Molecular and field characterization of exotic spring wheat germplasm for disease resistance genes and yield

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DECLARATION

I would like to declare that the data presented in this thesis "**Molecular and field characterization of exotic spring wheat germplasm for disease resistance genes and yield**" is generated by myself from the original research work in under the supervision of **Dr Armghan Shehzad** at the National Institute for Genomics and Advanced Bio-Technology (NIGAB), National Agricultural Research Centre, Pakistan. The results and material used in this thesis are genuine and never presented anywhere else earlier.

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Affaq Aslam

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Signature

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ABBREVIATIONS

NARC	National Agriculture Research Centre
NIGAB	National Institute of Genomics and Advanced Biotechnology
ARI	Agricultural Research Institute
AUDPC	Area Under Disease Progress Curve
$(NH_4)_2SO_4$	Ammonium sulfate
°C	Degree Celsius
CDRI	Crop Diseases Research Institute
cM	Centi Morgan
Conc.	Concentration
CTAB	Cetyl Trimethyl Ammonium Bromide
ddH_2O	Double distilled water
dH_2O	Distilled water
DNA	Deox*yr*ibonucleic Acid
dNTPs	Dinucleotide Triphosphates
EDTA	Ethylenediaminetetraacetic acid
ESTs	Expressed Sequence Tags
g	Gram
Lr	Leaf rust resistance gene
MAS	Marker Assisted Selection
mg	Milligram
ng	Nano gram
NILs	Near Isogenic Lines
PCR	Polymerase Chain Reaction PIC
pmol	Picomole
QTL	Quantitative trait loci
Yr	Yellow Rust
SNPs	Single Nucleotide Polymorphism
SSR	Simple Sequence Repeats
STS	Sequence Tagged Sites
Taq	*Thermus aquaticus*
TBE	Tris borate EDTA

TE	Tris EDTA
U	Unit
ul	microliter
PIC	Polymorphic Information Content

Abstract:

Wheat (*Triticum aestivum* L.) is major cereal crop that contributes the nutrient requirements of about 35% world population having high contents of starch, proteins, and vitamins. Wheat in Pakistan is prone to many biotic and abiotic factors. Stripe rust (biotic factor) caused by pathogen *Puccinia striiformis* f. sp. *tritici* (*Pst*) is a major threat for wheat cause loss in yield and grain quality in many countries. To control the disease, genetic resistance is the most effective method that can be attained by identifying the resistant genetic regions through wheat genome mapping. Mapping the resistant loci and their association with traits is highly exploited in this era. Major aim of current research was to evaluate 200 exotic spring wheat advanced lines with 4 local check varieties in field for yield characteristics and disease reaction. Also, the gene postulation for stripe rust resistant genes with the help of DNA markers was carried out. The DNA markers genotyped were also used for association mapping with yield related traits. The 200 exotic spring wheat advance lines were evaluated for phenotypic correlation of fifteen morphological traits. PCA analysis revealed positive correlation among yield per plant with Flowers per spike and number of tillers. Total 38 *SSRs* markers were applied on 204 wheat genotypes against fifteen stripe rust resistance genes; *Yr5, Yr15, Yr17, Yr18, Yr26, Yr36, Yr46, Yr48, Yr54, Yr59, Yr60, Yr61, Yr62, Yr64* and *Yr65* genes Markers revealed higher diversity based on Polymorphic Information Content (PIC) value. In addition to disease resistance, 23 out of 38 markers were also associated with growth and yield traits of wheat genotypes studied. Clustering based on virulence data grouped contemporary isolates together and revealed high genetic diversity among lines. The present research findings can be exploited for increased yield and disease resistant capability of studied wheat lines.

1 Introduction:

Wheat (*Triticum aestivum* L.) is one of the predominant food crops of world. It is a member of the Poaceae family. On the basis of chromosome number, it is subdivided into few species: Diploid (2n=2x=14; with 7 chromosomes pair e.g., einkorn wheat); Tetraploids (2n=4x=28; with 14 chromosomes pair e.g., durum wheat; Hexaploids (2n=6x=42) with 21 chromosomes pair e.g., Bread wheat (*T. aestivum*). Hexaploid wheat is developed by crossing tetraploid wheat *Triticum turgidum* (AABB) and diploid *Aegilops tauschii* (genome DD). Doubling of chromosome transform this cross into a diploid wheat. As a result, it now has three homologous chromosomes, one for each of genomes A, B, and D. All of them contribute 7 chromosomes. AABBDD with 2n = 42 (Ekboir, 2002; Levy & Feldman, 2002). It is nearly 5 time bigger than human chromosome with a size of 17 Gb (Keller, Feuillet, & Yahiaoui, 2005)

