, less rate of disease development, minimum values of AUDPC, longer latent period, lower pustule density and smaller size of uredinium (Table 14, 15, 16 and 17).

A total of 14 genotypes showed for single known gene with unknown gene (*Lr2a+, Lr10+, Lr13+, Lr23+, Lr24+, Lr26+* and *Lr28+*) and double known genes with unknown 14 gene combinations. Only 3 genotypes found triple known genes (*Lr23+26+1+)* with unknown gene combination. Among the single gene or in combinations of *Lr26* was observed in highest frequency among Indian bread wheat genotypes. In the seedling resistance and susceptibility test genotypes *viz*., VL-616 and KRL-210 were found susceptible, in contrast HI-1500, HD-2888, AKAW-4627, PBW-343 (*Lr24+Lr28+*) and HW-2004 showed resistant reaction and remaining genotypes displayed variable reaction to the all pathotypes inoculated. The isogenic lines *viz*, *Lr9* and *Lr19* did not matched by any of the 13 pathotypes tested at seedling stage. The group 77 had higher frequency for virulence, of which 77-5 had maximum of 82.4 per cent. The minimum was noticed in 12-9 as of 47.1 per cent.

Molecular characterization of selected Indian bread wheat genotypes for slow rusting pleiotrophic resistance genes using closely linked STS and microsatellite markers showed 12 genotypes (Raj-4083, DBW-16, VL-892, HS-533, PBW-596, PBW-590, VL-829, VL-907, HI-1563, HI-1584, RAJ-4229 and RAJ-4245) with *Lr34/Yr18/Pm38,* seven genotypes (GW-322, HD-2932, Sonalika, HS-240, HS-420, HD-2864 and HD-3093) with *Lr46/Yr29/Pm39* and none of the genotypes showed *Lr*67/*Yr*46.

Interestingly, 5 genotypes carrying both *Lr34/Yr18/Pm38* and *Lr46/Yr29/Pm39* were identified from the selected Indian bread wheat genotypes and 7 genotypes (VL-924, UAS-326, NI-5439, HI-977, DBW-17, KRL-210 and MACS-2496) did not show any of the above slow rusting genes but they

showed slow rusting mechanisms in the field unveiled the unknown slow rusting genes need to be identified.

This study indicates that these genotypes have potential durable resistance and multiple disease resistance genes (nearly 6 diseases of wheat) which can be used for durable rust resistance breeding programme as well as they can be grown in a larger area without affecting wheat production.

The studies on peroxidase and polyphenol oxidase (PPO) with wheat host and leaf rust pathogen interaction noticed that, activity of both the isozymes higher in response to inoculation of the pathogen and at adult plant stage appearance and expression of peroxidase were prominent than that of respective seedling growth stage. Overall results of this study indicated activity of peroxidase higher in resistant genotype and less in slow rusting and susceptible genotypes. In case of slow rusting genotype differences for both stages as well as both races were noticed but in case of susceptible genotype differences observed only to the stage but not to the races was documented.

In the present investigation, in comparison of resistant, slow rusting and susceptible genotypes, it was found that differences between seedling and adult plant stages in the expression of PPO in the resistant genotype. Whereas, differences were not observed among slow rusting and susceptible genotypes at seedling stage for both the races. At adult plant stages these two genotypes revealed exactly reverse expression of PPO for both races and mixture of races in the field.

Overall results on expression of isozymes revealed that, among resistant, slow rusting and susceptible genotypes, higher variation between peroxidase and PPO expression at different stages of growth under inoculated and uninoculated conditions were observed.

The histology study revealed that, spore germination was not observed at 24 h after inoculation in both races. It reveals that, upto 24 h no spore germination among all the genotypes tested. The spore germination started between 24 and 48 h interval, whereas, average maximum per cent spore germination was observed between 72 and 96 h after inoculation for both the races. The appressorial formation (%) was not observed upto 48 h after inoculation in both races. The appressorial formation started between 48 and 72 h interval, whereas, average maximum per cent appressorial formation was observed between 96 and 192 h after inoculation for race 77-5 and for race 104-2 it was observed between 72 and 96 h after inoculation among all the genotypes tested. The differences were observed between the genotypes but not between the group of genotypes, indicated that pre penetration processes of uredospore are not good components to identify slow leaf rusters.

In the evaluation of quality among slow rusters in comparison with resistant and susceptible genotypes found that, loss of grain protein content (GPC) was either increased or decreased or no significant changes obtained when compared with protected and unprotected conditions among all the three group of genotypes. The average mean losses of GPC showed gain of GPC in unprotected condition for resistance genotype (-3.29%). For slow rusting 3.53 per cent losses and susceptible genotypes showed 4.28 per cent losses.

