गेहूँ में पर्ण रतुआ प्रतिरोधी जीन "एलआर सिन ५५" का आनुवंशिक विश्लेषण

GENETIC ANALYSIS OF LEAF RUST RESISTANCE GENE "*LrSyn55*" IN WHEAT

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Genetic Analysis of Leaf Rust resistance Gene "*LrSyn55*" in Wheat

By

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CERTIFICATE

This is to certify that the thesis entitled **"Genetic Analysis of Leaf Rust resistance Gene "*LrSyn55*" in Wheat'** submitted to the Faculty of Post Graduate School, Indian Agricultural Research Institute, New Delhi, in partial fulfillment of requirement of degree of **Master of Science in GENETICS AND PLANT BREEDING,** embodies the result of a bonafide research work carried out by **Mr. Anuj Kumar** under my guidance and supervision. No part of the thesis has been submitted for any other degree or diploma.

It is further certified that any help or source of information, as has been availed during the course of the investigation, has been duly acknowledged.

(S. K. Jha)

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Place: New Delhi

Dedicated to
My Beloved
Parents and my Brother
Shri Subhash Chandra
Smt. Neelam Rani
and Rajat Arora

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ABBREVIATIONS

Barc	USDA-ARS Beltsville Agricultural Research Center
BC	Backcross
CIMMYT	Centro International de Mejoramiento de Maíz y Trigo (International Maize and Wheat Improvement Center)
cM	Centi Morgan
CTAB	Cetyl trimethyl ammonium bromide
DES	Directorate of Economics and Statistics
f. sp.	Formae speciales
F_1	Filial 1
F_2	Filial 2
FAO	Food and Agriculture Organization
FYM	Farm yard manure
IIWBR	Indian Institute of Wheat & Barley Research
ITs	Infection Types
IWGSC	International Wheat Genome Sequencing Consortium
Lr	Leaf Rust
Ltn	Leaf tip necrosis
MoA	Ministry of Agriculture
NEPZ	North eastern Plain Zone
NFSM	National Food Security Mission
NILs	Near isogenic lines
Pm	Powdery mildew
RP	Resistant parent
SBLs	Synthetic derived backcross lines
SHW	Synthetic Hexaploid Wheat
SP	Susceptible parent
Sr	Stem rust
SSR	Simple Sequence Repeats
Syn45	Synthetic 45
Syn55	Synthetic 55
TC	Thatcher
Yr	Yellow rust

Chapter 1 Introduction

Wheat is also known as the King of Cereals and is one of the primarily consumed cereal crop followed by rice and maize. Wheat is staple food for 36% (around 2 Billion people) of the population and supplies nearly 20% of the protein and calories worldwide (Braun *et al.,* 2010). The requirement of wheat is probable to rise between 30% and 40% by 2030. The present rate of increment of wheat production is around 1.2% annually, and it is supposed to increase at the rate of 1.6%–1.8% to fulfill the upcoming needs. Of the likely 1.6% increase, 1% must be derived from genetic gain through breeding (Ogbonnaya *et al.,* 2013). Currently, the global wheat yield on an average is 3 t/ha but there are substantial differences among different regions of the globe (Hawkesford *et al.,* 2013). To guarantee food security, genetic diversity and germplasm improvement are key to the reliable and sustainable production of crops under the changing climatic scenario.

The major wheat producing countries are China with 24.5 mha area with 134.34 million tons production and India with 30.4 mha area with 98.5 mt production (FAO 2017). During 2018-19 wheat production in India will reach a record of 101.20 mt (3rd advance estimate DES, Ministry of agriculture and farmer's welfare) and area to a record 30.8 mha (MoA India). Wheat is cultivated as an irrigated crop in north west plane zone that includes i.e. Punjab, Haryana, Western U.P. and planes of Uttarakhand States and yields more than 4.5 t/ha, Punjab and Haryana being the major producers (Farook *et al*., 2019), whereas in area of central zone such as Gujarat, Madhya Pradesh, Rajasthan, and parts of Uttar Pradesh are less than 3.0 t/ha (NFSM 2016). Wheat cultivated in central and western regions of the country have relatively higher protein and gluten in contrast to wheat from northern India as they are grown in relatively drier conditions (https://farmer.gov.in/M_ cropstatics wheat.aspx). Along with bread wheat, India also produced about 1.0 to 1.20 million tons of durum wheat in Madhya Pradesh, Rajasthan, and Maharashtra, mostly for food processors (https://farmer.gov.in/M_ cropstatics wheat.aspx). In spite of this, considering ever growing population of the country we need to produce more than current production to meet out the future demand.

In recent decades, genetic uniformity among the cultivars has been increased to a very high extent which is mainly due to common parents of most of the varieties grown presently and this uniformity increases their vulnerability to insect pest and diseases (Warburton *et al.*

2006) and to combat this problem genetic variability should be incorporated continuously, this will provide buffer against prevalent risks associated. So, to augment the genetic variability novel sources needs to be identified. The sources of variation can be elite lines, obsolete varieties, land races, wild relatives and lines developed using introgression from diverse sources.

Among the several abiotic and biotic constraints, wheat rusts are predominant and present throughout the wheat cultivated zones (Roelfs *et al.,* 1992) and causes maximum damage to wheat yield globally. Wheat rusts belongs to genus *Puccinia*, a genus of reasonably greatest detrimental fungi (Hooker, 1967). This comprises "leaf/orange/brown rust (*Puccinia triticina*), stem/black rust (*P. graminis tritici*) and stripe/yellow rust (*P. striiformis*)". Rusts essentially require living host (biotrophic parasites) and have a limited host range. Leaf rust is the most frequent among rusts and can infect wheat at all growing conditions (Samborski, 1985). Brown rust is the cause of substantial economic damages because of its recurrent infection cycles in the favourable environmental conditions throughout the crop growth period. (Huerta-Espino *et al.*, 2011). Global annual economic damages to wheat yields only due to rust fungi are valued at US $ 4–5 billion (Figueroa *et al*., 2018).

Significant losses have been stated by leaf, stripe and stem rusts in congenial conditions (Roelfs *et al.,* 1992) across the wheat growing countries (Joshi and Palmer, 1973; Wiese, 1977; Green and Campbell, 1979; Roelfs, 1978; Kolmer, 2001; Park, 2007). In India, wheat's rust outbreaks have been stated continuously (Joshi, 1976). Yearly damages vary from 15-20 % globally, owing to the rusts that is roughly about twenty to thirty million tons reduction in the production of the wheat (Hanson *et al.,* 1982), based on the time of rust outbreaks and the stage of crop growth (Chester, 1946; Joshi and Palmer, 1973).

Owing to its obligate parasitic nature rust has intrinsic capability of rapid development of novel pathotypes, which have the capacity to overcome the existing resistance genes (Mcintosh *et al.,* 1995). Across India, almost 120 races were identified systematically for all of the rusts, 52 of which belongs to the brown rust documented during 1931to 2015. Sixteen different pathotypes of *P. triticina* were recognized in last fifteen years (from 2000 to 2015). Several useful seedling resistance *Lr* genes including *Lr9*, *Lr19* and *Lr28* have become susceptible (Nayar *et al.,* 2003; Bhardwaj *et al.,* 2005, 2010a and 2011). Majority of the *T.*

aestivum based leaf rust resistance genes have been rendered ineffective by the most virulent and prevalent pathotype 77-5 in the country (Prasad *et al.*, 2017). Commonly used *Lr* resistance genes in rust resistance breeding programs in India are "*Lr1, Lr3, Lr9, Lr10, Lr13, Lr14a, Lr17, Lr19, Lr23, Lr24, Lr26, Lr28* and *Lr34"* (Bhardwaj *et al.*, 2010b), but only *Lr24* is still an effective all stage/Seedling resistance gene. Although, numerous virulent pathotypes have been identified to have virulence against *Lr24* in other parts of the world. (Singh, 1991). The present scenario clearly dictates that there is an urgent need for the broadening of rust resistance genes in Indian wheat breeding program. Hence, there is a requirement for the constant exploration of unique and novel sources of resistance genes. There have been regular initiatives by geneticists and breeders around the world to look for new and effective sources of resistance. Till now, 79 leaf rust resistance (*Lr*) genes have been designated to date and several of them have been utilised in the wheat improvement projects (McIntosh *et al.*, 1995, 2008, 2017, Qureshi *et al.*, 2018).

Alternate sources of resistance consisting primarily of various *Triticum* species and their descendants are believed to have huge quantities of unexploited genetic variation for useful characteristics such as biotic and abiotic stresses, yield, biomass etc. (Khush and Brar, 1992; Jiang *et al.*,1994). Common wheat, D genome donor *Triticum tauschii* have been considered as a possible source of valuable genes and many comprehensive efforts have been made to incorporate these valuable genes into modern wheat (Cox *et al.*, 1994).

Goat grass (*Trticum tauschii* Coss. Schmal), is known to provide resistance to several major wheat diseases like Rust, Karnal bunt, Powdery mildew, and to significant abiotic stresses like salinity, heat, drought and cold sensitivity (Dyck and Kerber, 1970; Kerber and Dyck, 1978; Hatchett and Gill, 1981; Martin *et al.*, 1982; Sharma and Gill 1983; Kerber, 1984; Gill *et al.*, 1985 and Gill and Raupp, 1987; Assefa and Fehrmann, 2004; Ryan *et al*., 2010; Pradhan *et al*., 2012; Ogbonnaya *et al*., 2013; Kalia *et al*., 2017; Nevo *et al.*, 2010; Rasheed *et al.*, 2018; Rawat., 2018).

At International Maize and Wheat Improvement Center (CIMMYT), several diverse accessions of *T. tauschii* and *T. turgidum* were crossed followed by chromosomes doubling of F_1 using colchicine resulted in 2n = 6X= 42 chromosome possessing Synthetic Hexaploid Wheats (SHWs) (Mujeeb-Kazi *et al.*, 1995) for effective gene transfer from D genome progenitor. CIMMYT has developed more than 1000 SHWs line (Das, 2016). However, due to the existence of

agronomically unwanted traits such as tenacious glumes and non-threshability, SHWs itself cannot be used as for cultivation. The SHWs can be directly crossed with common bread wheat deprived of any sterility and cytological interference. The SHWs hold tremendous genetic variability for resistance related to biotic/abiotic stresses, morpho-agronomic traits (Cox *et.al*., 1994). The resistance to biotic stresses identified in these SHWs include Hessian fly (Yu *et al.* 2009, 2010, 2012), greenbug (Weng *et al.* 2005), tan spot (Siedler *et al.* 1994; Xu *et al.* 2004; Tadesse *et al.* 2007), Spot blotch (Mujeeb-Kazi *et al.* 2001a), Karnal bunt (Villareal *et al.* 1996; Mujeeb-Kazi *et al.* 2001b, 2008), stem rust (Marais *et al.* 1994), leaf rust (Assefa and Fehrmann 2000; Gyani *et al., 2017),* Septoria tritici leaf blotch (STB) (Arraiano et al. 2001), Fusarium head blight (Mujeeb-Kazi et al. 2001b), stripe rust (Ogbonnaya *et al.* 2008), cereal cyst nematode (Eastwood *et al.* 1991).

Considering the effectiveness of resistance to various pathotypes, establishment of genetic nature and novelty is essential for exploiting new sources of resistance in wheat improvement programme. Division of Genetics, IARI, New Delhi is maintaining a set of 95 SHWs procured from CIMMYT. Previous studies on SHWs maintained at division with selected pathotypes of leaf rust revealed the presence of broad spectrum and effective resistance in Synthetic55. Further, genetic analysis has identified leaf rust resistance gene in Synthetic55 found a dominant gene to be located on chromosome 1DS of wheat (Singh, 2017). As there are four other genes (*Lr21, Lr42, Lr60* and *LrSyn45*) known to be located on similar position. Therefore, to understand the allelic relation and novelty of leaf rust resistance gene "*Lrsyn55*", the M.Sc. thesis problem titled, **"Genetic analysis of leaf rust resistance gene "*LrSyn55*" in Wheat"** was commenced comprehending these objectives:

1. Analysing effectiveness of leaf rust resistance genes present on chromosome 1D (*Lrsyn55*, *Lrsyn45*, *Lr21*, *Lr42* and *Lr60*) against diverse leaf rust pathotypes.

2. Understanding allelic relationship of leaf rust resistance gene '*Lrsyn55'* with other known leaf rust resistance on chromosome 1D

Chapter 2 Review of Literature

Wheat origin took place around 10,000 years ago in the Fertile Crescent and from there it is distributed to the other parts of the world. Several species have evolved from the primitive diploid progenitor that have 7 pairs of chromosomes, now mostly tetraploid (4X) and hexaploid (6X) species are under cultivation. *Triticum aestivum*, common bread wheat (2n=6X= 42, AABBDD), is an annual grass in the Poaceae (grass family) having a center of origin from Mediterranean region and southwest Asia (Matsuoka., 2011). According to FAO approximations that global marketable production of all types of wheat was 771.71 million tons in 2017, harvested from 218.54 million hectares. Major producers are China, India, the U.S., the Russian Federation, and France. Depending on the growth pattern, wheat is grouped into classes i.e., winter wheat and spring wheat, covering about 35% and 65% of the world's total area of wheat production, correspondingly (Braun *et al*., 2010; Sãulescu and Braun, 2001). At present, three species of wheat are grown commercially in India these are "*Triticum aestivum* (common bread wheat), *Triticum durum* (macaroni wheat) and *Triticum dicoccum* (emmer wheat)". *Triticum aestivum* supplies 95% of the total production followed by macaroni wheat, 4% and emmer wheat 1% (Gireesh *et al.*, 2014). Bread wheat is mainly cultivated in Punjab, Haryana, Uttar Pradesh, Punjab, Rajasthan, Bihar, and some regions Madhya Pradesh, Gujarat, and Maharashtra. Durum Wheat is grown in central and peninsular India whereas Emmer Wheat is grown in the Tamil Nadu, Karnataka, and Maharashtra.

2.1. Host: Wheat

2.1.1. Taxonomy

Wheat is taxonomically placed in phylum *Angiospermatophyta*, class *Monocotyledonopsida*, order *Poales*, family *Poaceae*, sub-family *Pooideae*, carrying around 500 species in 26 genus (Bálint., 2000). Carl Linnaeus designated common wheat as *Triticum aestivum* L. All of the species in the genus Triticum are categorized into three groups: einkorn, emmer, and spelt. (Schulz., 1913). The number of chromosomes in various cultivated wheat species of genus *Triticum* were reported as 2n=14, 28 and 42 (Sakamura and Sax 1918). Alone genus *Triticum* involves about 300 species (Clayton and Renvoize, 1986). Bread wheat is a "normal disomic allohexaploid (2n=6x=42) with A, B, and D as its three diverse genomes"

(Sears, 1954; Okamoto, 1962) each of them is donated from a different progenitor. The amount of DNA in the gamete of common wheat is nearly 1.7 x 10^{10} bp, with a mean of 810 Mb for each chromosome. The draft sequence of wheat contains 1,24,201 annotated gene loci (Ling *et al.*, 2013; Choulet *et al.*, 2014, Meyer *et al.*, 2014). Physical maps for all chromosomes were completed by International Wheat Genome Sequencing Consortium (IWGSC) in 2015. From 2018, reference sequence resources are freely available for public use (IWGSC 2018).

2.1.2. Evolution

The cultivation of wild einkorn and emmer wheat about 10,000 years back marked the evolution of human from a hunter-gatherer to an agrarian (Eckardt,2010). Common Bread wheat is evolved by two polyploidization events, the first neotetraploidization event took place between *Triticum urartu* (AA genome) as a male parent and an *Aegilops speltoides*-associated species (BB) as a cytoplasm donor 0.5 million years ago, giving rise to *Triticum turgidum ssp. diccocoides* (2n=4x=28, AABB genome), and the second hexaploidization event took place between *Triticum turgidum ssp. durum* (AABB genome) as a cytoplasm donor and *Aegilops tauschii* (DD genome) as a pollinator about 10,000 years ago, resulting in the modern hexaploid bread wheat (AABBDD genome) (Feldman 1995, Ozkan *et al*., 2001; Huang *et al*., 2002, Salse *et al.*, 2008). Enhancement in grain size and the advancement of non-shattering seeds are two main features in the evolution of bread wheat and various cultivated grasses (Eckardt, 2010). Species like *Triticum zhukovskyi* Menabde and Ericz. ($A^mA^mA^tA^tGG$ genome), *Triticum urartu* Tumanian ex Gandilyan (AA genome), *Triticum timopheevii* Zhuk. (A^tA^tGG genome), *Triticum aestivum* L. (AABBDD genome), *Triticum turgidum* L. (AABB genome), and *Triticum monococcum* L. (AA genome) is believed to have its origin in the 'Fertile Crescent' encompassing South-East Turkey, Eastern Mediterranean, Northern and Western Iran, Northern Iraq (Matsuoka, 2011).