In the marketing year of 2020/2021, the global production volume of wheat amounted to over 772.64 million metric tons. Evaluation revealed that only 10 percent wheat of developed country is exported to feed 3rd world countries (Ekboir, 2002). Pakistan ranked 8th among them with annual production of 25.4 million tons. Beyond any doubt, wheat provide carbohydrates, mineral, vitamins etc. Probably, 20 percent daily dietary calories are supplied by wheat. From 1947 to 2014, its cultivation area is increased up to 129% while production is elevated up to 612%. Around 10% of total value addition and 2% in gross domestic product (GDP) is shared by this single crop.

1.1 Rust:

Pathogens are utmost threat to the production of wheat and are continuously affecting our economy. Among all type of bacterial, fungal and viral attacks occur on wheat, the rust is the most threatening (Boyd, 2005; Wellings, 2011). It has its optimum temperature and humidity; and developed in epidemic form in maritime and temperate regions. As compare to lower altitude, the higher ones provide it a vital chance to spread rapidly (Johnson, 1992). That's why, it becomes obligatory to know the exact biology, ecological behavior, genetics etc, to develop a resistance cultivar (Ali et al., 2014). No less than 5.5 million tons yield losses occur only due to the stripe rust worldwide (Beddow et al., 2015). 70% of Pakistani wheat is susceptible to yellow rust (Ravi P Singh, William, Huerta-Espino, & Rosewarne, 2004).

The rust is of 3 kinds. Leaf rust (*Puccinia triticina* f.sp. *tritici),* stem rust (*Puccinia graminis* f.sp. *tritici*) and stripe rust (*Puccinia striiformis* f. sp. *tritici*). Stripe rust is the most common of them, and it has been found all throughout the globe excluding Antarctica. Stripe rust infection has been observed in over sixty countries worldwide (XM Chen, 2005). Many rust epidemics have been recorded throughout history, having severe impacts on the economy. It was once thought that this was a cool-climate disease (Ravi P Singh et al., 2004). However, recent outbreaks have been observed in warmer climates, indicating that this disease has adapted to the warmer temperature and has shifted from a colder to a hotter temperature (Case et al., 2014). Historically, disease was first identified in Europe during the 18th century by Gadd who termed it stripe rust.