The mean sedimentation value of resistant, slow rusting and susceptible genotypes showed non significant differences under protected plots (44.03, 40.0 and 42.40 ml respectively) compared to the unprotected plots (42.54, and 41.68 and 44.45 ml respectively)

The results of damaged starch revealed that, all the slow ruster and susceptible genotypes under unprotected condition had higher damaged starch in the flour. Whereas, resistant genotypes displayed significantly lower starch damage under unprotected condition. Results of damaged starch under protected condition showed higher damage in case of resistant group and lower starch damage in slow rusters as well as susceptible group of genotypes. The mean damaged starch of resistant and slow rusting genotypes showed non significance between unprotected and protected condition. Whereas, susceptible genotypes showed significant differences under unprotected and protected conditions.

In the present investigation, the studies on effect of wet gluten content (%) was affected by leaf rust. The average wet gluten content under protected condition of slow rusters (38.85) was higher than resistant (37.34) and susceptible (36.58) genotypes. Whereas, the average wet gluten content under unprotected condition was significantly higher in resistant (37.48) genotypes followed by slow rusters (34.70) and lowest in susceptible (33.35) genotypes. Results of dry gluten content (%) showed similar trend as in the wet gluten content. The results of gluten index showed very negligible gain and loss among majority of genotypes selected. The overall alveograph results on the rheological quality of the test dough indicated that highly susceptible genotype Agra local under diseased condition

showed very poor rheological quality whereas, slow ruster not affected much of the rheological quality. Slow ruster genotypes showed an average highest content of three micro-nutrients *viz*., zinc, iron and copper. Whereas, lowest was observed in case of manganese and magnesium content as compared to resistant and susceptible genotypes.

In the crop losses assessment, average yield of protected plots were recorded higher in slow ruster (37.28 q/ha) as compared to resistant (34.09 q/ha) and susceptible (36.79 q/ha) genotypes. It indicates higher yield potentiality of slow ruster genotypes. Under unprotected condition average yield was significantly higher in resistant (33.67 q/ha) followed by slow ruster genotypes (33.18 q/ha) which were on par with each other. Whereas, lowest was observed in susceptible (25.80 q/ha) genotypes. The average yield loss for resistance genotype was very low (1.57 %), whereas, for slow ruster was 10.62 per cent and susceptible genotypes showed very high (25.80 %) yield losses.

In the chemical management study, the best alternative to the propiconazole in the management of leaf rust was identified based on observing lower disease score (ACI), highest yield and highest thousand grain weight. The fungicide combination pyraclostrobin 13.3% + epoxiconazole 5% (Opera 18.3% SE) @ 0.1% two spray between 45 and 60 DAS is the best alternative to the propiconazole.

To combat the ever evolving shifty enemy of wheat crop, the present investigation revealed three strategies *viz*., long, medium and short term strategies. Identified slow rusters will offer long term strategy as they allow slow development of the pathogen without exerting selection pressure there by creating less variability of the pathogen but in the present study we observed cent percent variability of this pathogen. In the current study majority of the bread wheat genotypes displayed immune to resistant reaction, those genotypes will act as medium term strategy and as short term strategy, new molecule which is alternative to the propiconazole have greater significance in the chemical management of leaf rust pathogen.