The "A genome" is the fundamental genome of common wheat that is mainly concerned with the growth, cultivation under human management and genetic advancement of wheat. T. *urartu* (wild einkorn) is the plausible ancestor species of the A genome as it is nearly similar to common bread wheat particularly regarding to morphology and spike development (Johnson and Dhaliwal, 1976; Ling *et al*., 2013). *T. monococcum* and *T. urartu* are the lineages of diploid AA genome species (Huang *et al*., 2002). Crossing with "*Aegilops* species followed

by the chromosome doubling of subsequent hybrid eventually led to allopolyploidization and diversification of *Triticum* species, *T. timopheevii* (AtAtGG genome) and *T. turgidum* (AABB genome) are the outcomes of two major separate polyploidization events backed up by crossing (Sarkar and Stebbins, 1956; Shands and Kimber, 1973; Chapman *et al*., 1976; Dvořák, 1976). After that, these species were domesticated and grown in the Fertile Crescent of the Middle East (Feldman, 2001; Salamini *et al.*, 2002)". Massive studies by McFadden and Sears (1944) and Kihara (1944) proposed that *T. aestivum* is originated from the cross of *T. turgidum* with *Aegilops squarrosa.*

2.1.3. Gene Pool

Common wheat is a typical example for characterization of structural, functional, and epigenetic changes in an allopolyploid genome (Li A. *et al*., 2018). The genus "*Triticum* consists of six species: *Triticum monococcum L*. (AA genome); *Triticum urartu Tumanian ex Gandilyan* (AA genome); *Triticum turgidum L.* (AABB genome); *Triticum timopheevii Zhuk*. (AAGG genome); *Triticum aestivum L.* (AABBDD genome); and *Triticum zhukovskyi Menabde & Ericz*. ($A^mA^mA^tA^tGG$ genome). These species are classified into three sections: Sect. Monococcon (comprising of diploid species); Sect. Dicoccoidea (comprising of tetraploid species); and Sect. Triticum (comprising of hexaploid species)" (Bálint., 2000). Among the above-mentioned species, only *T. urartu* persists in its undomesticated form, while *T. aestivum* and *T. zhukovskyi* persists only as cultivated forms. The other species, *T. turgidum*(AABB), *T. monococcum*(AA) and *T. timopheevii* (AAGG), have both a wild and a domesticated form (Matsuoka 2011).

The primary gene pool comprises of the species that have chromosomes homologous to bread wheat that allows easy pairing and recombination between these chromosomes. The species carrying "AA genome can be simply crossed with tetraploid AABB and hexaploid AABBDD species but may require particular intrusions like cytology, backcrossing and embryo rescue as their F_1 are sterile". Therefore, the primary gene pool can be utilized for crop enhancement.

The "secondary gene pool of wheat comprises of species that have a minimum of one genome homologous to common wheat such as *Triticum* and *Aegilops* species, and the diploid *Aegilops* with S-genome (associated to the B sub-genome). Gene transmission is feasible

among homologous chromosomes via precise recombination with little to moderate difficulties". Current findings *in Ae. Tauschii* has recognized agronomically useful traits that may be lacking in bread wheat (Mondal *et al*., 2016; Vikram *et al*., 2016, Arora *et al*., 2018).

Tertiary gene pool includes species that have genomes not associated to any of the genomes of the cultivated wheat. Thus, gene transfer is very difficult. Different techniques such as irradiation, *Ph*1 manipulations (using monosomic of 5B and *Ph1* mutants). It consists of those species of the *Triticeae* which do not fits in the primary or secondary gene pools e.g. A*egilops triuncialis* (UUCC), *Aegilops caudata* (CC), *Thinopyrum* (EE), Secale (RR).

2.1.4. Synthetic hexaploid wheat and its constitution

Synthetic hexaploid wheat (SHW) is taken into account as a very important tool for transferring numerous useful genes from *Aegilops tauschi* Coss. to cultivated wheat. *Aegilops tauschi*, the patron of the D genome of cultivated wheat has several attractive genes for wheat improvement (Mujeeb-Kazi and Rajaram 2002). Genomic regions of *Aegilops tauschii* can subsidize to nearly 10% upsurge in grain weight (Röder *et al*.,2008) and increase grain yield (Börner *et al*., 2015). Kihara *et al*., 1957 and Kerber and Dyck, 1978 advised a way for the creation of synthetic hexaploid wheat to use *T. tauschii* as a contributor of resistance genes for biotic and abiotic stresses and supplementary agronomically useful traits. They produced "synthetic hexaploid wheat by crossing *T. tauschii* as a male parent to the *Triticum turgidum L. var. durum* and doubling the chromosomes of subsequent sterile F_1 hybrid from the cross. Another technique devised by Mujeeb-Kazi *et al*., (1987) involved crossing, embryo rescue followed by treatment with colchicine for the transfer of gene(s) from *T. tauschii* to common wheat and had developed synthetic hexaploid wheat". For the very first time SHW was used to improve germplasm for resistance against biotic stresses (Mujeeb-Kazi and Asiedu 1990) and explicitly to the Karnal bunt (Warburton *et al*., 2006). During the period from 1987 to 2003 nearly 1000 of SHW lines were established at CIMMYT from an extensive collection of *A. tauschii* accessions (Mujeeb-Kazi *et al.*, 1995, 2001b).

Synthetic Hexaploid Wheat is described to hold resistance for many biotic stresses. These comprise resistance to "Hessian fly (Yu *et al.*, 2012, Morgounov *et al*., 2018), greenbug (Weng *et al.*, 2005, Crespo-Herrera *et al*., 2019), Karnal bunt (Mujeeb-Kazi *et al.*, 2008, Emebiri *et al.*,2019), stem rust (Ogbonnaya *et al.*, 2008, Periyannan *et al*.,2014), leaf rust

(Assefa and Fehrmann, 2000, Gyani et al., 2017), Septoria nodorum blotch (Loughman *et al.*, 2001), Septoria tritici leaf blotch (Arraiano *et al.*, 2001), Fusarium head blight (Szabo-Hever *et al.*, 2018), tan spot (Tadesse *et al.*, 2007), cereal cyst nematode (Eastwood *et al.*, 1991). Greater variability of HMW-GS (William *et al*., 1993, Doneva *et al*., 2018), Salinity (Schachtman *et al*., 1992, Pritchard *et al*.,2002), Cereal cyst nematode(Mulki *et al*., 2012) and root lesion nematodes" (Thompson, 2008, Mulki *et al*. 2012).

SHW is poor in agronomic performance and harbor many undesirable genes such as tenacious glumes and non-threshability (Li A. *et al.*, 2018). However, due to their hexaploid nature, they make crossing work simple and fruitful with the bread wheat as compared to diploid and tetraploid progenitors in breeding programs. These SHW can be tested for desirable traits and the selected accessions can be used to develop synthetic derived backcross lines (SBLs). SBLs are agronomically comparable to the enhanced recurrent parents but carry the introgressed characters under selection. SBLs have been enumerated as new sources for disease resistance including against Karnal bunt, *Septoria tritici* blotch, Helminthosporium leaf blight (Mujeeb-Kazi *et al*. 2001a, 2001b), and for higher micronutrient concentration in the grain (Calderini and Ortiz-Monasterio 2003).

SBLs show a substantial expansion in diversity in contrast to their parents. Many countries around the world have registered as many as 62 SBLs as cultivars. China released Chuanmai 42 variety at commercial scale and it was the first CIMMYT synthetic derivative variety released in the world. Since 2008, India has released 10 synthetics derived cultivar for breeding purpose. India has released 2 cultivars namely HPBW 01 and PBW 677 for breeding purpose in 2016 (Li A. *et al.*, 2018).

Around one-fourth of the world's population is affected by severe health problems triggered due to deficiency of Fe (Mclean *et al*., 2009). Synthetic wheat can be used for developing micronutrient-rich "biofortified" wheat (Calderini and Ortiz-Monasterio 2003). Both micronutrients and macronutrients are present in higher concentrations in many synthetic hexaploids. CIMMYT has produced Zinc-rich and superior-yielding SBLs (Guzmán *et al.*, 2014), and many wheat cultivars are released in India namely "Zinc Shakti, WB2, and HPBW 01" (Li A. *et al.* 2018).

SHWs forms the potential basis of genetic variability for abiotic stress tolerance in wheat. SBLs may offer up to 45% increase in yield as compared to their common wheat parents under drought conditions (Trethowan and Mujeeb-Kazi 2008). Synthetic varieties have thicker roots that can develop deeper roots into the soil.

Synthetic hexaploid wheat is a very useful genetic tool that should be exploited to transfer agronomically superior genes from a broad array of tetraploid or diploid donors, as well as wild species, for increasing the genetic diversity of common wheat. Synthetic hexaploid wheat, furnished with its broad genetic base from wild donor species, is expected to play a greater role to meet upcoming environmental challenges (Li A. *et al.* 2018).

2.2 Pathogen: Wheat rust fungi

2.2.1. Taxonomy and initial identification

Rust pathogens of wheat fall under *Puccinia* genus of the family Pucciniaceae of order Uredinales that is placed in the class Basidiomycetes (Littlefield 1981). Rusts are obligate biotrophs and requires a living host for their multiplication (Voegele & Mendgen, 2009; Kemen *et al*., 2015; Dracatos *et al*., 2018). Rust spread by airborne urediniospores from one graminae plant to another plant and from the field to field and rapidly grow under conducive weather conditions. Primary inoculum originates from volunteer plants (Singh *et al.* 1991). The three rust diseases affecting wheat are stem rust, leaf rust, and stripe rust. In India, due to the presence of diverse climatic conditions all kinds of rusts are identified to be existent in diverse agro-climatic regions. “Stripe/Yellow rust of wheat is common in cool climates of Northern India. Stem/Black rust of wheat is limited to Peninsular and Central India. Among all the rusts, leaf/brown rust is prevalent and infect the wheat crop wherever it is grown”.

In India during 1786 to 1956, 17 significant epidemics of wheat rusts were recorded of which 1786, 1805, 1828-29, 1831-32, 1879, 1887 and 1907 were extremely devastating (Nagarajan and Joshi.,1975). In India during “1946-47, a serious stem rust epidemic caused an economic damage of around 2 million tons of wheat in the central India” (Joshi *et al.*, 1986). Globally, overall estimated loss due to rust fungi is the US $4–5 billion to wheat production annually (Figueroa *et al*., 2018). Rust fungi are put under thorough investigation and

supervision against their possibly damaging effects on global crop production (Singh *et al*., 2011; Bueno-Sancho *et al*., 2017).

Dr. Karam Chand Mehta in 1931 started organized study of wheat rusts in India. “Stem rust spores sustain in southern India's Nilgiri and Palney hills under adverse circumstances, that in turn become the cause of disease in central and coastal areas during the growing season (Nagarajan and Joshi 1975). The P. striiiformis that flourish at lower temperature conditions survive throughout the year in the Himalayan regions and acts as a major source of infection in Punjab, Himachal Pradesh, Uttarakhand, Western Uttar Pradesh, Haryana, and northern Rajasthan wheat growing areas. Leaf rust fungi thrives under moderate climatic conditions and thus survives and spreads throughout India” (Nagarajan and Joshi, 1985).

Extreme leaf rust infestations took place in northwestern India during 1971–1972 and 1972–1973. K68 suffered as loss of 24.1% whereas losses for Kalyansona and Sonalika were estimated 5.9 and 2.0%, respectively (Joshi *et al*., 1976). In 1978, WL711 the most popular variety of northwest India suffered the epidemic a leaf rust (Nagarajan and Joshi 1975). In 1993, severe epidemics of leaf rust occurred in North-West India over an area of 4 million hectares under HD2285 and HD2329 varieties that lead to the substitution of the above varieties with UP2338, PBW343, and WH542.

2.2.2. Lifecycle of Pathogen and its Host range

Rusts are placed in phylum Basidiomycota under the class Basidiomycetes under the order *Uredinales* and family *Pucciniaceae* that encompasses 17 genus and 4121 species. Most of them belong to the genus *Puccinia* (Kirk *et al.,* 2001). Rust is an obligate biotrophic fungus having a macrocyclic life cycle. Cereal Rusts are heteroecious fungi producing five different kinds of spores during asexual reproduction. Sexual reproduction only takes place during the resting spore stage. Based on host range and spore size, varieties and *formae speciales* have been proposed for numerous rust pathogen attacking cereals (Dinoor *et al.,* 1988; Leonard and Szabo 2005, Aime *et al*.,2018).

John Craigie was the first person to study the rust pathogen in 1927. There are 5 kinds of spores of rust namely Pycniospore(I), Aeciospore(II), Urediospore(III), Teliospore(IV) and Basidiospore(V). “Teliospores are the resting spores of most of the rusts that may stay alive up

to few months in absence of a host plant. Wheat rusts because of its heteroecious nature requires two unrelated host plants to complete its life cycle. Barberry (*Berberis vulgaris L.*) is a well-recognized alternate host for stem rust (*P. graminis*) while *Thalictrum* spp. for leaf rust (*P. triticina*), alternate host for stripe rust is not known yet. Stem and leaf ruts are called macrocyclic rusts as they produce all five spore stages i.e. urediniospores (2n), teliospores (2n), basidiospores (n), pycniospores (n) and aeciospores (2n)" whereas Stripe rust is a microcyclic rust because its life cycle involves only urediniospores (2n) and teliospores (2n) stage and urediniospores are the only source of inoculum (Singh *et al.,* 2004).

2.2.3. Occurrence

Leaf Rust is a key disease of wheat causing great economic losses all over the world (Samborski 1985; Roelfs *et al*., 1992). Among all the three types of wheat rusts, Brown rust is the most widespread and most frequent in occurrence. Wheat leaf (Brown) and stem (Black) rusts causes loss up to 30% in leaf rust susceptible cultivars in Australia (Rees and Platz 1975) and up to 55% in cultivars susceptible to both Black and Brown rusts (Keed and White 1971). During 1992, Leaf rust epidemic caused yield loss up to 37% in susceptible cultivars in Australia.

Brown rust is presently the most prevalent and severe disease of common wheat in South America. Loss caused due to leaf rust can be over 50% in case of serious epidemics if proper management practices are not used. From 1996-2003 damages caused by leaf rust valued was the US $ 170 million on 10 commonly grown cultivars. In 1976–1977 at Northwest Mexico, a leaf rust epidemic damaged the cultivated area of the variety Jupateco 73. Highly infected seedlings caused a reduction in harvest by up to 40% (Dubin and Torres, 1981). Leaf rust is an extensively distributed and regularly occurring disease in the Indian sub-continent (Joshi, 1976). The first ever Black rust outbreak reported during 1786 A.D. in central India (Joshi and Nagarajan 1975). In 1946 - 47 rust epidemics caused a loss of nearly 2 million tons of wheat (Asthana, 1948). Brown and Yellow rust appeared in a widespread form in the Northwestern region of the country resulted in the loss of 8 to 15 lakh tons of wheat in 1971-72 an 1972-73 (Joshi, 1976). During the early 1980s, Bihar suffered damage of nearly 1 million ton (Joshi *et al.,* 1986). Leaf rust spreads from both Northern and Southern Indian hills (Mehta, 1952). The long-distance dissemination of leaf rust towards Central India from the southern

foci is reported to be related with cyclonic rains (Nagarajan and Singh 1973, 1976). Cultivation of Kalyansona was withdrawn from Northwestern India due to stripe and leaf rusts epidemic of 1971-72 and 1972-73 and was replaced by resistant cultivars such as WL 711, Sonalika, and others.