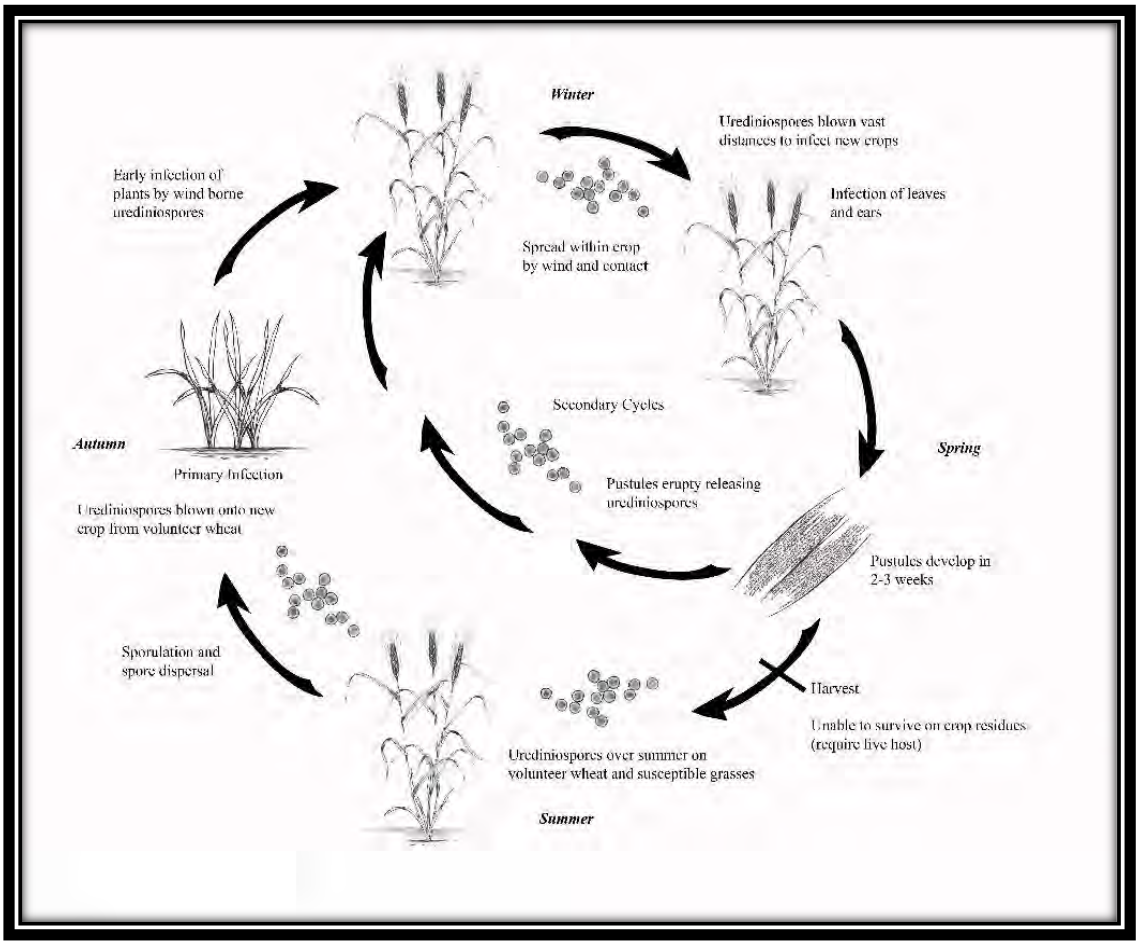

Figure 1. 1 Complete Cycle of Stripe Rust

The genus Puccinia, which includes the Pucciniaceae family, belongs to the Basidiomycetes class, which includes the order Uredinales (XM Chen, 2005). Stripe rust is found in bread wheat and durum wheat, triticale, and a few kinds of barley. Jin

et al. have identified a new host for stripe rust (Jin, Szabo, & Carson, 2010). Barberis spp. offer sexual recombination in the off-season in the manner of pycnial and acial phases, as well as asexual uredial and tellial stages on accessory hosts. The Himalayan region of the Indian subcontinent is recognized as a center of variety for sexual recombination with numerous Barberis species.(Ali et al., 2014).

Table1. 1 *Puccinia striiformis* f. sp. *tritici* has a wide spectrum of hosts (Pst).

Source: (W. Chen, Wellings, Chen, Kang, & Liu, 2014)

Primary hosts	**Pycnial or aecial (alternate) hosts**	**Accessory hosts**
Triticum 4 spp. E.g. wheat crops (cultivated) (*T. aestivum* L., *T. dicoccum* Schrank, *T. turgidum* var. *durum* L., *T. dicoccoides* Korn)	*Berberis* spp. (*B.atrocarpa*$_1$, *B. stenostachya* , *B. shensiana1, B. soulieana, B. wangii1*, *B. phanera1*, *B. davidii1*, *B. poiretii1*, *1*, *B. potaninii1*, *B. jamesiana1*, *B. aggregata* var. *integrifolia, B. ferdinandi-coburgii*$_1$, *B. brachypoda1*, *B. circumserrata*$_1$, , *B. dasystachya1*, *B. aggregata1*, *B. chinensis*$_2$, B. *platyphylla1B. holstii2*, *B. koreana2, B. vulgaris2* and *B. guizhouensis*$_1$)	*Mahonia aquifolium*3 (Oregon grape) observed as under artificial inoculation
Hordeum vulgare L. (Cultivated barley)	*Mahonia aquifolium*3 (Oregon grape) observed as under artificial inoculation	
Triticosecale (Triticale) *Secale cereale* L. (Cultivated rye)		

Stripe rust affects all sections of the plant, but mainly green leaves, leaf sheaths, glumes, and awns. These diseases have a direct impact on seedling germination, growth processes, height decline, leaf damage, floral set lessen, low quality fodder, grain shriveling, and grain yield loss. Striped or orange pustules produce a lengthy stripe between veins and are organized in a systemic fashion. When a susceptible wheat variety is cultivated, all the following sections are severely harmed. On the resistant

varieties, symptoms ranged from no visible signs to hypersensitive specks surrounded by necrosis or chlorosis (Chen, 2005). When the circumstances are favorable and a susceptible host is present, infection can occur at any moment during the life cycle of the plant (Ahmed et al., 2014). The pathogen's ideal temperature for disease development is between 12 and 15°C, while 3°C is the minimal temperature for growth (Chen, 2005).

1.1.1 Mode of Transmission:

Rust fungus are obligate parasitic biotrophs that cannot grow on artificial culture mediums. These spores can infect the host over a great distance. Pathogens have a remarkable capacity to spread across nature. Rusts are the most common cause of wheat loss, and they do more damage in a shorter amount of time than any other disease (Chen et al., 2014).