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*original not seen

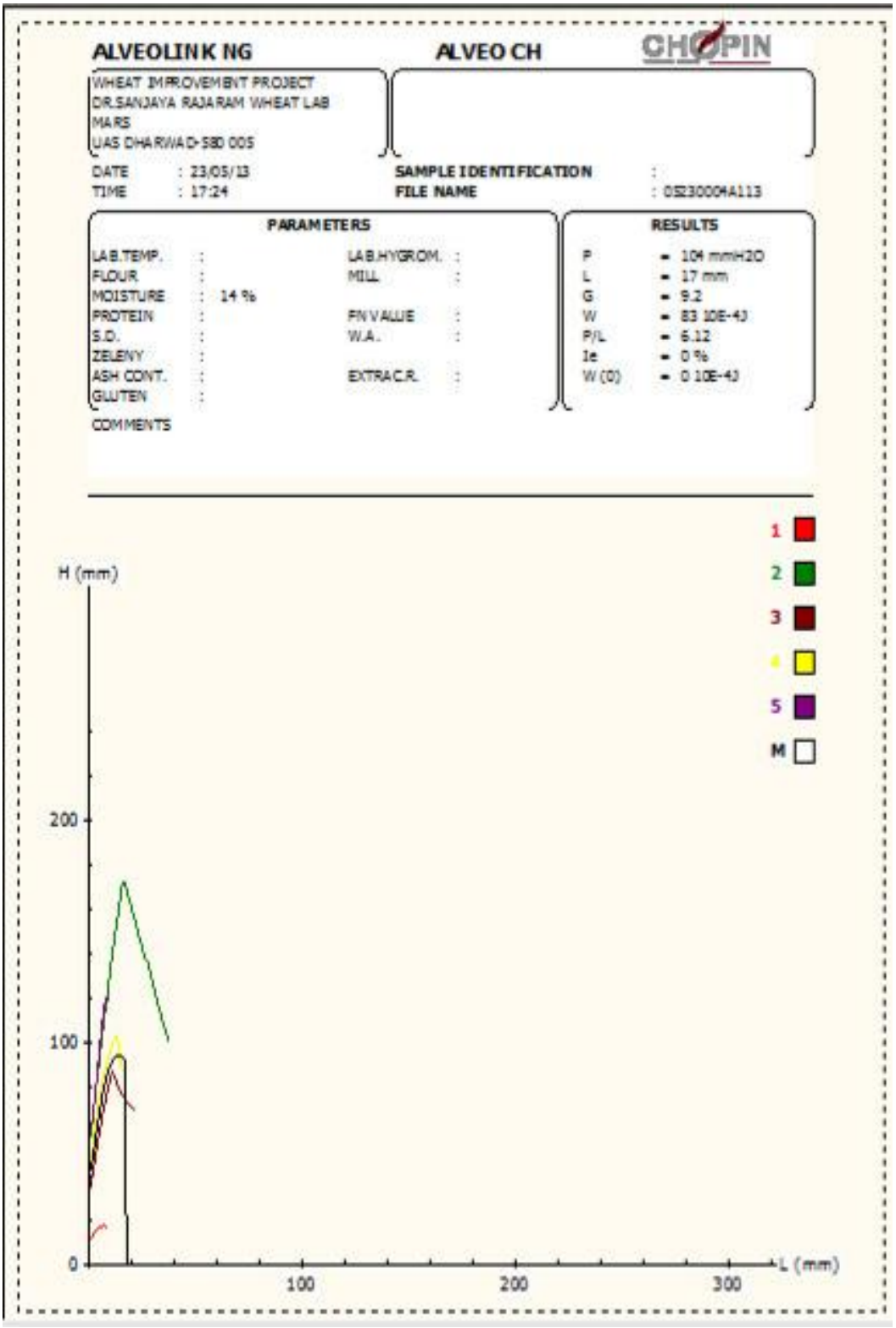
Appendix – I: Weekly Meteorological Observations of Main Agricultural Research Station, University of Agricultural Sciences, Dharwad during *Rabi* 2011-12

Meteorological Week		Temperature (^{0}C)		Rainfall (mm)	Rainy days	Relative humidity (%)	
		Mean Max	Mean Min			Morning	Evening
36	Sep 3-Sep 9	26.0	20.7	45.8	6	95	81
37	Sep 10-Sep 16	28.9	20.3	4.8	1	91	67
38	Sep 17-Sep 23	28.2	19.5	7.0	1	90	64
39	Sep 24-Sep 30	30.0	18.5	5.4	1	88	53
40	Oct 1-Oct 7	30.0	19.5	173.3	7	90	64
41	Oct-8-Oct 14	29.9	20.2	175.5	7	92	58
42	Oct 15-Oct 21	30.0	19.7	174.0	7	91	58
43	Oct 22-Oct 28	29.8	19.0	170.9	7	84	50
44	Oct 29-Nov 4	30.0	18.3	73.9	3	89	50
45	Nov 5-Nov 11	30.6	15.7	0.0	0	70	31
46	Nov 12-Nov 18	30.3	15.0	0.0	0	63	31
47	Nov 19-Nov 25	29.6	13.0	0.0	0	68	29
48	Nov 26-Dec 2	29.1	19.0	3.4	1	87	53
49	Dec 3-Dec 9	30.5	14.7	0.0	0	75	36
50	Dec 10-Dec 16	29.9	15.0	0.0	0	81	41
51	Dec 17-Dec 23	28.3	11.8	0.0	0	78	35
52	Dec 24-Dec 31	29.1	11.9	0.0	0	65	32
1	Jan 1-Jan 7	30.8	17.5	0.0	0	87	51
2	Jan 8-Jan 14	29.4	13.4	0.0	0	77	46
3	Jan 15-Jan 21	29.2	11.2	0.0	0	67	37
4	Jan 22-Jan 28	30.0	13.4	0.0	0	85	46
5	Jan 29-Feb 4	29.8	14.4	0.0	0	82	52
6	Feb 5-Feb 11	32.1	15.2	0.0	0	66	37
7	Feb 12-Feb 18	33.0	16.5	0.0	0	62	28
8	Feb 19-Feb 25	34.1	17.3	0.0	0	41	16
9	Feb 26-Mar 3	34.2	16.7	0.0	0	59	22
10	Mar 4-Mar 10	35.2	17.6	0.0	0	47	18
11	Mar 11-Mar 17	35.2	18.3	0.0	0	56	19
12	Mar 18-Mar 24	35.5	18.7	0.0	0	72	23
13	Mar 25-Mar 31	35.9	20.3	0.8	0	83	28