2.2.4. Nomenclature and Pathotypes

Leaf rust of wheat was documented as a different species from other species of rusts in 1815 by Augustin de Candolle, he designated it as *Uredo rubigo-vera* (DC). "Later Winter (1884) placed wheat brown rust (leaf rust) in *Puccinia rubigo-vera*. Eriksson (1899) described wheat leaf rust as a single species. Mains and Jackson (1921, 1926) established a physiological specialization in leaf rust". *P. rubigo-vera* was further classified into 56 *formae speciales* (*f. sp.*), one of them, *f. sp*. tritici, matched to Eriksson's *P. triticina* (Mains, 1926). Cummins and Caldwell proposed the name for the leaf rusts of grasses based on spore morphology and telial hosts of several species of grasses, wild wheat and rye they called it as *Puccinia recondita* (Cummins and Caldwell, 1956). Now, it is renamed as "*P. triticina* Eriks. due to specificity to wheat" (Bolton *et al.,* 2008, Anikster *et al.,* 1997).

2.2.5. Avirulence/Virulence formula

Wheat leaf rust caused by *P. triticina* can be further partitioned by the responses of genetically diverse lines of wheat (host) to pure isolates of the pathogen. Mains and Jackson (1921) were the first to report such physiologic specialization in wheat leaf rust. They identified 12 physiologic races on 11 differential cultivars (Mains and Jackson, 1926). The cultivars were Malakof, Hussar, Democrat, Webster, Norka, Turkey 47, Mediterranean, Carina, Brevit, Similis, and Loros. Out of these 11 differentials, 3 were dropped later in 1932 (Johnston and Mains, 1932). Revised differential sets for leaf rust were developed by Johnston and Browder (1966) and it was named as "1966 standard differentials or international differential set". Afterward, backcross lines encompassing single genes for resistance to leaf rust were established and used as differential hosts for race investigations. These backcrossed single-gene lines provided an efficient means of characterizing the parasite populations. "Most of the countries in the world use a standard set of near-isogenic lines derived from Thatcher developed by P. L. Dyck at Winnipeg, Canada (McIntosh *et al.,* 1995). United States of America and Canada uses a set of 12 near-isogenic lines (NILs) in the background Thatcher

as differentials" (Long and Kolmer 1989). In India Nagarajan *et al.*, (1983) modified the differentials based on the binomial system and developed a new differential set. This involves three differential sets namely Set 0, Set A, Set B. Set 0 comprises the nine cultivated varieties, Set A consists of nine NILs containing one gene for resistance in the background of Thatcher and Set B comprises seven International differential lines given by Mains and Jackson (1921).

2.3. Genetics of leaf rust resistance

Genetic studies have led to the discovery of individual genes for leaf rust resistance. Ausemus *et al.,* gave the designation of these genes by *Lr* number in 1946. In 1926 Mains *et al.,* were the first to determine the wheat varieties "Malakof and Webster" bears a gene that imparts leaf rust resistance, those were given name *Lr1* and *Lr2*, correspondingly (Ausemus *et al.*, 1946). The *Lr* genes differ in their effectiveness from mild-effective to high-effective (Odintsova, 1988). To date, around 210 rust resistance genes (including leaf, stem and stripe rust) and associated markers for several genes are available for the use of breeders. Some of the linked gene combinations like "*Lr34/Sr57/Pm38/Ltn1*; *Lr46/Yr29/Sr58/Pm39/Ltn2*; *Sr2/Yr30*; *Lr67/Yr46/Sr55/Ltn3"* are known to confer durable resistance to different rusts (Gupta *et al*., 2017).

Till now, 79 Leaf rust resistance genes entitled *Lr1* to *Lr79* have been categorized in bread wheat, durum wheat, and diploid wheat species. *Lr79* is resistant to current common wheat *Puccinia triticina* pathotypes in India and Australia but is not effective against durum-specific Pt races, CA 1.2 and ETH 125.2 (Qureshi *et al*. 2018). Most *Lr* genes provide seedling resistance and follow the gene-for-gene concept and give a hypersensitive response (Flor 1942). Recognition and incorporation of the new leaf rust resistance genes into germplasm helps in avoidance of genetic uniformity and vulnerability of cultivars to the concerned pathogen.

Initially, Leaf rust resistance genes were identified by inoculation with diverse leaf rust pathotypes with well-known avirulence and virulence reactions on wheat differential sets (Loegering *et al*., 1971 and Browder, 1980), but unable to evaluate the existence of genes when the resistance is given for more than one gene, the interaction of genes, or for race-nonspecific resistance genes (Knott, 1989; McCartney *et al*., 2005). Cytogenetic investigations were exercised to detect the rust resistance genes on respective chromosomes. Soliman *et al*., (1964)

identified the chromosome carrying *Lr1*, *Lr3*, and *Lr11* leaf rust resistance genes. Dyck and Samborski, (1968), have described that the *Lr2* locus have three alleles. They were shown to be present on a single locus in *P. triticina* that impart avirulence or virulence to exclusive leaf rust resistance genes. *Lr14a* and *Lr14b* leaf rust resistance genes were also found to be allelic (Dyck 1970). Now with development of sequencing and advanced molecular biology techniques, it is easy to locate the gene precisely on a particular locus. Several genes like *Lr67* (Herrera-Foessel *et al.*, 2011), *Lr68* (Herrera-Foessel *et al.*, 2012), *LrBi16* (Zhang *et al*.,2015), *Lr75* (Singla *et al*., 2017), *Lr76* (Bansal *et al*., 2017), *Lr79* (Qureshi *et al*., 2018) were mapped and located precisely based on analysis of molecular markers.

2.4. *Lr* genes on Chromosome 1DS

Four leaf rust resistance genes have been known to be located on the short arm of chromosome 1D. *Lr21* was identified in the *T. tauschii* Iranian accession TA1599 (Rowland and Kerber 1974; McIntosh *et al*., 1995). It is a dominant gene and was cloned (Huang *et al*., 2003) and reported to be a simple (single-copy) locus encoding a nucleotide-binding site–leucine-rich repeats (NBS–LRR) protein of 1080 amino acids. *Lr42* gene is also introgressed from the *T. tauschii* (accession TA450) the D genome donor of common bread wheat. *Lr42* was first reported to be situated on chromosome 1DS using monosomic analysis in wheat (Cox *et al.,* 1994). *Lr42* inheritance pattern has been reported as partially dominant (Cox *et al.,* 1994), dominant (Czembor *et al.*, 2008, Gill *et al.*, 2019) and recessive (Liu *et al.*, 2013), *Lr42* is mapped between Xwmc432 and Xgdm33 SSR markers on chromosome 1DS (Liu *et al.*, 2013). *Lr42* is now fine mapped in hexaploid wheat using RIL population to a 3.7 cM genetic interval flanked by TC387992(KASP marker) and Xwmc432(SSR marker) (Gill *et al.*, 2019). *Lr60* gene is a dominant gene positioned on the short arm of 1D, at a genetic distance of 8.4 cM distal to the SSR marker Barc149 (Hiebert *et al.*, 2008). *Lr60* is about 8 cM distal to *Lr21*. Recently one of the undesignated recessive leaf rust resistance gene *LrSyn45* has been found to be located on chromosome 1DS in Synthetic45 (Gyani *et al.*, 2017).

Chapter 3 Materials and Methods

Synthetic hexaploid wheat 'Synthetic55' derived through crossing and chromosome doubling from *Triticum turgidum* var. *durum* (2n=28) and *T. tauschii* (2n=14) stated to have a leaf rust resistance. The leaf rust resistance gene carried by Synthetic55 is monogenic and dominant in nature and has been mapped on short arm of chromosome 1D of wheat (Singh, 2017). As already, four leaf rust resistance genes are known to be placed on the short arm of chromosome 1D namely *Lr21, Lr42, Lr60,* and *LrSyn45*. Therefore, the novelty of putative "*LrSyn55*" needs to be confirmed. "Genetic analysis of leaf rust resistance gene "*LrSyn55*" in wheat" was carried to understand allelic relation with other leaf rust resistance genes located on chromosome 1DS. All the experiments related to the proposed research work were carried out in the glass house, farm and wheat molecular biology laboratory of Division of Genetics and National Phytotron Facility, Indian Agricultural Research Institute, New Delhi. The plant materials used and methods followed to accomplish the objectives of the study are elaborated below.

3.1. Materials:

3.1.1. Host material

Seeds of Thatcher, Thatcher+*Lr21,* KS91WGRC11 (*Lr42),* Synthetic45 Synthetic55, HD2932, and HD2733 were available at Division of Genetics, Indian Agricultural Research Institute, New Delhi. The seed of Thatcher+*Lr60* (RL6172, NBPGR Acc. EC920986) was procured from Modern Research and Development Centre, Manitoba, Canada. The description about the parental lines used in the study are given below.

3.1.1.1. Synthetic 55:

The SHWs were produced from hybridization between "*Triticum turgidum* var. *durum* lines and several accessions of *T. tauschii* at the International Maize and Wheat Improvement Center (CIMMYT), Mexico" (Mujeeb-Kazi and Hettel, 1995). Synthetic 55 is one of SHWs maintained at division that exhibits broad spectrum resistance towards leaf rust pathogen under artificial rust epiphytotics in field (Ram *et al.*, 2002). Parentage of Synthetic 55 is "GAN/*Ae. squarrosa* (180) with CIMMYT Synthetic ID: 221, Cross ID: CIGM 90-799".

3.1.1.2. Synthetic 45:

Synthetic 45 is also one of the SHWs and it also exhibits broad spectrum resistance against leaf rust pathogen in artificially created rust epiphytotic in field and glass house condition (Gyani *et al.*, 2017). The parentage of Synthetic 45 is "68.111/RGB-U//WARD/3/FGO/4/RAB/5/*Ae. squarrosa* (878) with CIMMYT Synthetic ID: 160 Cross ID: CIGM 89-559".

3.1.1.3. Thatcher:

Thatcher is a hard red spring wheat variety and was once the leading variety in the Canadian Prairies from 1939 to the mid-1960s due to resistance to stem rust and outstanding baking characteristics (Hiebert *et al*., 2016). The Heads are white chaffed and beardless having the pedigree as Marquis/Iumillo Durum//Marquis/Kanred winter wheat. It has been used extensively to develop isogenic lines for leaf rust resistance genes.

3.1.1.4. RL6043 (Thatcher+*Lr21*):

This is one of the near-isogenic line (NIL) in thatcher background containing *Lr21* gene. The Pedigree of line is Thatcher*6//R.L.5406 (Browder, 1980).

3.1.1.5. **KS91WGRC11 (*Lr42)***:

This is the reference genetic stock for *Lr42* gene. The pedigree of KS91WGRC11 (*Lr42*) is "Century*3/*Ae. tauschii* (TA2450)" (Cox et al., 1994).

3.1.1.6. RL6172 (Thatcher + *Lr60*):

This is also one of the NIL containing *Lr60* gene in the background of Thatcher. The pedigree of line is "Thatcher*3/V860" (Dyck, 1994).

3.1.1.7. HD 2932:

It was released through the national system in 2007 for irrigated late sown conditions of the central zone and peninsular zone having yield potential of 41-45 q/ha. This is one of the popular varieties released by Indian Agricultural Research Institute, New Delhi having the pedigree of "Kauz/Star//HD2643".

3.1.1.8. HD 2733:

This variety was released through the national system for irrigated timely sown conditions of the north eastern plains zone (NEPZ) of India in 2001. The average yield potential of this variety is around 50 q/ha. This is also one of the national checks in AICRP trials and popular variety released by Indian Agricultural Research Institute, New Delhi having the pedigree of **"**Attila/3/TUI/CARC/CHEN/CHTO/4/Attila".

3.1.2. Pathogen

Twenty-one pathotypes of leaf rust pathogen *Puccinia triticina* Eriks. were used in the current research work for which inoculum was procured from IIWBR, Regional Station, Flowerdale, Shimla during 2017-18 and 2018-19. These twenty-one pathotypes were used for multipathotype testing. The pathotypes together with their binomial designation and avirulence/virulence formulae based on Nagarajan *et al.*, (1983) are given in Table 1. The commonly used old pathotypes nomenclature is also mentioned in the same table. The two most prevalent leaf rust pathotypes in India are 77-5 and 77-9. Therefore, these pathotypes were also used for multipathotype testing and pathotype 77-5 was used for genetic studies.

3.2. Methods:

3.2.1. Development of populations:

Resistant parental line Synthetic55 and susceptible parental lines Thatcher, HD2932, and HD2733 were screened against leaf rust pathotype 77-5 under glasshouse condition in trays and later they were transplanted to the field. The seedlings tested for rust reaction were transplanted in the field, were further used for attempting cross to get F_1 seeds. The F_1 seeds were used to grow seedlings and were screened against pathotype 77-5, and after the recording of disease score, they were transplanted to the field. The F_1 seedlings were confirmed for hybridity through the molecular marker and morphological traits. The confirmed F_1 plants were selfed to get F_2 seed and were also backcrossed with recessive parent to get BC_1F_1 seed for validation of inheritance through test cross. The detail of F_2 and BC_1F_1 populations are provided in Table 2.

3.2.2. Hybridity confirmation of F_1s through molecular and morphological markers

Hybridity was confirmed for F_1 plants between resistant parent Synthetic55 and susceptible parent Thatcher, HD2932 and HD2733. Leaf samples were collected from all F_1 plants and DNA was extracted using the Cetyl trimethylammonium bromide method of DNA isolation (Murray and Thompson, 1980). Purification of genomic DNA was done by using DNase free RNase (10 mg/ml) at 70^0C. DNA was quantified with reference to known quantity of λ uncut DNA (50ng, 100ng, and 150ng) stock. A working stock with 25 ng/µl of DNA was prepared from DNA master stock and used for setting PCR reactions. The linked SSR marker CFD15 was used in 10 µl reaction volume containing 4 µl of GoTaq® master mix (Promega), 1 µl of each primer, 1 µl of genomic DNA (25 ng) and 3 µl of water with PCR profile: initial denaturation step of 94^0C for 5 min, followed by 30 cycles of 94 ^{0}C for 30 seconds (denaturation), at specific annealing temperature 60^0C for 1 min, and 72^0C for 30 seconds (primer extension), with a final extension of 72^0C for 10 min and further cooling at 4^0C. The amplicon was loaded in 4.5% Agarose gel and was documented using GelDoc (SynGene). Brown glume and awn color is a dominant morphological marker present in Synthetic55. This marker was also used to confirm the hybridity of F_1s. The true hybrid plants were then selfed to produce F_2 seeds for the inheritance studies.

3.2.3. Multiplication of inoculum of rust:

Iinoculum of 21 pathotypes of leaf rust fungus (*Puccinia triticina*) procured from IIWBR, Regional Station, Flowerdale, Shimla, was multiplied following procedure (Figure 1) used by Joshi et al. (1988) in the rust phenotyping glasshouse. Agra local was sown in 4 inches plastic pots filled with thoroughly mixed garden soil and farm yard manure (FYM) in a ratio of 5:1. Seeds were sown in bulk of 10-15 seeds at center of the pot after filling $2/3^{rd}$ of the pot and then were properly covered with soil mix. Optimum moisture was maintained in pot to ensure proper germination and growth of seedlings. Properly grown seedlings were inoculated after 8-10 days of sowing depending upon the climatic condition and leaf expansion. Rust inoculum was properly mixed with inert talc and applied on the surface of leaves. After inoculation with uredospore, water was sprayed using fine mist sprayer in such a way that water has appeared as dew deposited on the surface of leaves. The inoculated and water sprayed seedlings were incubated in a chambers with 80-100% humidity (Figure 1) for 48 hrs.