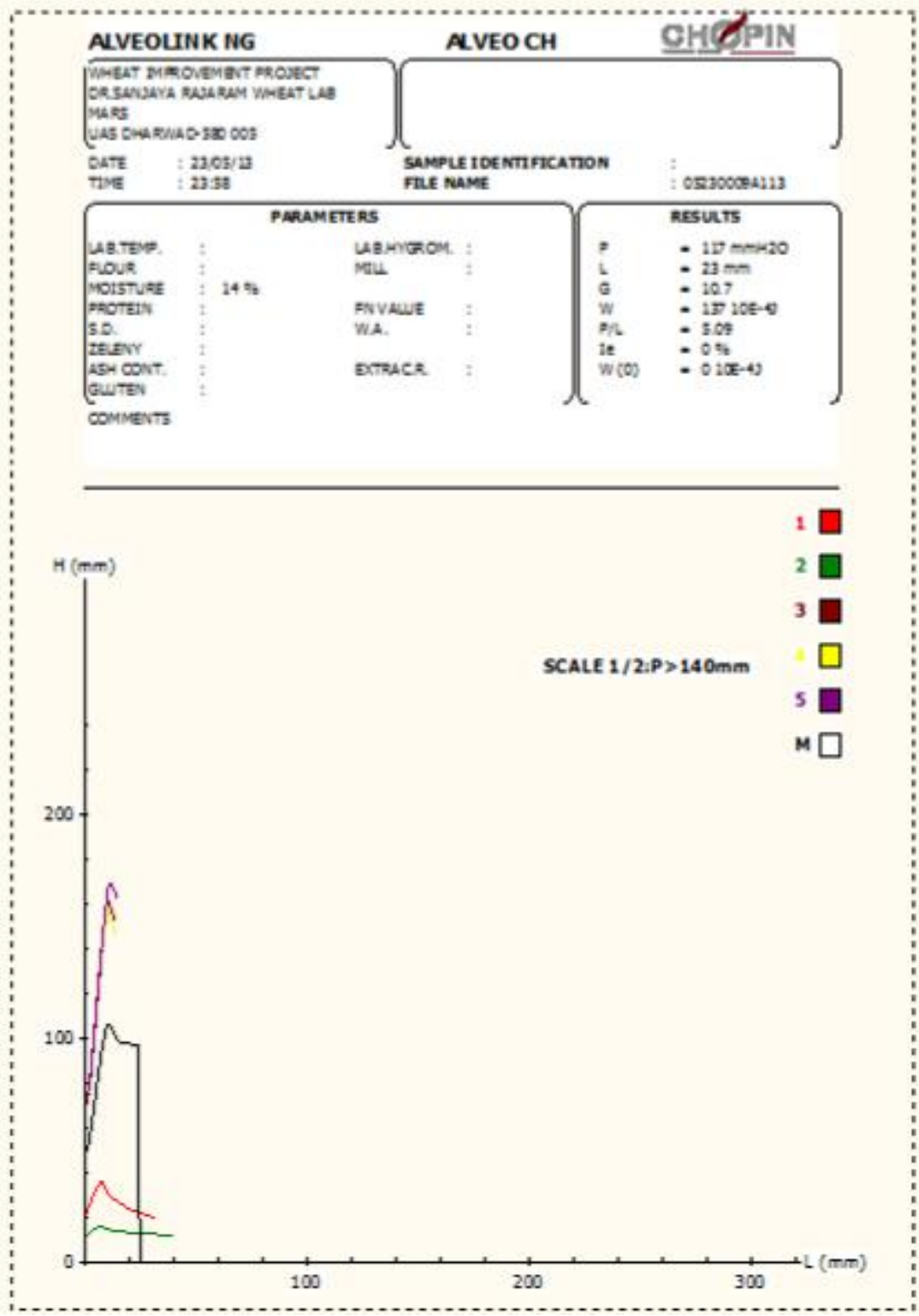
Appendix – II: Weekly Meteorological Observations of Main Agricultural Research Station, University of Agricultural Sciences, Dharwad during *Rabi* 2012-13