Table 1. Binomial designation and avirulence/virulence formula of pathotypes of leaf rust (*P. triticina*) used in the study

S.No.	Pathotype Designation		Avirulence/virulence formula
	Old	New	
1	12-3	49R37	***Lr1,2a, 9,10,13,18,19,20,23,24,28/*** *Lr2c,3a,14a,15,17,26*
2	12-4	69R13	***Lr1,2a, 9,13,15,17,19,23,24,26,28/*** *Lr2c,3a,10,14a,18,20*
3	12-5	29R45	***Lr1,2a, 9,10,15,19,23,24,28 /*** *Lr2c,3a,13,14a,17,18,20,26*
4	77	45R31	***Lr9,10,17,19,23,24,26,28/*** *Lr1,2a,2c,3a,13,14a,15,18,20*
5	77A	109R31	***Lr9,17,19,23,24,26,28/*** *Lr1,2a,2c,3a,10,13,14a,15,18,20*
6	77A-1	109R23	***Lr9,17,19,20,23,24,26,28/*** *Lr1,2a,2c,3a,10,13,14a,15,18*
7	77-2	109R31-1	***Lr9,17,19,24,26,28/*** *Lr1,2a,2c,3a,10,13,14a,15,18,20,23*
8	77-3	125R55	***Lr9,19,20,23,24,28/*** *Lr1,2a,2c,3a,10,13,14a,15,17,18,26*
9	77-4	125R23-1	***Lr9,19,20,24,26,28/*** *Lr1,2a,2c,3a,10,13,14a,15,17,18,23*
10	77-5	121R63-1	***Lr9,18,19,24,28/*** *Lr1,2a,2c,3a,10,13,14a,15,17,20,23,26*
11	77-6	121R55-1	***Lr9,18,19,20,24,28/*** *Lr1,2a,2c,3a,10,13,14a,15,17,23,26*
12	77-7	121R127	***Lr18,19, 23,24,28/*** *Lr1,2a,2c,3a,9,10,13,14a,15,17,20,26*
13	77-8	253R31	***Lr9,23,24,26,28/*** *Lr1,2a,2c,3a,10,13,14a,15,17,18,19,20*
14	77-9	121R60-1	***Lr2a,2c,9,18,19,24,28 /*** *Lr1,3a,10,13,14a,15,17,20,23,26*
15	77-10	377R60-1	***Lr2a,2c, 9,18,19,24/*** *Lr1,3a,10,13,14a,15,17,20,23,26,28*
16	104-1	21R31-1	***Lr9,10,13,15,19,24,26,28/*** *Lr1,2a,2c,3a,14a,17,18,20,23*
17	104-2	21R55	***Lr9,10,13,15,19,20,23,24,28/*** *Lr1,2a,2c,3a,14a,17,18,26*
18	104-4	21R57	***Lr2a,3a,9,10,13,15,19,23,24,28/*** *Lr1,2c,14a,17,18,20,26*
19	107-1	45R35	***Lr1,3a,9,10,17,19,20,23,24, 28/*** *Lr2a,2c,1314a,15,18,26*
20	108	13R27	***Lr3a,9,10,15,17,19,23,24,26,28/*** *Lr1,2a,2c,13,14a,18,20*
21	162A	93R15	***Lr1,9,15,19,23,24,26,28/*** *Lr2a,2c,3a,10,13,14a,17,18,20*

Table 2: Details of populations developed for confirmation of Inheritance of *LrSyn55*

Resistant Parent	**Susceptible Parent**	**F_1**	**F_2**	**BC_1F_1**
Syn55	Thatcher	Thatcher/Syn55	F_2	Thatcher/Syn55//Thatcher
	HD2932	HD2932/Syn55	F_2	HD2932/Syn55//HD2932
	HD2733`	HD2733`/Syn55	F_2	HD2733`/Syn55//HD2733

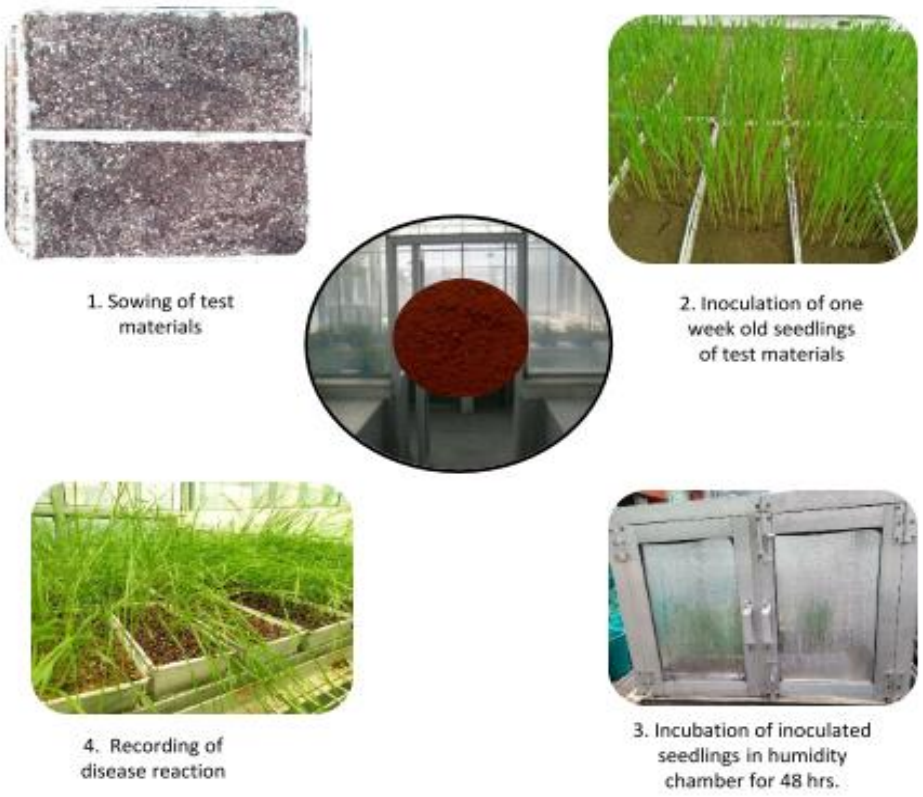

Figure 1. Screening against leaf rust in glass house

The seedlings were taken out after incubation and kept in the glasshouse for sporulation under natural condition. The spores appeared after 10-14 days of inoculation was collected by gently tapping the seedlings on butter paper. The care was taken to avoid any contamination. The collected uredospore inoculum was kept under room temperature for a day and further stored in the freezer for screening of test materials.

3.2.4. Screening of test material against rust for multipathotype testing:

For screening of test materials viz. Synthetic55, RL6043 **(**Thatcher+*Lr21*), KS91WGRC11 (*Lr42)*, RL6172 (Thatcher + *Lr60*), Synthetic45, Thatcher, HD2932 and HD2733 against 21pathotypes of leaf rust pathogen seedlings were grown in rectangular trays (11″×4″×3″). Trays were filled with garden soil and FYM mix, equidistant lines were opened in trays with help of a wooden marker and 10 seeds were sown in each line. 10 days old seedlings were inoculated with inoculum of 21 test pathotype in water suspension using fine mist water sprayer. The urediniospores were mixed in water to make a suspension and a drops of Tween 20 (Polysorbate 20) was also added in mixture. Tween 20 is a surfactant that helps is adhesion of spore to the leaves of the seedlings. The inoculated seedlings were kept in chambers with 80-100% humidity for 48 hours and subsequently they were transferred in glass house workbenches. Proper care was taken to maintain the purity of each pathotypes and any chance of cross contamination was avoided by maintaining them in separate chambers. Infection types were recorded after appearance of pustule at 10-12 days of inoculation following the Stakman *et al.,* (1962) 0-4 scale classification (Table 3). The variation in each infection type were indicated by the use of – and + on disease score.

3.2.5. Screening of segregating materials for inheritance study:

For inheritance studies, Resistant Parent (RP), Susceptible Parent (SP), F_1, and F_2 from a cross involving Synthetic 55 (RP) with Thatcher, HD2932, and HD2733 (SP) were sown in rectangular trays using a similar method as explained for multipathotype testing. Ten F_2 seeds were sown in each row and along with one row each of both the parents. The inoculated seedlings were scored after appearance of infection at 10-12 days of inoculation according to the Stakman *et al.,* (1962) 0-4 scale (Table 3). The infection type 0, ; , 1 and 2 were clustered as a resistant individuals while infection types 3 and 4 were clustered as susceptible individuals. The screening of F_2 populations along with F_1s and parental lines were performed

in the glass house of National Phytotron Facility having a temperature regime of 18^0C at night and 22^0C at day time.

3.2.6. Development of intercross F_2 and screening for understanding allelic relation

To understand the allelic relation of leaf rust (*Lr*) resistance gene *LrSyn55* with other effective *Lr* genes located on chromosome 1DS, parental line Synthetic55 was crossed with Synthetic45 and KS91WGRC11 (*Lr42).* The F_1 seeds were sown and seedlings were transplanted after screening with pathotype 77-5. F_1 plants were selfed to get intercross F_2 seed. F_2 seeds were sown in rectangular trays using a similar method as explained for inheritance study. The inoculated seedlings were scored against pathotype 77-5 after 10-12 days of inoculation using similar method adopted for inheritance study.

3.2.7. Statistical Evaluation:

The Chi-square was performed to determine the goodness-of-fit of the observed segregation ratio recorded from the study and to relate it with the expected segregation ratios.

$\chi^2 = \sum$ {(Observed number of individuals -Expected number of individuals $)^2$/ Expected number of individuals}

Table 3. Infection type classification given by Stakman *et al.*, (1962)

Class	ITs #	Description of symptoms
Immune	0	No uredia nor other indications of infection
Very resistant	;	No uredia, but hypersensitive flecks present
Resistant	1	Minute uredia; surrounded by necrotic areas
Moderately resistant	2	Uredia small to medium; usually in green island surrounded by a decidedly chlorotic or necrotic border
Mesosthetic	X	Uredia variable ,sometimes including all infection types and intergradations between them on same leaf; no mechanical separation possible; on reinoculation small uredia may produce large ones, and vice versa
Moderately susceptible	3	Uredia medium in size; coalesces infrequent; no necrosis, but chlorotic areas may be present , especially under unfavorable conditions
Susceptible	4	Uredia larger, and often coalescing; no necrosis , but chlorosis may be present under unfavorable conditions

Plus and minus signs are used to indicate variations within a given infection type;
'=' :Uredia much smaller than typical and at the low limit for the infection type
'-' :Uredia smaller than normal
'+' :Uredia larger than normal
'++' :Uredia much larger than typical and at the upper limit for the infection type
'C' indicates exceptionally pronounced chlorosis
'N' indicates more than usual degree of necrosis

Chapter 4 Results

Rapid emergence of new virulent pathotypes in wheat rust pathogens necessitates the search for novel and broad-spectrum sources of resistance by exploring diverse gene pool. Diversification of rust resistance in new cultivars through broad-spectrum genes helps in sustainable yield enhancement. It is always useful to know the effectiveness and genetic nature of a resistance gene for efficient incorporation in breeding program. The evidence about the worth of a known resistance gene to diverse pathotypes aids the breeder to deploy a resistance gene based on the predominance of pathotypes in the target geographical region. The knowledge regarding nature of genes and their mode of action will help in designing the strategy to be followed in resistance breeding program.

Synthetic hexaploid wheat 'Synthetic55' is one of the stocks identified to carry a dominant broad-spectrum leaf rust resistance on chromosome 1DS. As there are 4 other leaf rust resistance genes reported on same chromosome. Therefore, current study was commenced to determine the effectiveness of leaf rust resistance gene located on chromosome 1DS and to understand the allelic relation of *LrSyn55* with other leaf rust resistance genes located on same chromosome. The experiments were conducted during 2017-2019 using facilities available at Indian Agricultural Research Institute, New Delhi.

In view of above facts, the leaf rust resistant lines carrying *Lr* genes on chromosome 1DS (*Lrsyn55*, *Lrsyn45*, *Lr21*, *Lr42*, and *Lr60*) were screened against diverse pathotypes of leaf rust pathogen and to understand the allelic relationship of leaf rust resistance gene '*Lrsyn55'* with other effective leaf rust resistance genes on chromosome 1DS, intercross populations were screened. Results of both objectives are presented hereunder:

4.1. Multipathotype testing to understand effectiveness of *Lr* genes on 1DS

Leaf rust resistant lines known to carry *Lr* genes on chromosome 1DS viz. Synthetic55, Synthetic45, RL6043 (Thatcher+*Lr21*), KS91WGRC11 (*Lr42*), RL6172 (Thatcher+ *Lr60*) along with three susceptible varieties Thatcher, HD2932, HD2733 were evaluated against 21 pathotypes (Table 4) of leaf rust pathogen *Puccinia triticina* at seedling stage under glass house conditions.

Infection types (ITs) recorded against each pathotype on all the test lines are presented in Table 4. It was observed that Synthetic55, Synthetic45 and KS91WGRC11 (*Lr42*) expressed resistant infection type against all 21 pathotypes used in investigation. The infection type of Synthetic55 varied from IT '0;' to IT ';1', for Synthetic 45 varied from IT ';' to IT 'X^{-}' while for KS91WGRC11 (*Lr42*) from IT '0;' to IT ';1$^{=}$'. The presence of necrosis was observed in KS91WGRC11 (*Lr42*) against pathotypes 12-4, 12-5, 77A, 77-3, 77-6, 77-8, 77-9 and 107-1. The reaction on RL6043 (Thatcher+*Lr21*) was mostly susceptible except for pathotype 162A, while RL6172 (Thatcher+*Lr60*) was susceptible to all the pathotypes. The presence of susceptibility on *Lr21* was reported earlier but reaction on *Lr60* was aberrant. Therefore, the purity of RL6172 was confirmed through SSR markers (Cfd15, Cfd61, Gdm33 and Barc149) reported to be located on chromosome 1DS (Hiebert *et al*., 2008). It was found that the stock is not correct, as the banding pattern in RL6172 and Thatcher is similar (Figure 2). Therefore, the effectiveness of *Lr60* cannot be determined. In contrast to resistant lines, the susceptible lines namely Thatcher, HD2932 and HD2733 exhibited susceptible response with infection type ranging from '3' to '3^{+}'against all 21 pathotypes used in the study. The representative reaction against some races are depicted in Figure 3. Results suggested the broad-spectrum nature of leaf rust resistance genes, *LrSyn55*, *LrSyn45* and *Lr42*. The susceptibility of HD2932 and HD2733 against all the pathotypes of leaf rust used in study advocated its use as contrasting susceptible parent with superior agronomic background for genetic analysis of leaf rust resistance.

4.2. Validation of Genetic nature of *LrSyn55* in diverse F_2 populations

The genetic analysis of resistance to leaf rust inherited by Synthetic55 in F_2 population with contrasting susceptible parent Agra local was found to be monogenic dominant in nature (Singh., 2017). Therefore, to confirm its genetic nature in diverse populations, Synthetic55 was crossed with 3 susceptible varieties namely Thatcher, HD2932 and HD2733 to develop 3 F_2 populations. The hybridity of F_1 seedlings for each cross was confirmed through polymorphic SSR marker CFD15. The resistant parents have an amplicon size of 179 bp while the susceptible parents have an amplicon size of 160 bp. The presence of both the amplicons in all the F_1 plants confirmed the hybridity of F_1 seedling (Figure 4). Along with SSR marker, the difference in glume color and awn trait have also confirmed hybridity of F_1 plants (Figure 5).