Meteorological Week		Temperature (^{0}C)		Rainfall (mm)	Rainy days	Relative humidity (%)	
		Mean Max	Mean Min			Morning	Evening
36	Sep 3-Sep 9	26.36	20.67	28.4	5	94	85
37	Sep 10-Sep 16	27.79	20.06	1.8	0	93	75
38	Sep 17-Sep 23	28.83	18.57	1.2	0	85	59
39	Sep 24-Sep 30	30.69	19.29	49.8	3	88	55
40	Oct 1-Oct 7	27.33	20.53	43.2	2	93	74
41	Oct-8-Oct 14	30.46	18.43	37.6	1	77	48
42	Oct 15-Oct 21	31.23	16.99	0.0	0	69	40
43	Oct 22-Oct 28	29.86	17.90	8.4	1	83	47
44	Oct 29-Nov 4	27.61	17.83	34.5	1	82	58
45	Nov 5-Nov 11	30.11	20.07	0.0	0	90	56
46	Nov 12-Nov 18	28.94	13.53	1.2	0	65	36
47	Nov 19-Nov 25	30.10	14.97	0.0	0	76	45
48	Nov 26-Dec 2	30.40	16.07	0.0	0	76	41
49	Dec 3-Dec 9	29.79	16.90	0.0	0	76	49
50	Dec 10-Dec 16	31.40	14.57	0.0	0	74	37
51	Dec 17-Dec 23	30.60	12.80	0.0	0	80	33
52	Dec 24-Dec 31	29.43	13.04	0.0	0	78	39
1	Jan 1-Jan 7	32.06	17.11	0.0	0	82	32
2	Jan 8-Jan 14	30.67	13.80	0.0	0	66	29
3	Jan 15-Jan 21	31.09	13.29	0.0	0	61	22
4	Jan 22-Jan 28	31.13	13.76	0.0	0	62	24
5	Jan 29-Feb 4	30.81	14.10	0.0	0	62	27
6	Feb 5-Feb 11	32.13	16.64	0.0	0	70	33
7	Feb 12-Feb 18	32.01	17.31	2.2	0	71	36
8	Feb 19-Feb 25	33.84	17.64	0.0	0	60	38
9	Feb 26-Mar 3	35.27	17.54	0.0	0	68	30
10	Mar 4-Mar 10	34.93	19.36	0.0	0	73	25
11	Mar 11-Mar 17	35.41	19.60	42.0	1	82	26
12	Mar 18-Mar 24	35.09	19.63	0.0	0	71	23
13	Mar 25-Mar 31	36.37	20.02	0.0	0	72	28