Table 4. Infection types on Syn55, *Lr21*, *Lr42*, *Lr60*, Syn45, Thatcher, HD2932 and HD2733 against 21 pathotypes of leaf rust (*P. triticina*) at seedling in glass house

S. No.	Pathotype	Syn55	*Lr21*	*Lr42*	*Lr60*	Syn45	TC	HD2932	HD2733
1	12-3	**;1**$^{=}$	3^{-}	;1$^{=}$	3^{+}	;1^{+}	3	3+	3
2	12-4	**;**	3^{-}	;N	3	;1	3	3	3
3	12-5	**;1**$^{=}$	3^{-}	;N	3	;1	3	3	3
4	77	**0;**	3^{-}	0;	3	;1	3+	3+	3
5	77A	**;**	3	;N	3	;1$^{=}$	3	3+	3+
6	77A-1	**;1**$^{=}$	3^{-}	0;	3	;1$^{=}$	3	3+	3
7	77-2	**0;**	3	0;	3	;1^{-}	3+	3+	3+
8	77-3	**;1**$^{=}$	3^{-}	;N	3	1	3+	3+	3+
9	77-4	**;1**$^{-}$	3	0;	3	X^{-}	3	3-	3+
10	77-5	**;1**$^{=}$	3	0;	3	;1	3	3	3
11	77-6	**;1**$^{-}$	3	;1$^{=}$N	3	;	3	3	3
12	77-7	**0;**	3^{-}	0;	3	X$^{=}$	3	3	3+
13	77-8	**;1**$^{=}$	3^{-}	;1$^{=}$N	3	;1	3	13+	3
14	77-9	**0;**	3	;N	3	X^{-}	3	3	3
15	77-10	**;1**$^{-}$	3	0;	3	;1^{-}	3+	3+	3+
16	104	**;**	3^{-}	0;	3	;1$^{=}$	3+	3+	3+
17	104-2	**;1**	3^{-}	0;	3	;1$^{=}$	3	3	3
18	104-4	**;**	3^{-}	0;	3	;1$^{=}$	3+	3+	3+
19	107-1	**;1**$^{-}$	3^{-}	;N	3	;1$^{=}$	3	3	3
20	108	**;**	3^{-}	0;	3	;1$^{=}$	3	3	3
21	162A	**0;**	;1	0;	3	;	3+	3-	3

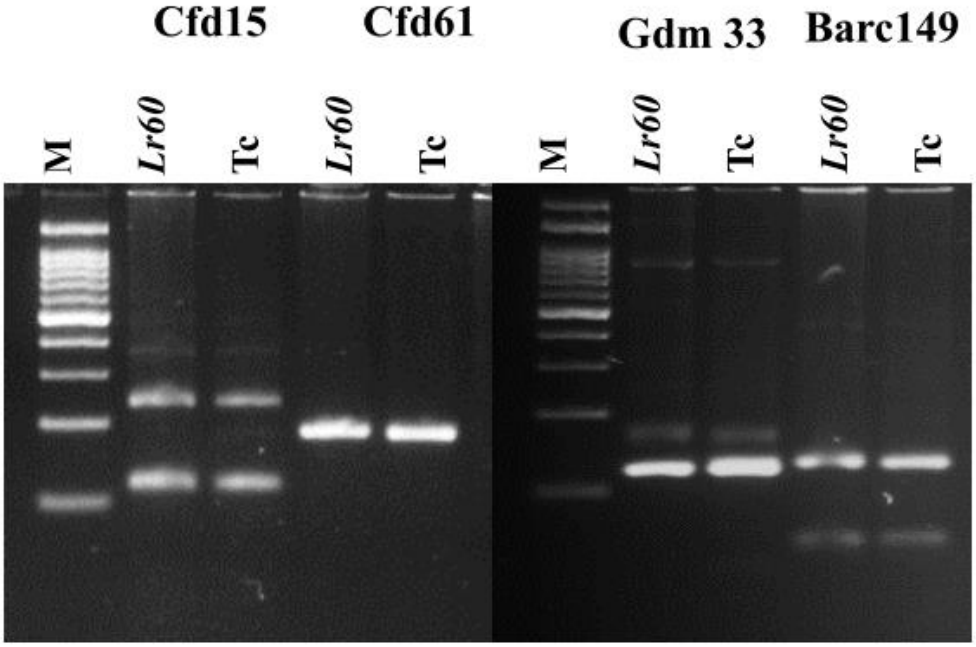

Figure 2. Confirmation of wrong stock of *TC+Lr60* through SSR markers located on 1DS

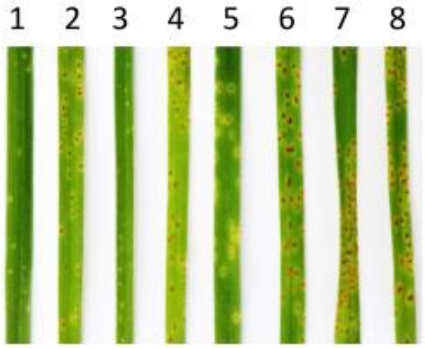

Figure 4A. SRT with Race 77-2

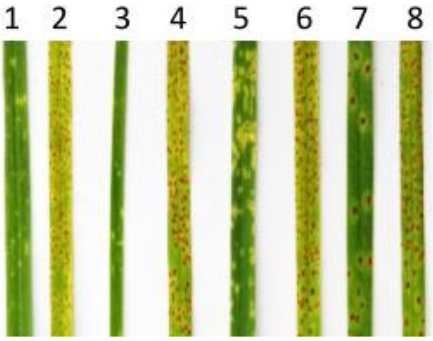

Figure 4B. SRT with Race 77-A1

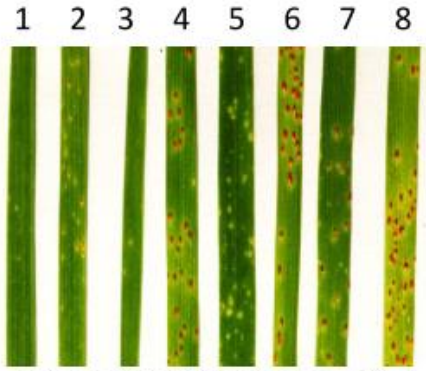

Figure 4C. SRT with Race 104-4

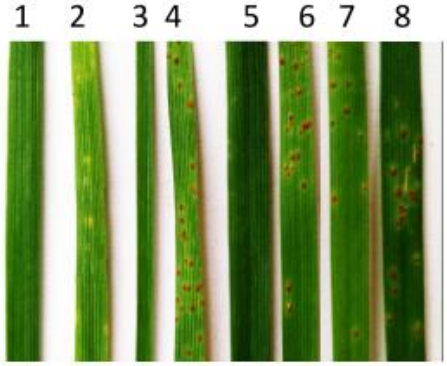

Figure 4D. SRT with Race 162A

Figure 3. Infection type of (1) Syn55, (2) TC+ *Lr21*, (3) *Lr42*, (4) TC+ *Lr60* (5) Syn45 (6) TC (7) HD2932 and (8) HD2733 against different Leaf rust pathotypes

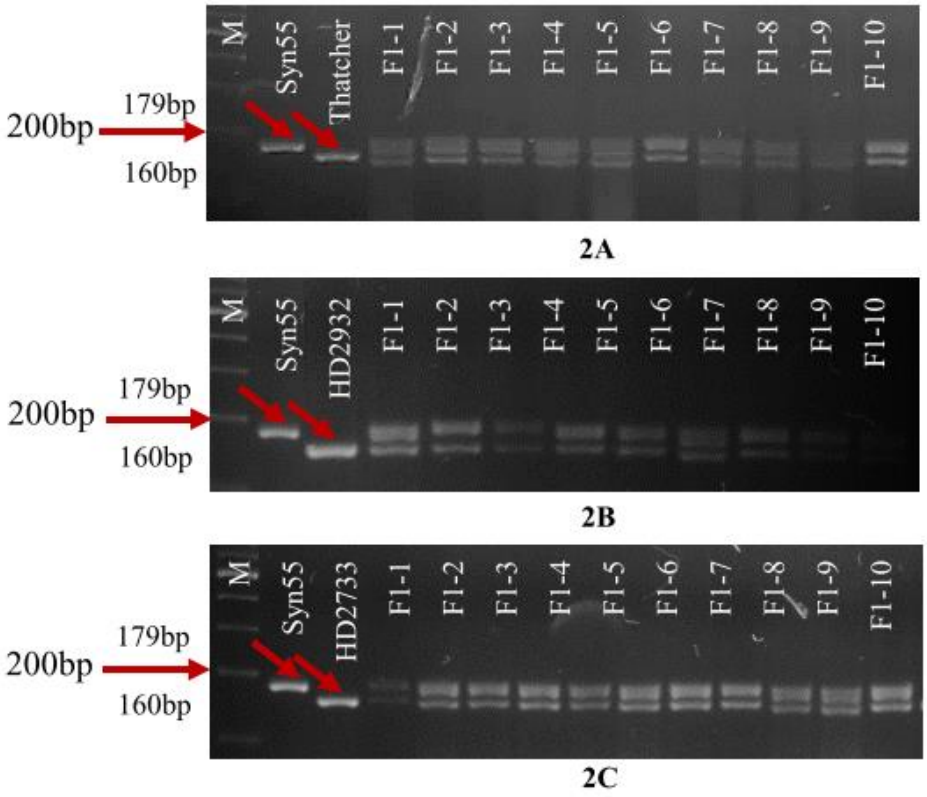

Figure 4. Confirmation of hybridity of F_1 plants using polymorphic marker CFD15 (2A-2C)

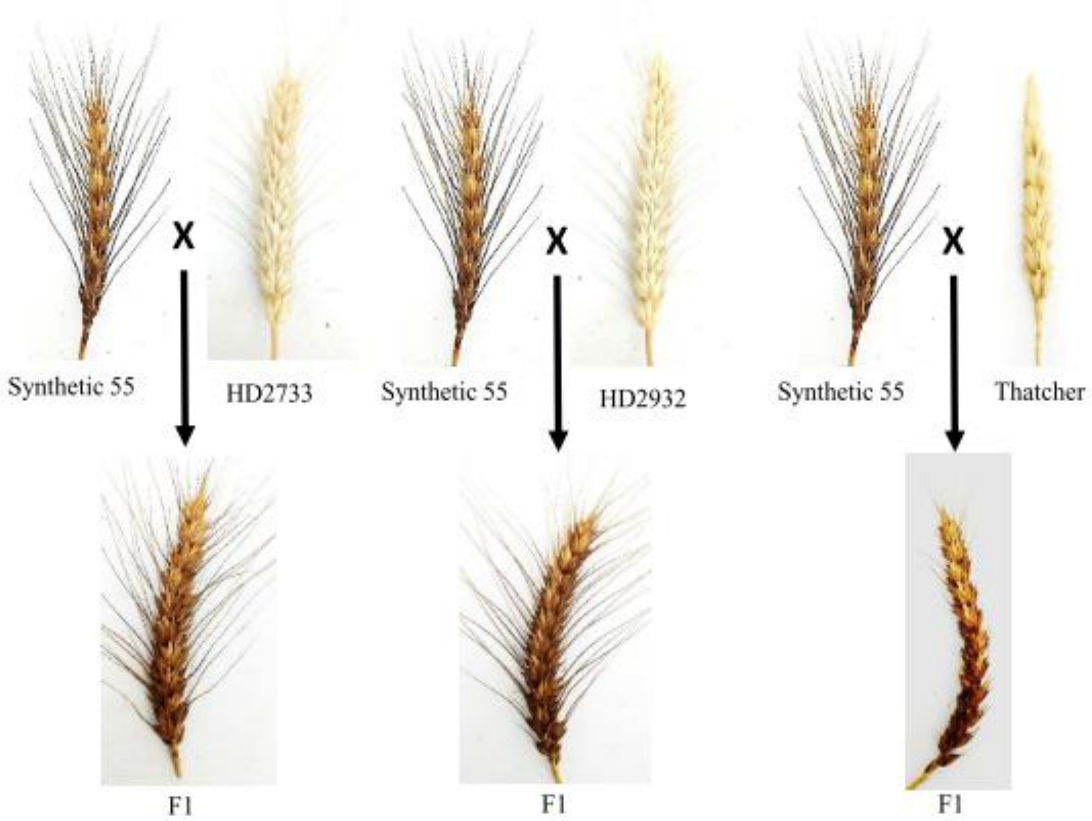

Figure 5. Spikes of Syn55, HD2733, HD2932, Thatcher and F_1 between them to confirm hybridity of F1 based on glume colour

All the true F_1 plants were selfed and sufficient F_2 seeds have been harvested from each plant separately for genetic analysis. Parental lines, F_2 populations along with 3 F_1s from crosses Synthetic55/Thatcher, Synthetic55/HD2932 and Synthetic55/HD2733 were tested at seedling stage by inoculating one of the most virulent and predominant leaf rust pathotype 77-5, under glasshouse conditions.

The RP 'Synthetic55' has shown hypersensitive resistant reaction with infection type ';1' whereas contrasting susceptible parents Thatcher, HD2932 and HD2733 exhibited susceptible reaction with IT '3^+'. The F_1s of all the crosses, Synthetic55/Thatcher and Synthetic55/HD2932, Synthetic55/HD2733 produced 'resistant reactions with IT ';1' to IT ';1^+''. The reaction of F_1 seedlings confirmed the previous report of "dominant nature of resistance in Synthetic55".

A total of 366 F_2 seedlings from two different F_1 plants from Synthetic55/Thatcher were tested with pathotype 77-5. The F_2 population from first F_1 plant with a size of 180 seedlings showed a segregation of 128 resistant and 52 susceptible plants. The observed F_2 frequency in two phenotypic classes fits well in the expected ratio of 3R:1S ($\chi2$=1.451, P=0.228 at 1 d.f.). Similarly, F_2 population from second F_1 plant with a size of 186 seedlings also segregated in expected genetic ratio of 3R:1S (132 resistant: 54 susceptible; $\chi2_{(3:1)}$ =1.612, $P_{(1d.f)}$ = 0.204). The representative image of parents, F_1 and F_2 are shown in Figure 6. Analysis of pooled data from both F_2 populations segregated in 260 resistant and 106 susceptible individuals with a segregation of 3R:1S ($\chi2_{(3:1)}$ =3.063, $P_{(1df)}$ =0.080) with non-significant heterogeneity ($\chi2$=0, P=1 at 1 d.f.) among the individual F_1 plant derived F_2 populations (Table 5).

In second population involving Synthetic 55/HD2932 as parents, a total of 316 seedling from two independent F_1 plants were screened against pathotype 77-5. The F_2 population from first F_1 plant having 138 seedlings segregated in 106 resistant and 32 susceptible with a segregation ratio of 3R: 1S ($\chi2$=0.241, P=0.623 at 1 d.f.), similarly F_2 population with 178 seedlings from second F_1 plant also segregated in 3R: 1S ($\chi2$=0.906, P=0.341 at 1 d.f.). The representative image of parents, F_1 and F_2 are shown in Figure 7 The pooled F_2 population from both F_1 plants segregated in 234R:82S which fits well in one dominant gene segregation

of 3R and 1S with $\chi 2_{(3:1)} = 0.151$, $P_{(1df)}=0.696$) with non-significant heterogeneity ($\chi 2= 0.996$, P= 0.318 at 1 d.f.) (Table 5).

In third F_2 population from Synthetic 55/HD2733 a total of 332 seedlings from 2 independent F_1 plants were evaluated against pathotype 77-5. The F_2 from first F_1 plant with 174 seedlings segregated in 128R and 46S with a segregation ratio of 3R:1S ($\chi 2= 0.191$, P= 0.661 at 1 d.f.), similarly F_2 from second F_1 plant also segregated in ratio of 3R and 1S ($\chi 2= 0.210$, P= 0.646 at 1 d.f.). The representative image of parents, F_1 and F_2 are shown in Figure 8 The pooled analysis from both population with a sum of 332 seedlings segregated in 244R and 88S, which fits in a segregation ratio of 3R:1S ($\chi 2_{(3:1)}=0.401$, $P_{(1df)}=0.526$) with non-significant heterogeneity ($\chi 2=0$, P=1 at 1 d.f.) (Table 5).The segregation ratio in all the F_2 populations have validated the "monogenic dominant nature of *LrSyn55*".

4.3. Validation of Genetic nature of *LrSyn55* in diverse test cross populations

In addition to F_2 populations, 'three test cross populations involving three susceptible lines as recurrent parent i.e. Synthetic55/Thatcher//Thatcher, Synthetic55/HD2932//HD2932 and Synthetic55/HD2733//HD2733 were also tested against the pathotype 77-5 (Table 6). The test cross population of 38 seedlings from Synthetic55/Thatcher//Thatcher segregated in 16 resistant and 22 susceptible plants and fits in test cross ratio of 1R:1S ($\chi 2=0.947$, P=0.330 at 1 d.f.). The second test cross population with 58 seedlings from (Synthetic55/HD2932// HD2932) segregated in 24R and 34S with a test cross ratio of 1R:1S' ($\chi 2=1.724$, $P_{(1df)}=0.189$). Further, the segregation of 52 test cross seedlings (22R: 30S) from Synthetic55/HD2733//HD2733 in 1R:1S test cross ratio with $\chi 2=1.230$, $P_{(1df)}=0.267$ supported the conclusion that Synthetic55 carries a single dominant gene tentatively named as *LrSyn55* for leaf rust resistance.