Appendix III: Alveograph parameters of NIAW-917 under protected condition



Appendix IV: Alveograph parameters of NIAW-917 under unprotected condition



Appendix V: Alveograph parameters of HD-2189 under protected condition

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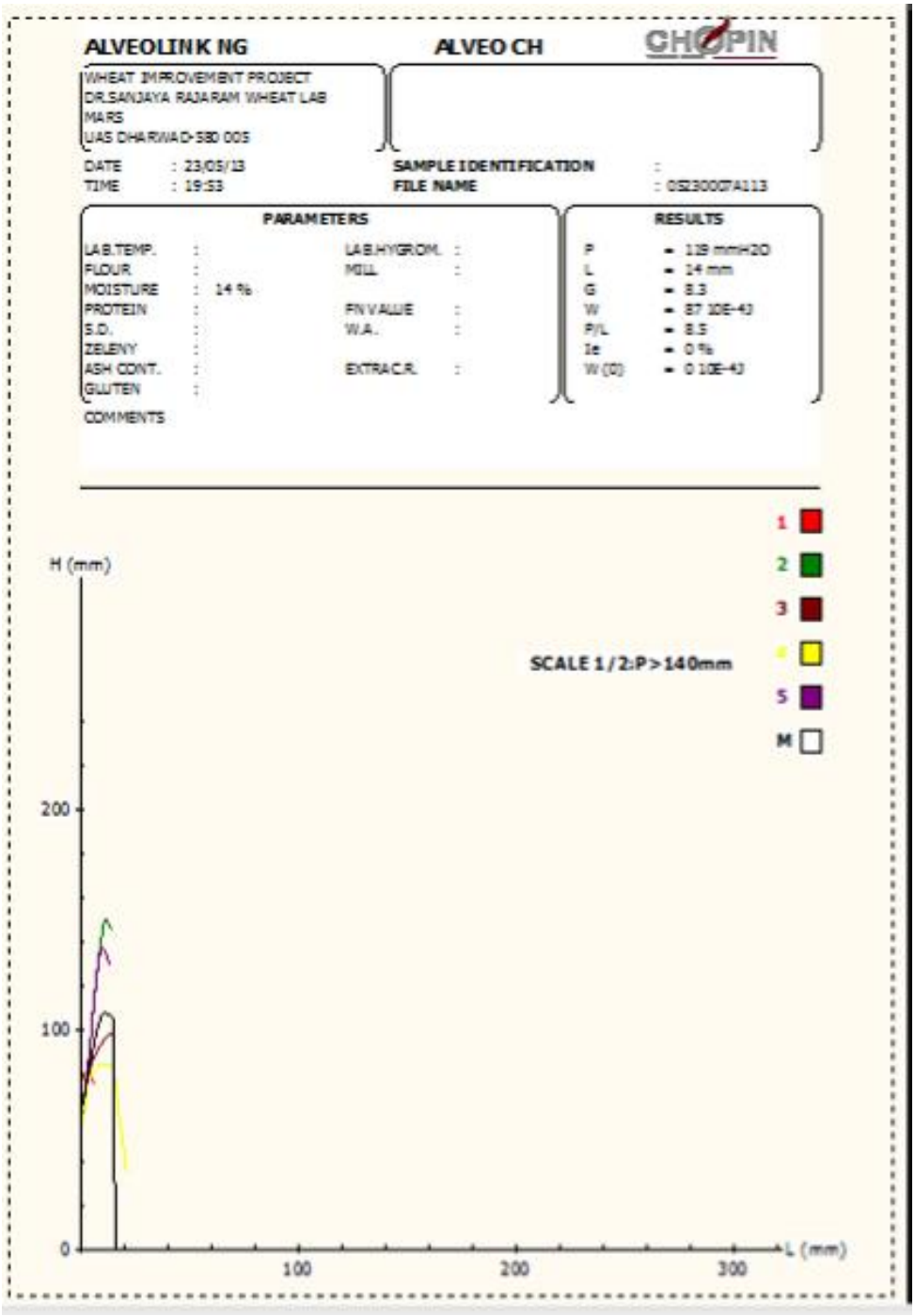
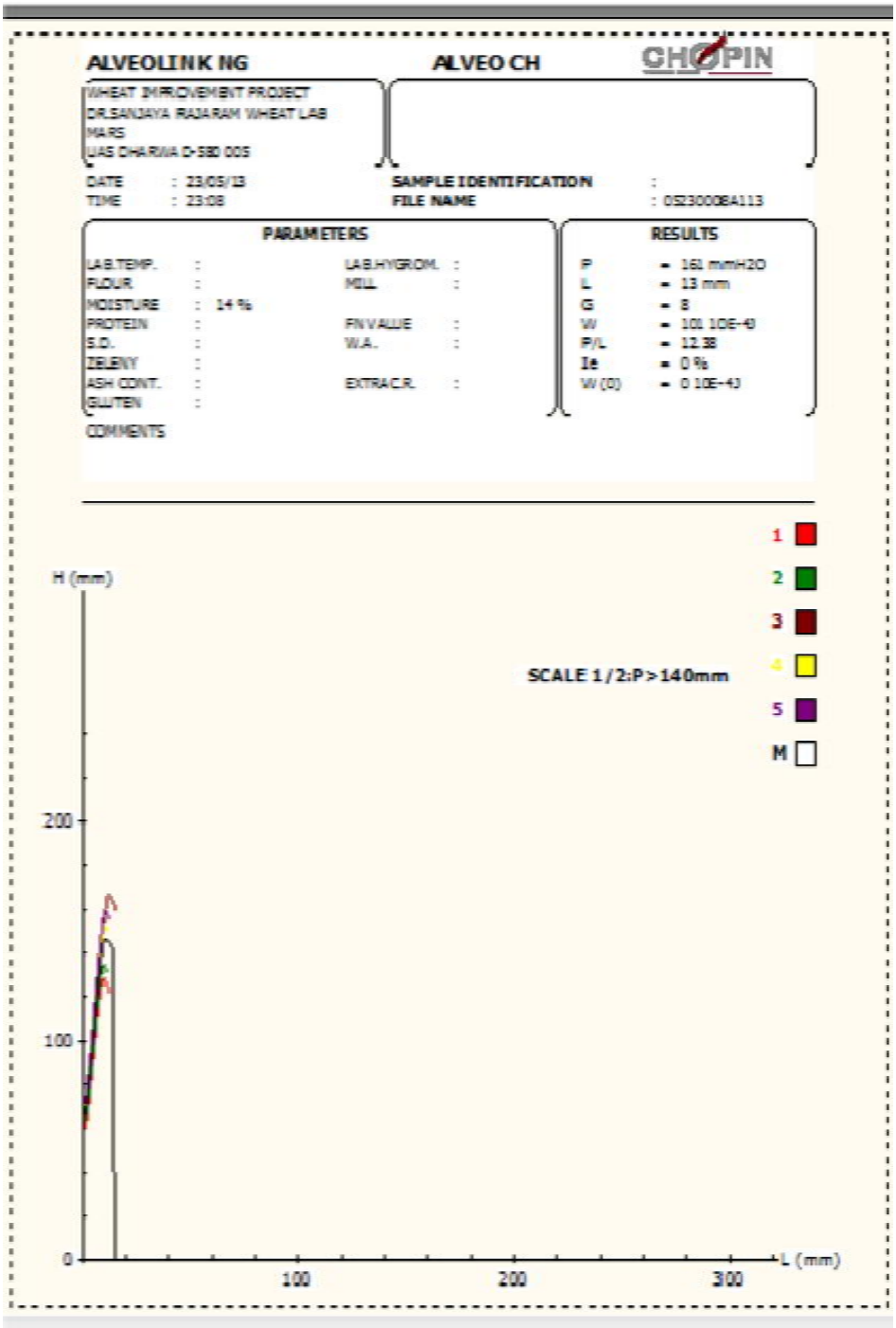
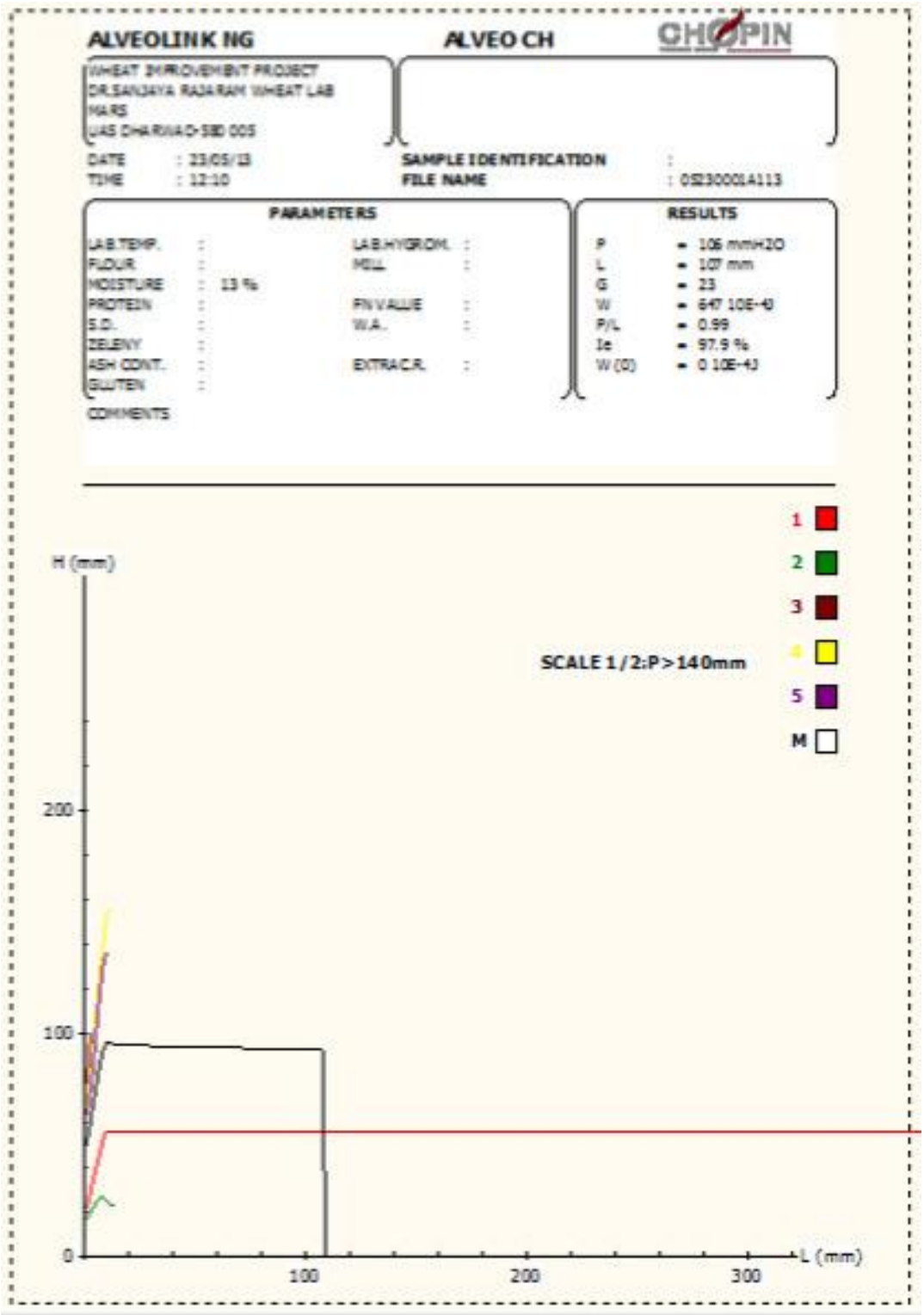