4.4 Understanding the Allelic relationship between different *Lr* genes located on 1DS

As there are four other leaf rust resistance genes known to be located on the short arm of chromosome 1D i.e., *Lr21*, *Lr42*, *Lr60* and *LrSyn45*. Therefore, to understand the novelty of *LrSyn55* crosses were developed between the Synthetic55 with lines carrying effective genes namely *Lr42* and *LrSyn45*. The chances of *LrSyn55* being *Lr21* was ruled out based on multipathotype testing, where *Lr21* was found to be effective against only pathotype 162A and

Table 5. Segregation ratio in F_2 of crosses involving Synthetic 55, Thatcher, HD 2932, HD 2733 against pathotype 77-5 of leaf rust (*P. triticina*) at seedling stage in glass house

Line/ Population	No. seedlings			Expected ratio	χ2	P-Value
	Resistant	Susceptible	Total			
Synthetic 55(Syn55)	10	0	**10**			
Thatcher(Tc)	0	10	**10**			
HD 2932	0	10	**10**			
HD 2733	0	10	**10**			
Syn55/Tc $–F_1$	10	0	**10**			
Syn55/ HD 2932 $–F_1$	10	0	**10**			
Syn55/ HD 2733 $–F_1$	10	0	**10**			
Syn55/Tc $–F_2$ (from F_1 Plant No.1)	128	52	**180**	3:1	1.451	**0.228**
Syn55/Tc $–F_2$ (from F_1 Plant No.2)	132	54	**186**	3:1	1.612	**0.204**
Total	**260**	**106**	**366**	3:1	3.063	**0.080**
Homogeneity χ2 at 1 d.f. Syn55/Tc					0	**1**
Syn55/ HD 2932-F_2 (from F_1 Plant No.1)	106	32	**138**	3:1	0.241	**0.623**
Syn55/ HD 2932-F_2 (from F_1 Plant No.2)	128	50	**178**	3:1	0.906	**0.341**
Total	**234**	**82**	**316**	3:1	0.151	**0.696**
Homogeneity χ2 at 1 d.f. Syn55/ HD 2932					0.996	**.318**
Syn55/ HD 2733-F_2 (from F_1 Plant No.1)	128	46	**174**	3:1	0.191	**0.661**
Syn55/ HD 2733-F_2 (from F_1 Plant No.2)	116	42	**158**	3:1	0.210	**0.646**
Total	**244**	**88**	**332**	3:1	0.401	**0.526**
Homogeneity χ2 at 1 d.f. Syn55/ HD 2733					0	1

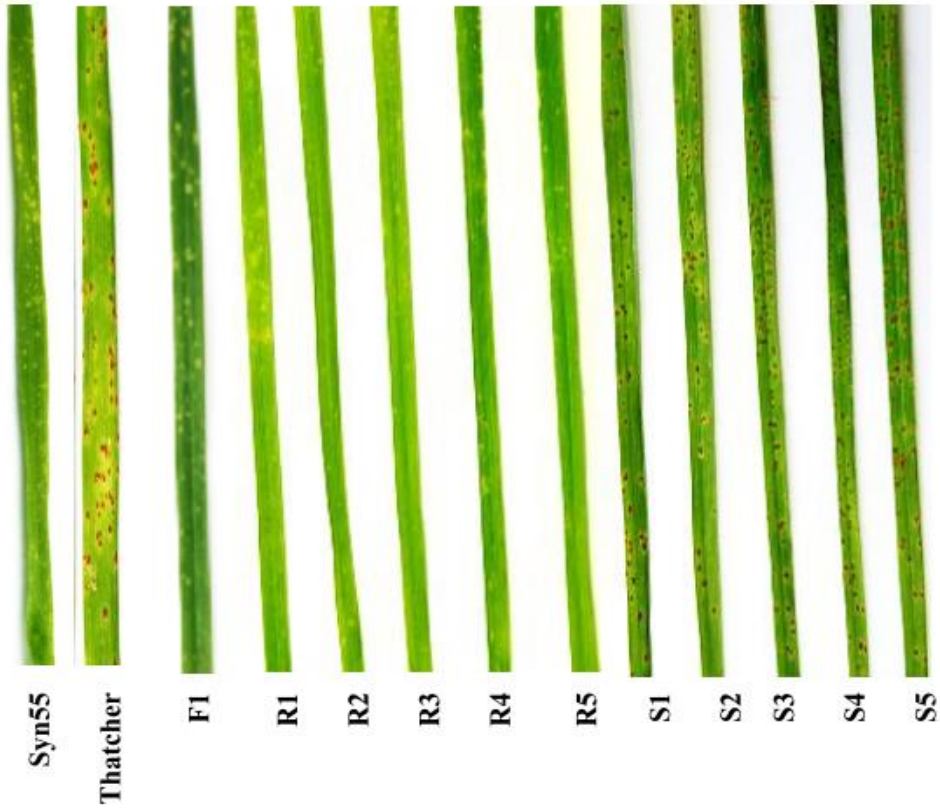

Figure 6. Infection types on Syn55, Thatcher, F_1 and F_2 (Res. R1-R5, Sus. S1-S5) from cross Syn55/Thatcher against Leaf rust pathotype 77-5

Figure 7. Infection types Syn55,HD-2932, F_1 and F_2 (Res. R1-R5, Sus. S1-S5) from cross Syn55/HD2932 against Leaf rust pathotype 77-5

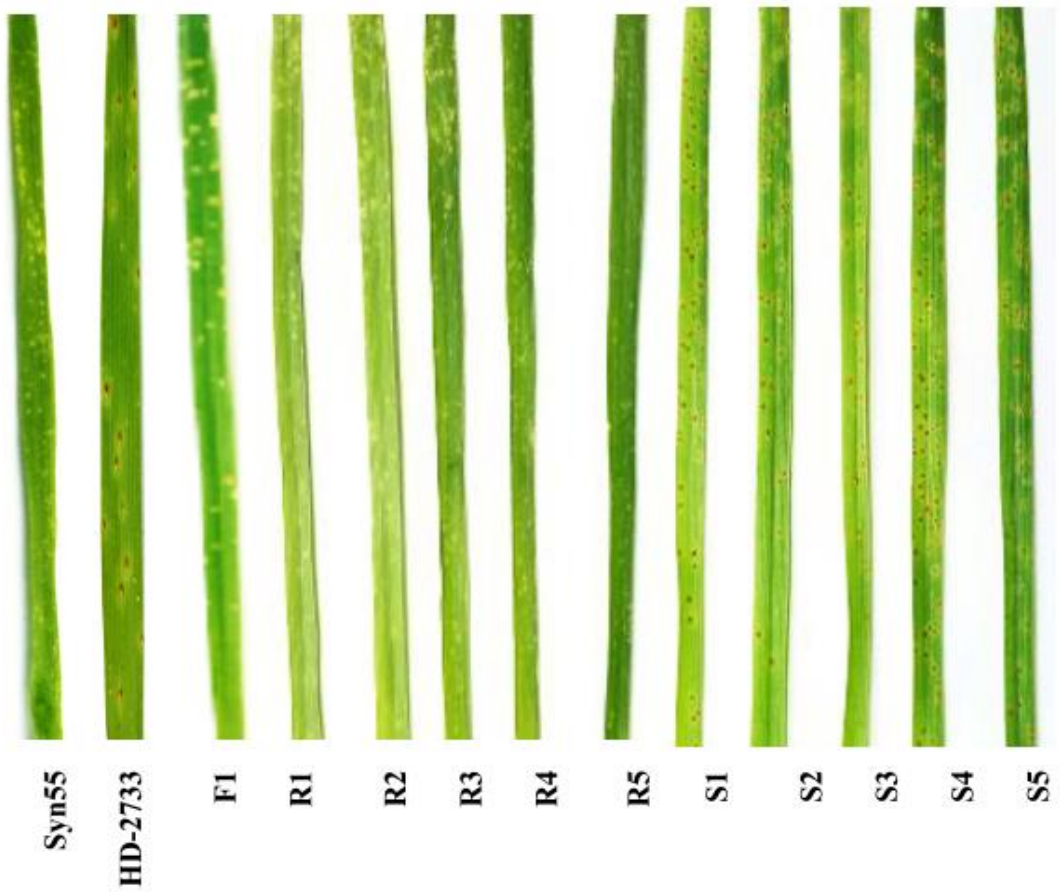

Figure 8. Infection types Syn55,HD-2733, F_1 and F_2 (Res. R1-R5, Sus. S1-S5) from cross Syn55/HD2733 against Leaf rust pathotype 77-5

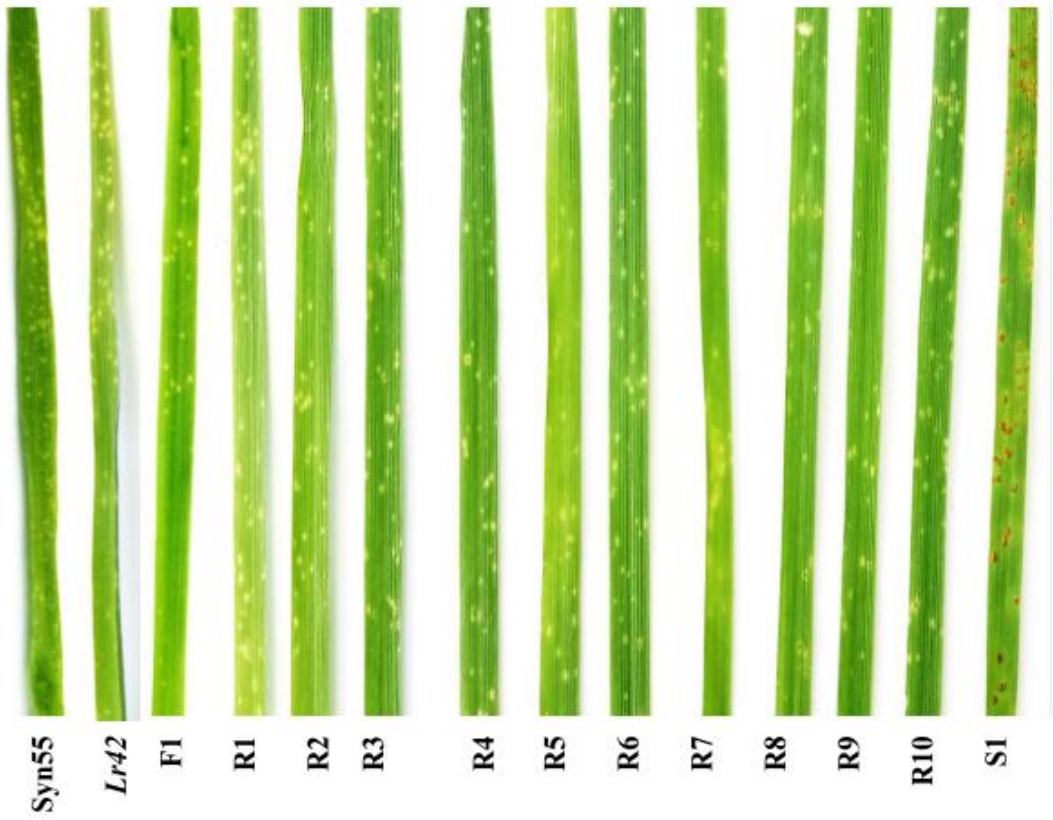

Figure 9. Infection types Syn55, *Lr42*, F_1 and F_2 (Res. R1-R10, Sus. S1) from cross Syn55/*Lr42* against Leaf rust pathotype 77-5

Figure 10. Differential reaction of KS91WGRC11 (*Lr42*) and Synthetic 55 against pathotype 77A and 77-6

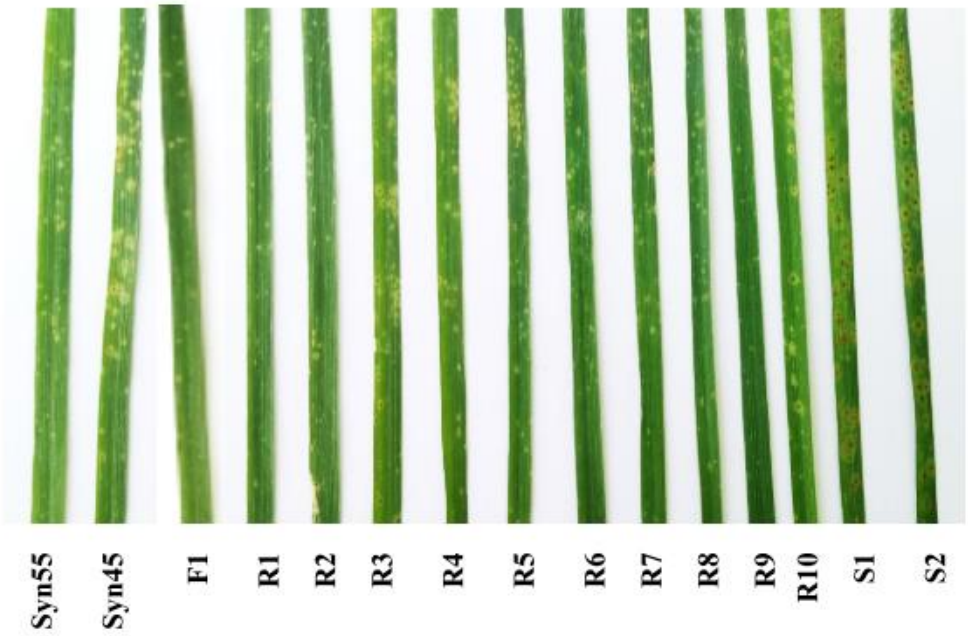

Figure 11. Infection types Syn55,Syn45, F_1 and F_2 (Res. R1-R10, Sus. S1-S2) from cross Syn55/Syn45 against Leaf rust pathotype 77-5

Table 6. Segregation ratio in BC_1F_1 of crosses involving Synthetic 55, Thatcher, HD 2932, HD 2733 against pathotype 77-5 of leaf rust (*P. triticina*) at seedling stage in glass house

Line/ Population	No. seedlings			Expected ratio	$\chi 2$ (1:1)	P-Value
	Resistant	Susceptible	Total			
Synthetic 55(Syn55)	10	0	**10**			
Thatcher(Tc)	0	10	**10**			
HD 2932	0	10	**10**			
HD 2733	0	10	**10**			
Syn55/Tc//Tc -BC_1F_1	16	22	**38**	1:1	**0.947**	**0.330**
Syn55/HD 2932//HD2932- BC_1F_1	24	34	**58**	1:1	**1.724**	**0.189**
Syn55/HD 2733//HD2733- BC_1F_1	22	30	**52**	1:1	**1.230**	**0.267**
Total	62	86	**148**	1:1	**3.891**	**0.048**
Homogeniety $\chi 2$ at 2 d.f.					**0.01**	**0.920**

Table 7. Segregation ratio in F_2 of intercrosses involving Synthetic 55, KS91WGRC11 (*Lr42*) and Synthetic 45 against pathotype 77-5 of leaf rust (*P. triticina*)

Line/ Population	Expected ratio	No. seedlings		
		Resistant	Susceptible	Total
Synthetic 55 (*Syn55*)		10	0	**10**
KS91WGRC11 (*Lr42*)		10	0	**10**
Synthetic 45 (*Syn45*)		10	0	**10**
Syn55/KS91WGRC11 (*Lr42*)–F_1		10	0	**10**
Syn55/Syn45 –F_1		10	0	**10**
Syn55/KS91WGRC11 (*Lr42*) -F_2	All Resistant	559	7	**566**
Syn55/Syn45- F_2	All Resistant	243	31	**274**

Synthetic 55 effective against all the races used in study. While chances of *LrSyn55* being *Lr60* cannot be ruled out based on multipathotype testing because of non-availability of correct stock. But the reported reaction of *Lr60* (; 1 2) (Hiebert *et al*., 2008) is different from that of *LrSyn55* (0 ;) against pathotype 12-3 and 77-2, which rules out the *LrSyn55* being *Lr60*. But the final conclusion can be inferred only after evaluating Synthetic55 and *Lr60* against diverse pathotypes in similar condition or getting a susceptible recombinant, if found to have similar avirulence/virulence pattern. To rule out the possibility of *LrSyn55* being *Lr42* and *LrSyn45* having the similar location and similar resistance pattern against 21 pathotypes used in study, intercross F_2 populations were developed by crossing Synthetic55 with KS91WGRC11 (*Lr42*) and Synthetic45. F_1 plants from both the cross have been confirmed to be hybrid based on phenotypic appearance. True F_1 plants were selfed to get sufficient amount of F_2 seed. F_2 seedlings from both the crosses, (Synthetic55/KS91WGRC11 (*Lr42*) and Synthetic55/Synthetic45) were tested against pathotype 77-5 along with parental lines and F_1. The F_1 seedling of both the crosses were resistant. All F_2 seedlings were expected to be resistant in case of their identical nature. Out of 566 F_2 seedling from Synthetic55/KS91WGRC11 (*Lr42*), 559 were resistant and 7 were susceptible (Table 7). The presence of susceptible recombinant in F_2 has ruled out the possibility of *LrSyn55* being *Lr42* (Figure 9). In addition to susceptible recombinant, multipathotype testing showed presence of necrosis in *Lr42* line against pathotype 12-4, 12-5, 77A, 77-3, 77-6, 77-8, 77-9 and 107-1. The representative image for the pathotype 77A and 77-6 are shown in Figure 10. This is also an indication of their non-identical nature. Further, to rule out the possibility of *LrSyn55* being *LrSyn45,* again a F_2 population between Synthetic55/Synthetic45 was evaluated against pathotype 77-5 along with parents and F_1. The presence of 31 susceptible seedlings (Table 7) from a population size of 274 again ruled out the identity of *LrSyn55* being *LrSyn45* (Figure 11).