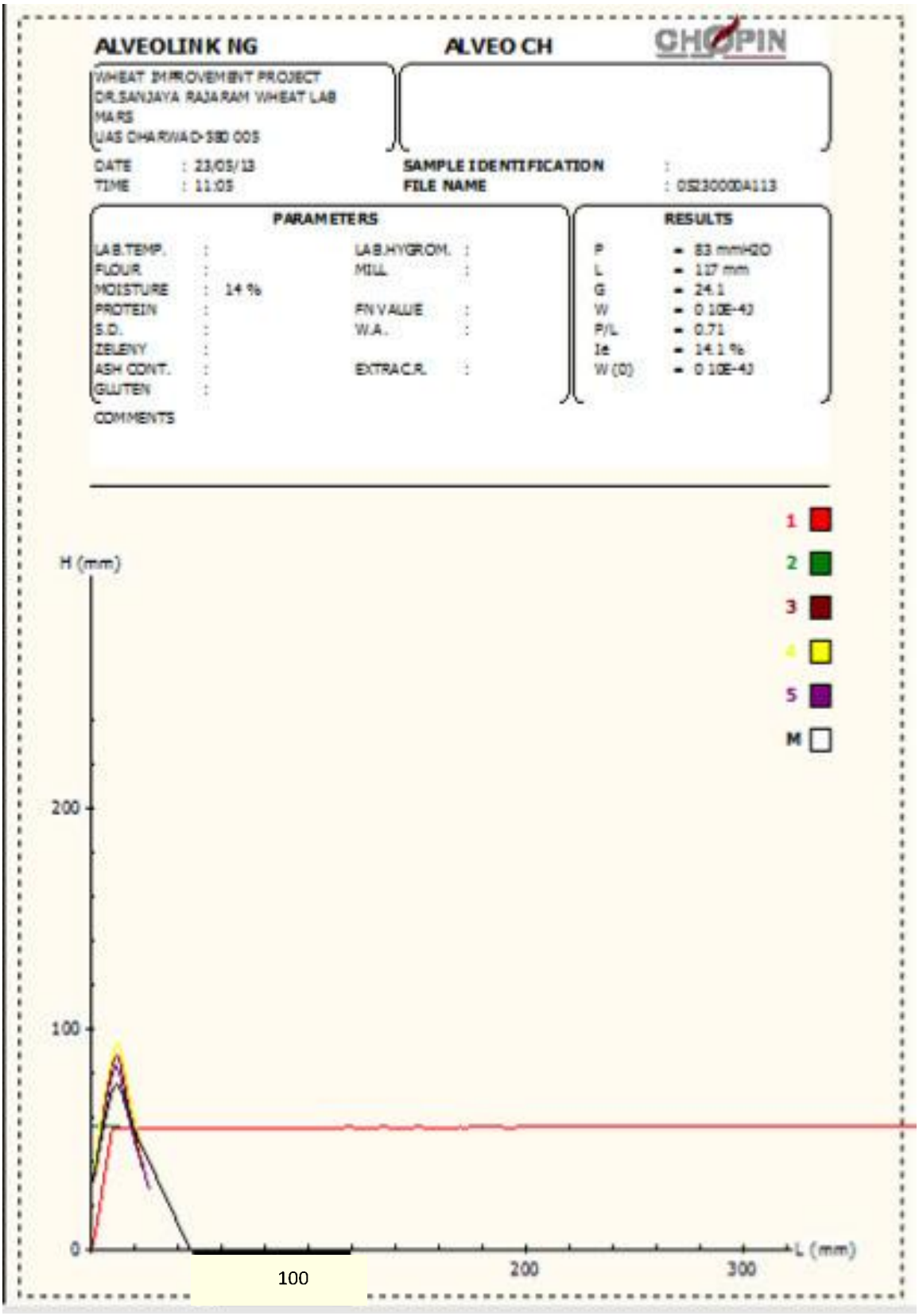
Appendix VI: Alveograph parameters of HD-2189 under unprotected condition