The results from multipathotype testing indicated broad effectiveness of *LrSyn55*, *LrSyn45* and *Lr42*. The avirulence/virulence pattern ruled out the possibility of *LrSyn55* being *Lr21*. The chances of *LrSyn55* being *Lr60* can be ruled out based on their differential of resistance reaction, but can be confirmed only after testing under similar condition or after getting a recombinant susceptible plant in case of similar disease reaction.The chances of *LrSyn55* being *Lr42* or *LrSyn45* was ruled out in present study by getting susceptible recombinant seedlings.

Chapter 5 Discussion

Wheat is an important source of protein and calorie, consumed as a staple food for 30 percent of mankind (Eversole *et al.,* 2014). Three species of wheat viz. bread wheat, macaroni wheat, and emmer wheat are majorly under cultivation in India. Out of these, bread wheat (*Triticum aestivum* L.) occupy maximum area and production. Bread wheat is allohexaploid (2n=6X=42) in nature with B, A and D as its three diverse genomes (Sears, 1954; Okamoto, 1962) and formed through natural hybridizations followed by speciation (Marcussen *et al*., 2014).

Globally, India occupies second position as wheat producer following China. In 3rd Advance approximations (2018-19) from Directorate of Economics and Statistics (DES), Ministry of Agriculture & Farmers Welfare, India will realize the maximum wheat yield of 101.20 million tons. Due to genetic enhancement for yield components and resistance/tolerance to biotic and abiotic stresses, production and productivity of wheat have progressively improved in many agro-climatic zones. The chief limiting factors distressing the production of wheat are high temperature and drought in abiotic and rusts in biotic stresses.

The causal organism of Wheat rust is an obligate biotrophic fungal pathogen belonging to genus *Puccinia* of family *Pucciniaceae* under the order *Uredinales* of class Urediniomycetes and phylum Basidiomycota. Rust diseases are prevalent and cause substantial economic damages to wheat yield (Roelfs *et al.,* 1992). All the three rusts *viz.* leaf rust (*Puccinia triticina* Eriks.), stripe rust (*Puccinia striiformis* Westend) and stem rust (*Puccinia graminis* Pers. f. sp. *tritici* Eriks. & Henn.) are reported to cause severe damage periodically in conditions favorable for disease development (Roelfs *et al.,* 1992). Among 3 rusts, brown rust is more prevalent and cosmopolitan (Bolton *et al.,* 2008) as it can survive and grow at medium and broader temperature ranges compared to stripe and stem rusts which require cooler and warmer temperatures respectively. Therefore, leaf rust can be the major contributor in overall annual losses than other rusts all around the world (Marasas *et al.,* 2004, Kolmer, 2005). The level of disease damage is dependent on the crop stage when infection happens, the resistance genes deployed in the region and frequency of virulence in the pathogen population (Nagarajan and Joshi, 1975; McIntosh *et al.,* 1995). In India, brown rust is of common in entire wheat growing

region and cause penalty in wheat production. Development of cultivars having inherent resistance to rust pathogen is the best way to control yield penalty (Kolmer, 1996).

Across India, almost 120 races were identified systematically for all of the rusts, 52 of which belongs to the brown rust documented during 1931to 2015. Sixteen different pathotypes of *P. triticina* were recognized in last fifteen years (from 2000 to 2015)**.** Till date, 79 different *Lr* genes have been catalogued (McIntosh *et al*., 2017; Qureshi *et al.,*2018). Though, many of the known genes are ineffective because of the prevalence of virulent pathotypes. Most of the *T. aestivum* derived resistance genes have become ineffective in India to the pathotypes of group 77 and several valuable alien resistance genes like *Lr9, Lr19* and *Lr28* have also become ineffective (Nayar *et al*., 2003; Bhardwaj *et al*., 2005, 2010a & 2011) even before their extensive deployment in new cultivars. Owning to rapidly evolving nature of the leaf rust pathogen, it is necessary to explore novel sources of resistance.

The ancestors and wild species of cultivated wheat has been an outstanding source of resistance to several diseases in the past apart from many other useful traits *viz.* tolerance to abiotic stresses, quality characteristics, gained overall biomass and Harvest Index that were utilized for wheat improvement (Dyck *et al.,* 1970, Khush and Brar, 1992, Jiang *et al.,* 1994) which encouraged inter-specific transfer of desirable genes from wild species into wheat cultivars (Dhaliwal *et al.,* 1993). Effective efforts have been made to transfer desirable genes from wild relatives of wheat and many resistance genes have been introgressed into bread wheat(Cox *et al.,* 1994; Huang and Gill 2001; Marais *et al*., 2005; Oliver *et al*., 2005; Kuraparthy *et al*., 2007; Trethowan and Mujeeb-Kazi 2008; Riar *et al*., 2012; Liu *et al*., 2017; Mago *et al*., 2019).

Goat grass (*Triticum tauschii* (Coss.) Schmal), the "D genome donor of hexaploid wheat (*T. aestivum*)" is a comparatively unexploited germplasm pool for expanding the genetic diversity of common wheat. This species have been evaluated in the past by numerous researchers and testified to be a source of resistance to many diseases such as Karnal bunt, scab, spot blotch, leaf rust, stripe rust and abiotic stresses like cold temperature, salinity and drought (Dyck and Kerber, 1970; Kerber and Dyck, 1978; Hatchett and Gill, 1981, 1984, Martin *et al.,* 1982; Sharma and Gill, 1983; Kerber, 1984; Gill *et al.,* 1985, 1986 and Gill and Raupp, 1987).

For effective utilization of *T. tauschii*, Kihara *et al.* (1957) and Kerber and Dyck (1978) gave a method to produce synthetic wheat. They produced "synthetic hexaploid wheat by crossing *T. tauschii* as a male parent to the *Triticum turgidum L. var. durum* and doubling the chromosomes of subsequent sterile F_1 hybrid from the cross. Mujeeb-Kazi *et al.,* in 1995 at CIMMYT produced synthetic hexaploid wheats by means of the same procedure by crossing diverse accessions of *T. tauschii* (2n=14) with *T. turgidum* (2n=28) and encouraging chromosome doubling of the subsequent F_1 hybrids with 21 chromosomes using colchicine to produce 42-chromosome" Synthetic Hexaploid Wheats (SHWs).

The Synthetic Hexaploid Wheats (SHWs) that were developed then, they can be directly used to donate traits in them to cultivated wheat without cytological interference. These Synthetic Hexaploid Wheats possesses huge diversity for tolerance/resistance to abiotic/biotic stresses and yield related agronomic traits (Valkoun *et al.,* 1990; Cox *et.al*., 1994). Varying "level of resistance to leaf rust has also been stated earlier in many accessions of Goat Grass by other workers (Kihara *et al.*, 1965; Kerber and Dyck, 1969, 1973; Dyck and Kerber, 1970; Kerber and Dyck, 1978; Innes and Kerber, 1994)".

The "95 SHWs produced at CIMMYT were available with the Division of Genetics, IARI, New Delhi" and were examined against particular pathotypes of Indian leaf rust flora by Singh *et al.,* (1998). Some of these SHWs showed a varying degree of resistance reaction to leaf rust. One of the SHW, Synthetic 55 has shown a high degree of resistance to leaf rust at seedling as well as at adult plant stage in the field under artificial epiphytotic of most virulent and prevalent pathotype 77-5. Further, the leaf rust resistance gene in Synthetic55 was found to be located on chromosome 1DS of wheat and has been tentatively named as "*LrSyn55"* (Singh, 2017). As there are four other leaf rust resistance genes namely *Lr21, Lr42, Lr60* and *LrSyn45* located on the same chromosome.

Therefore, to know whether *LrSyn55* is a novel leaf rust resistance gene or one among the known *Lr* gene located on the same chromosome, present M.Sc. research work entitled "**Genetic analysis of leaf rust resistance gene "*LrSyn55*" in wheat"** has been proposed with objectives of (1) Analyzing the effectiveness of leaf rust resistance genes present on chromosome 1D (*Lrsyn55, Lrsyn45, Lr21, Lr42*, and *Lr60*) against diverse leaf rust pathotypes

and (2) Understanding allelic relationship of leaf rust resistance gene '*Lrsyn55'* with other known leaf rust resistance on chromosome 1D.

5.1. Multipathotype Testing to understand the effectiveness of *Lr* genes on 1DS

The multipathotype test is an assessment of host response with a number of pathotypes separately. The reaction is recorded as infection type (IT) based on the host response. The test is regularly done at the seedling stage for wheat rust. Resistance expressed from the seedling stage is called seedling resistance, which is operative all through the life span of the plant. Multipathotype test of Synthetic55, Synthetic45, RL6043 (Thatcher+*Lr21*), KS91WGRC11 (*Lr42)* and RL6172 (Thatcher+*Lr60*) along with three susceptible varieties Thatcher, HD2932, HD2733 against 21 pathotypes provided knowledge regarding the effectiveness of given resistance to diverse pathotypes that is required for its utilization in the breeding program. This will also help the breeder to deploy effective gene based on the prevalence of pathotypes in a target geographical area.

Synthetic55, Synthetic45, and KS91WGRC11(*Lr42)* expressed a high degree of resistance against all 21 pathotypes with infection type ranging from IT '0;' to IT ';1^{+}' while *Lr21* was susceptible to most of the pathotypes (ITs 3^{-} to 3) except 162A. *Lr21* was also reported to be ineffective to most of the leaf rust pathotypes except 10, 11, 16, 63, 106, 162A pathotype group (Prasad *et al*., 2017). This indicates that *Lr21* is not very effective but can be used in combination with other genes to provide resistance against a set of pathotypes. The reaction of RL6172 (Thatcher+*Lr60*) against all the pathotypes used in the study were susceptible with ITs of 3 to 3^{+}. The observed reaction of *Lr60* was unlikely, as the gene was reported to be effective against several Canadian pathotypes (Hiebert *et al*., 2008). Therefore, to confirm its identity SSR markers (Cfd15, Cfd61, Gdm33, and Barc149) reported to be located on chromosome 1DS (Hiebert *et al*., 2008) was used. The stock of *Lr60* was found to show similar amplicon banding pattern compared to Thatcher. The SSRs are robust markers and can be used to confirm the identity of genotypes based on fingerprint generated for different genotypes (Song *et al*., 2005). The wrong stock for *Lr60* was the reason behind aberrant phenotypic response against different leaf rust pathotypes. Therefore, the effectiveness of *Lr60* needs to be verified on the procurement of correct stock. The contrasting

parents namely “Thatcher, HD2932, HD2733 were susceptible (ITs '33^{+}' to '3^{+}') to all the pathotypes” used in the study. The highly susceptible reaction of agronomically superior varieties like HD2932 and HD2733 advocates their use as a susceptible parent in genetic analysis and can provide an advantage over the use of landraces like Agra local and Kharchia local as a susceptible parent. The use of superior genotypes can provide some desirable recombinant with superior agronomic performance. This can be helpful in breeding for rust resistance by understanding the genetic nature of genes under study as well as simultaneous selection of desirable resistant recombinants.

High level of resistance was observed on Synthetic55 (*LrSyn55*), Synthetic45 (*LrSyn45*) and KS91WGRC11(*Lr42)* against all the pathotypes used in the study. The resistance of these stocks was evaluated earlier also (Gyani *et al*., 2017; Singh, 2017 and Prasad *et al*., 2017) against several other pathotypes and they were found to be resistant. This confirms their broad-spectrum nature of resistance and their usefulness in providing resistance against a large number of pathotypes.

5.2. Validation of the genetic nature of *LrSyn55* in diverse F_2 populations

The genetic nature of gene of interest under study can be known based on the phenotypic reaction of F_1, Segregation in F_2, $F_{2:3}$ and test cross progenies. The genetic nature should be validated in diverse populations to reconfirm its behavior, any likely effect of background and interaction with other genes. The genetic behavior of *LrSyn55* was reported to be monogenic dominant in F_1, F_2, $F_{2:3}$ and test cross progenies between Synthetic 55 and Agra local (Singh, 2017). To further validate its genetic nature, F_1, F_2, and test cross progenies were developed between Synthetic 55 and susceptible parents (Thatcher, HD2932 and HD 2733). The hybridity of F_1 seedlings was confirmed through polymorphic SSR marker CFD15, having amplicons from both the parents in F_1. The use of SSR markers in hybrid confirmation is being routinely used (Nandakumar *et al*., 2004). The morphological markers like glume colour and awn traits has also confirmed the hybridity of F_1. This confirms that stable morphological traits can also be used for confirming hybridity of F_1 (Muthuraj *et al*., 2019). The reaction of tested F_1 seedlings in all the crosses were resistant with an IT of ‘;1’ to ‘;1^{+}’. This confirmed the dominant nature of *LrSyn55*.

To further validate the monogenic nature of *LrSyn55*, the F_2 populations of cross Synthetic 55/Thatcher were examined with 'pathotype 77-5 and segregation' was analysed. Phenotyping of 366 F_2 seedlings against pathotype 77-5 found 260 resistant seedlings and 106 susceptible seedlings, which fits in an expected segregation ratio for a "single dominant gene for resistance" ($\chi 2_{3:1}$=3.063, $P_{(1df)}$ =0.080).

In the second F_2 population from cross Synthetic 55/HD2932, a total of 316 seedlings were screened against pathotype 77-5. The segregation of population in 234 resistant seedlings and 82 susceptible seedlings again fits in "one dominant gene segregation ratio" with $\chi 2_{(3:1)}$ = 0.151, $P_{(1df)}$ =0.69.

In the third F_2 population from cross Synthetic 55/HD2733, a total of 332 seedlings were screened against pathotype 77-5. The segregation of population in 244 resistant seedlings and 88 susceptible seedlings again fits in "one dominant gene segregation ratio" with $\chi 2_{(3:1)}$ =0.401, $P_{(1df)}$ =0.526

The results from 3 F_2 populations developed from Synthetic55/Thatcher, Synthetic55/HD2932 and Synthetic55/HD2733 confirmed the initial report of monogenic dominant nature of *LrSyn55* (Singh, 2017). Similarly, the other genes located on chromosome 1DS namely *Lr21* was reported to be monogenic dominant (Jones *et al*., 1990) and *Lr60* also as monogenic dominant (Hiebert *et al*., 2008). Although, *Lr42* had some disagreement for it's dominant (Czembor *et al.,* 2008) or recessive nature (Liu *et al.,* 2012), but recently it has been confirmed to be monogenic dominant in original *Ae. tauschii* accession from where it has been identified initially (Gill *et al.,* 2019). The other gene *LrSyn45* is reported to be monogenic recessive in the F_2 population between Synthetic45 and Agra local (Gyani *et al*., 2017).

5.3. Validation of the genetic nature of *LrSyn55* in diverse test cross populations

To further validate the monogenic dominant nature of *LrSyn55*, test cross populations developed from Synthetic55/Thatcher//Thatcher, Synthetic55/HD2932// HD2932, and Synthetic55/HD2733//HD2733 were evaluated against pathotype 77-5. The test cross population of 38 plants from Synthetic55/Thatcher//Thatcher segregated in 16 resistant seedlings and 22 susceptible plants with a test cross segregation ratio of 1R:1S ($\chi 2_{(1:1)}$ =0.947, P=0.330 at 1 d.f). Similarly, segregation of 58 test cross progenies from Synthetic55/HD2932//

HD2932 segregated in 24R:34S with $\chi 2_{(1:1)}$ =1.724, $P_{(1df)}$ =0.189. The test cross population of 52 seedlings from Synthetic55/HD2733//HD2733 segregated in (22R: 30S) 1R:1S test cross ratio with $\chi 2_{(1:1)}$ =1.230, $P_{(1df)}$ =0.267. All the test cross populations have segregated in a ratio of 1R and 1S, which confirmed the monogenic nature of *LrSyn55* through analysis of test cross progenies.