Appendix VII: Alveograph parameters of Agra local under protected condition



Appendix VIII: Alveograph parameters of Agra local under unprotected condition



MECHANISM OF SLOW LEAF RUSTER, MOLECULAR CHARACTERIZATION IN BREAD WHEAT AND VARIABILITY IN *Puccinia triticina* ERIKS.

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ABSTRACT

Off-season survey in Chikmagalur and Chitradurga districts of Karnataka revealed the absence of wheat crop. Three years normal season survey indicated the presence 20 different pathotypes. The pathotype 121R63-1 (77-5) was dominant and molecular profiling of 25 different isolates showed high genetic variability.

UAS-326, UAS-315, VL-616, VL-924, HD-2189, HD-2932, HD-3091, NI-5439, HI-977, HS-420, DBW-16, KRL-210, Pavon-76, RL-6077 and Parula were identified as slow leaf rusters and Agra Local, Lal Bahadur, Lok-1, Sonalika, C-306, DWR-162, PBW-343, DBW-17 and HS-240 were identified as fast leaf rusters. Molecular characterization of bread wheat showed 12 genotypes with *Lr34/Yr18/Pm38,* seven genotypes with *Lr46/Yr29/Pm39* and none of the genotypes showed *Lr*67/*Yr*46. However, five genotypes (HD-2189, UAS-315, VL-616, NW-4091 and RAJ-4270) carryed both *Lr34/Yr18/Pm38* and *Lr46/Yr29/Pm39* genes.

Isozymes study revealed a higher variation of peroxidase and polyphenol oxidase at different growth stages under inoculated and uninoculated conditions. Pre-penetration processes of uredospore were not a good criterion for selection of slow leaf rusters. Loss of total grain protein content (GPC) was either increased or decreased or no significant changes observed when compared with protected and unprotected conditions among all the three group of genotypes. No significant difference was observed in the mean damaged starch of slow leaf rusters, whereas susceptible genotypes showed significant differences. Wet gluten and dry gluten content (%) was affected by leaf rust. Slow leaf ruster genotypes showed an average highest content of three micro-nutrients, *viz* zinc, iron and copper. The average yield loss was minimum in resistant and slow leaf rusters. However, it was very high in susceptible genotypes. A combi- product (Pyraclostrobin 13.3% + Epoxiconazole 5%) @ 0.1 % was found to be the best alternative to the propiconazole.