5.4. Establishing the novelty of *LrSyn55*

5.4.1 Ruling out the possibility of *LrSyn55* being *Lr21*

Gene postulation is an established method of identifying genes based on the display of similar avirulence and virulence pattern. The difference in avirulence/virulence pattern establishes the difference between two genes (Kolmer, 2003, Randhawa *et al*., 2016). In the present study, RL6043 (Thatcher+*Lr21*) was found to be susceptible against most of the races. The similar observation has been reported earlier also (Prasad *et al*., 2017). It was found in the present study that *Lr21* is not effective against most of the pathotypes of 77 group, 12 group, 104 group, 107 and 108 group, while *LrSyn55* was highly effective against all these pathotypes. This clearly established the fact that *LrSyn55* is not *Lr21*.

5.4.2. Ruling out the possibility of *LrSyn55* being *Lr60*

RL6172 (Thatcher +*Lr60*) (NBPGR Acc. No. EC920986) received from Modern research and development center, Manitoba, Canada was also screened against 21 pathotypes of leaf rust pathogen. The reaction of the line was susceptible against all the pathotypes, which was not expected. As the line was reported to be resistant against several Canadian races (Hiebert *et al*., 2008). Therefore, the effectiveness of *Lr60* and postulation of *LrSyn55* being *Lr60* could not be confirmed. Although, the ITs reported by Hiebert *et al*., (2008) for *Lr60* against pathotype 12-3 and 77-2 is ';12', while the reaction of *LrSyn55* is IT ';$1^{=}$'. The difference in IT may be due to their non-identical nature, which can be confirmed only after evaluation of both of them under similar condition against diverse pathotypes or recovery of few susceptible plants in case of their similar display of disease reaction against all the pathotypes.

5.4.3. Ruling out the possibility of *LrSyn55* being *Lr42*

KS91WGRC11 the stock for *Lr42* was evaluated along with Synthetic 55 against 21 pathotypes of leaf rust pathogen. Both the lines were found to be resistant against all the pathotypes. Therefore, ruling out the possibility of *LrSyn55* being *Lr42* was not possible based on multipathotype testing. Although, presence of necrosis was evident on *Lr42* against pathotypes 12-4, 12-5, 77A, 77-3, 77-6, 77-8, 77-9 and 107-1. The difference in infection types between *LrSyn55* and *Lr42* indicted their likely difference. Further, to understand the allelic relation, an F_2 population was developed by the crossing of Synthetic 55 with KS91WGRC11. The presence of 7 susceptible seedlings in an F_2 population of 566 against pathotype 77-5 has confirmed their non-identical nature. Similar allelism test was also used to rule out the possibility of identical nature of *Lr21* and *Lr60* through screening of 1141 F_2 plants from the cross of RL6043 (*Lr21*)/RL6172 (*Lr60*), there were 29 seedlings with either higher infection types (2) or susceptible infection types (3–4) (Hiebert *et al*., 2008).

5.4.4. Ruling out the possibility of *LrSyn55* being *LrSyn45*

Synthetic 55 and Synthetic 45 were reported to carry leaf rust resistance gene on chromosome 1DS. The difference in the genetic nature of *LrSyn55* being dominant and *LrSyn45* being recessive suggests their likely difference (Singh, 2017; Gyani *et al*., 2017). Further, both the lines were found to be resistant against all the 21 pathotypes used in the study. But Synthetic 45 has an ITs of 'X^- to $X^{=}$' against 77-7 and 77-9, while Synthetic 55 has an IT of '0;'. This has again indicated the likely difference between them. But to confirm their ultimate difference, again an F_2 population between them has been developed and screened against pathotype 77-5. Presence of 31 susceptible seedlings from a total population of 274 has again ruled out the possibility of *LrSyn55* being *LrSyn45*.

The present study confirmed the monogenic dominant nature of *LrSyn55*, which is found to be a broad spectrum in nature along with *Lr42* and *LrSyn45*. Multipathotype testing ruled out the possibility of *LrSyn55* being *Lr21*, as later was susceptible to several pathotypes against which *LrSyn55* is resistant. Further, the possibility of *LrSyn55* being *Lr42* and *LrSyn45* is ruled out based on the presence of susceptible seedlings in the F_2 population between them.

Therefore, most likely Synthetic 55 carries a novel rust resistance gene which will be useful for diversifying the base of leaf rust resistance in wheat breeding.

Chapter 6 Summary and Conclusion

Wheat yield is primarily hampered by three rusts *viz.* leaf rust, stripe rust and stem rust in the world. Leaf rust is very widespread among wheat diseases causing economic damages to the wheat yield in all the wheat growing areas throughout the globe. Wheat rust is an obligate parasite which requires essentially a living host and evolves rapidly to develop novel pathotypes that offset the prevailing resistance in the extant varieties.

Therefore, to deal with quick evolving rust pathogen, there is a quest in all parts of the planet for the identification of novel resistance genes and incorporation of the novel genetic variation in wheat varieties. Cytogeneticists were targeting on the manipulation of the chromosome constitution using related species to introgress new variability for resistance. Mujeeb-Kazi *et al.,* (1995) in CIMMYT created Synthetic Hexaploid Wheats (SHWs) by hybridizing different accessions of *T. tauschii* (Goat Grass) with *T. turgidum* to obtain 42-chromosome fertile wheat, to utilize the unexploited variability of *T. tauschii* for resistance/tolerance to various biotic and abiotic factors, quality and yield-related traits without use of cytogenetic manipulation.

A set of these 95 SHWs were available at Division of Genetics, IARI, New Delhi. Preliminary studies suggested some of these SHWs having resistance to important Indian pathotypes of leaf rust. The leaf rust resistance gene present in Synthetic 55 is a dominant gene located on short arm chromosome 1D of wheat and has been tentatively designated as '*LrSyn55*' (Singh, 2017).

Already four *Lr* genes are reported to be present on the short arm of chromosome 1D namely *Lr21, Lr42, Lr60* and *LrSyn45.* Therefore, to test whether putative "*LrSyn55*" is a new gene or a previously existing gene, genetic analysis of leaf rust resistance gene "*LrSyn55*" in wheat was carried with objectives to analyze the effectiveness of leaf rust resistance genes present on chromosome 1D (*Lrsyn55, Lrsyn45, Lr21, Lr42,* and *Lr60*) against diverse leaf rust pathotypes and to understand the allelic relationship of leaf rust resistance gene '*Lrsyn55'* with other known leaf rust resistance genes on chromosome 1DS.

Complete experimentations related to the research took place at the glass house, field and molecular biology laboratory of Division of Genetics and National Phytotron Facility, Indian Agricultural Research Institute, New Delhi.

The monogenic dominant nature of *LrSyn55* was validated in 3 diverse F_2 populations developed by crossing of Synthetic 55 with susceptible varieties Thatcher, HD2932 and HD2733. The F_2 seedlings in 3 populations had segregated in a ratio of 3R and 1S. In addition, the test cross populations developed by crossing of F_1 with recessive susceptible parents have segregated in 1R and 1S. The segregation ratio in F_2 and test cross populations have confirmed the initial report of monogenic dominant nature.

Leaf rust resistant lines carrying *Lr* genes on chromosome 1DS namely Synthetic 55, Synthetic 45, RL6043 (Thatcher+*Lr21*), KS91WGRC11 (*Lr42)*, RL6172 (Thatcher+*Lr60*) along with three susceptible varieties Thatcher, HD2932, HD2733 were evaluated against 21 pathotypes of leaf rust pathogen *Puccinia triticina* at seedling stage under glasshouse conditions. Infection types appeared on different lines concluded that Synthetic 55, Synthetic 45, and KS91WGRC11 (*Lr42)* are effective sources of resistance and can be utilized in resistance breeding program, as they have been found to be resistant against all 21 pathotypes used in study.

Appearance of susceptible reaction on RL6043 (Thatcher+*Lr21*) against pathotypes of 77 group, 12 group, 104 group, 107 group and 108 group has ruled out the possibility of *LrSyn55* being *Lr21,* because Synthetic 55 was resistant to all these pathotypes.

Possibility of *LrSyn55* being *Lr60* could not be ruled out in present study, because of non-availability of correct stock. The wrong stock of *Lr60* was found to be susceptible to all the pathotypes, which was not expected as per reported reaction of RL6172. The line was also confirmed through SSR markers to be Thatcher. Even though, the chances of being *LrSyn55* not being *Lr60* can be predicted based on infection type of *Lr60* ‘;12’ against 12-3 and 77-2 by Hiebert *et al*., 2008 while the IT of Synthetic 55 ‘;1⁼’. But the final conclusion can be inferred only after multi pathotype testing in similar condition or appearance of recombinant susceptible seedling in case of similar avirulence/virulence pattern.

Synthetic 55, Synthetic 45 and KS91WGRC11 (*Lr42)* have displayed resistant reaction against all the pathotypes used in study. Although, appearance of necrotic infection type on *Lr42* against some of the pathotypes like 12-4, 12-5, 77A, 77-3, 77-6, 77-8, and 107-1 has indicated likely difference with *LrSyn55*, which was confirmed through presence of 7 recombinant susceptible seedlings out of 566 seedlings from a F_2 population between intercross of Synthetic 55 with KS91WGRC11. Further, the possibility of *LrSyn55* being *LrSyn45* was also ruled out based on presence of 31 recombinant susceptible seedling in F_2 population between Synthetic 55 and Synthetic 45.

Conclusion

The segregation of F_2 and testcross populations between Synthetic55 and susceptible lines (Thatcher, HD2932 and HD2733) has reconfirmed the monogenic dominant nature of *LrSyn55*. The contrasting infection types on Synthetic 55 and RL6042 (Thatcher+*Lr21*) against several pathotypes has ruled out their chances of being same gene. The chances of *LrSyn55* being *Lr60* could not be ruled out because of non-availability of correct stock. The chances of *LrSyn55* being *LrSyn45* and *Lr42* was ruled out based on presence of recombinant susceptible seedlings in F2 population between them. The results predict the *LrSyn55* being a new gene present on short arm of chromosome 1D of wheat, which is effective and broad spectrum in nature.

Abstract

Genetic Analysis of Leaf Rust resistance Gene "*LrSyn55*" in Wheat

Wheat productivity is severely impacted by incidence of rust infection across the globe. Among the wheat rusts, leaf rust is most common and causes economic loss to the wheat crop. Leaf rust of wheat caused by *Puccinia triticina* Eriks, is an 'obligate biotrophic parasite' that evolves frequently and develops new pathotypes to overcome major resistance genes being exploited in wheat breeding.

Therefore, to handle this rapidly evolving rust pathogen, there is a continuous quest for identifying new resistance genes throughout the world. In this effort, one of the Synthetic Hexaploid Wheats (SHW), Synthetic 55 [GAN/*Ae. squarrosa* (180), CIMMYT Synthetic ID: 221, Cross ID: CIGM 90-799] available at Indian Agricultural Research Institute, New Delhi was investigated for confirmation of inheritance of leaf rust resistance gene "*LrSyn55*", effectiveness of *Lr* genes located on 1DS and testing the allelic relationship of *LrSyn55* with the other four known genes located on short arm of chromosome 1D.

Leaf rust resistance gene carried by Synthetic55, *LrSyn55* was confirmed to be monogenic dominant in 3 F_2 and 3 Test cross populations involving Thatcher, HD2932 and HD2733 as susceptible parent. As *LrSyn55* was reported to be located on chromosome 1DS, having 4 other *Lr* genes viz., *Lr21*, *Lr42*, *Lr60* and *LrSyn45*. The multipathotype testing against 21 pathotypes suggested the resistance of *LrSyn55*, *LrSyn45* and *Lr42* to be effective against all the pathotypes. The chances of *LrSyn55* being *Lr21* and *Lr60* were ruled out based on infection types against different pathotypes, although multipathotype testing of *Lr60* could not be performed due to unavailability of true stock. The likelihood of *LrSyn55* being *Lr42* and *LrSyn45* was ruled out by observing recombinant susceptible seedlings in F_2 population developed by inter crossing the effective *Lr* genes on chromosome 1DS. The research work undertaken confirms the uniqueness of *LrSyn55* compared to other *Lr* genes located on chromosome 1DS. The study also identifies its broad spectrum effectiveness to be exploited for resistance breeding.

Keywords: Synthetic Hexaploid Wheat, Resistance, *LrSyn55*, Leaf Rust, Genetic Analysis

सार

गेहूँ में पर्ण रतुआ प्रतिरोधी जीन "एलआरसिन ५५" का आनुवंशिक विश्लेषण

विश्व भर में रतुआ संक्रमण की घटनाओ से गेहूँ की उत्पादकता बुरी तरह से प्रभावित होती है। गेहूँ रतुआ में, पर्ण रतुआ सबसे आम है। जो गेहूँ की फसल को आर्थिक हानि पहुँचाती है। गेहूँ का पर्ण रतुआ एक अविकल्पी जैवरूपक परजीवी "*पुक्सिनिआ ट्रिटिसीना* ईरिक्स" के कारण होता है। जोकि अक्सर तेजी से विकसित होता है, और गेहूँ प्रजनन में प्रमुख प्रतिरोध जीन का दोहन करने के लिए नये रोग प्रजनक प्ररूपों को उत्पन्न करता है।

इसलिए इस तेज़ी से विकसित होने वाले रतुआ रोगजनक को रोकने के लिये, विश्वभर में नये प्रतिरोधी जीन की पहचान करना एक निरंतर अनुसंधान है। इस प्रयास में भारतीय कृषि अनुसंधान संस्थान, नई दिल्ली में उपलब्ध संश्लेषित षट्गुणित गेहूँ में से एक, संश्लेषित ५५ [जीऐऍन / *एजेलोप्स इसकारोसा* (१८०), सीआईएमएमवाईटी संश्लेषित आईडी:२२१, संकर: सीआईजीएम ९०-७९९],का निरूपण पर्ण रतुआ प्रतिरोध जीन "एलआरसिन ५५" की वंशानुगति, १डीएस पर उपस्थित एलआर जीनो की प्रभाविता और १डी गुणसूत्र की छोटी भुजा पर उपस्थित अन्य चार जीनो के साथ एलआरसिन ५५ के विकल्पिता सम्बन्ध परीक्षण की पुष्टिकरण के लिए किया गया है।

एलआरसिन५५ पर्ण रतुआ प्रतिरोधी जीन जोकि संश्लेषित ५५ में उपस्तिथ है, को ३ एफ २ और ३ परीक्ष्यार्थ संकरण समष्टि जिसमें अतिसंवेदनशील जनक थेचर, एचडी२९३२ और एचडी२७३३ निहित है, में एकलजीन प्रभाविता के लिए सुनिश्चित किया गया है। जैसा कि एलआरसिन५५ को १डीएस गुणसूत्र पर स्थित होने की सूचना मिली थी, जिसमें चार अन्य एलआर जीन, एलआर२१,एलआर४२, एलआर६० और एलआरसिन४५ थे। इक्कीस रोगजनकप्ररूप के प्रति बहुरोगजनकप्ररूप परीक्षण ने एलआरसिन५५, एलआरसिन४५ और एलआर४२ को सभी रोगजनकप्ररूप के प्रति प्रभावी होने का सुझाव दिया। एलआरसिन५५ के एलआर२१ और एलआर६० होने की संभावना को अलग-अलग रोगजनकप्ररूप के प्रति संक्रमण प्रकारों के आधार पर नकारा गया था, हालांकि एलआर६० के बहुरोगजनकप्ररूप परीक्षण को यथार्थ वंश की अनुपलब्धता के कारण प्रदर्शन नहीं किया जा सका। एलआर४२ और एलआरसिन४५ के एलआरसिन५५ होने की संभावना को एफ २ समष्टि में पुनर्योजक अतिसंवेदनशील अंकुरों का अवलोकन करके विकसित किया गया था, जो १डीएस गुणसूत्र पर प्रभावी एलआर जीन को अंतरसंकरण करके विकसित किया गया था। यह अनुसंधान कार्य, १डीएस गुणसूत्र पर स्थित अन्य एलआर जीन की तुलना में, एलआरसिन५५ की विशिष्ठता की पुष्टि करता है । यह अध्यन, प्रतिरोध प्रजनन के लिए इसकी व्यापक वर्णक्रम प्रभावशीलता की भी अभिधारणा करता है।

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