The disease may spread by wind, water, birds, and even humans, although wind is the most essential mode of transmission. Rust has no geographical boundaries, and wind directions allow stripe rust to spread (Duveiller, Singh, & Nicol, 2007). There are three important variables to consider: Pathogen development; Time, pathotypes (microorganism). Wind direction enhances the opportunities for pathogen development (Ali et al., 2014).

Stripe rust virulence for *Yr*9 resistance gene was first reported in East Africa in 1986, then moved to S*yr*ia, Turkey, and finally Iran in North and Middle East Asia in 1991-92. The viral race's transmission channel to Pakistan and India during the 1986-87 wheat crop season and subsequently to Nepal is a perfect demonstration of wind dispersal (Ravi P Singh et al., 2004). Similarly, virulence that made *Yr*2 gene susceptible, was originally discovered in Turkey and was then tracked in Pakistan by wind. Another example of wind-borne spreading is the transfer of stem rust race *Ug-99* from Uganda to Iran. Within the period of six months, stripe rust expanded from northern Mexico through southern Texas to North Dakota, covering a span of over 2,000 kilometers (Xianming Chen, Penman, Wan, & Cheng, 2010).

1.1.2 Wheat Rust in Subcontinent:

In Pakistan, rust outbreaks have caused various degrees of devastation (Duveiller et al., 2007). Stripe rust is a major concern in Pakistan's wheat-growing region, which covers 70% of the country (Qamar, Gardezi, & Iqbal, 2012). Stripe rust hotspots include central Punjab, Khyber Pakhtunkhwa, and sections of Baluchistan. In Pakistan, thirteen outbreaks of wheat rust have been recorded in past (Afzal et al.,

2008). Pakistan suffered four major wheat rust epidemics in 1978, 1994-95, 1997-98, and 2005 (Bahri et al., 2011).

The major cultivar Mexipak was attacked by the leaf rust pandemic in its early stages in 1978, resulting in a massive loss of 10.1 percent, approximately US$86 million (Bux et al., 2012). The technique for producing wheat varieties in Pakistan was swiftly altered because of this epidemic. During the 1991-92 wheat growing season, a stripe rust outbreak was detected on local white wheat in the Baluchistan region, resulting in enormous losses. Pak-81 and Pirsabak-85 (*Yr*9), magnificent wheat varieties with comparable genetic origins, were badly damaged by stripe rust in 1995, resulting in an outbreak in Pakistan (Afzal, Haque, Ahmedani, Bashir, & Rattu, 2007). During the 2003-04 season the epidemic on the Inqilab-91 (*Yr*27) was serious, because 80% of the wheat land alone was planted with a single cultivar.

1.1.3 How to control Stripe Rust:

Fungicides and resistant cultivars are two alternative ways to tackle wheat rusts. First one is costly, non-environmental friendly, must be used at the right time during each crop season (Yong et al., 2015). Neighboring field's unprotected crops also provide continuous inoculum to the fungicide-protected field. Moreover, poor farmers may not be able to afford fungicide spraying (Qamar, Ahmad, Rabbani, Shinwari, & Iqbal, 2014).

The second option is to build genetic resistance through the development of resistant cultivars that are both environmentally favorable and reasonably long-lasting. There are two types of genetic resistance: race specific and non-race specific. Major genes govern race specific resistance or vertical type resistance, which offers total resistance (Parlevliet, 1975). Major genes limit the pathogen's capacity to multiply on the host plant and prevent the fungus from overcoming resistance (Rosewarne et al., 2013). Such type of resistance is manifested as hypersensitivity and eventually cause the death of fungus. Resistance gained by using major genes is not long termed solution because pathogens easily overcome such resistance via mutation. In Pakistan, majority of wheat cultivars have resistant genes like *Yr2, Yr5, Yr7, Yr10, Yr15, Yr27 etc.* (Begum et al., 2014).

Virulence has already developed in Pakistan, except for *Yr*5, *Yr*10 and *Yr*15. Recent observations in KPK indicate the presence of virulent isolates of the stripe rust

resistance genes *Yr*5 and *Yr*15 (Chen et al., 2014). A single gene-based rust pandemic was also observed for Mexipak and Inqilab-91, with significant area covered by monoculture and that was Pakistan's worrying situation (Afzal, Ul-Haque, Rauf, Ahmad, & Firdous, 2010).

Table1. 2 Type of Resistance with their basic descriptions

Type of Resistance	Description	Durability
Seedling or all-stage	Can be seen on initial stages but it stays effective in the entire plant life.	Usually not durable
Adult Plant resistance (APR)	Can't be observed at seedling stage and only expressed in adult plants	Usually observed as durable
Race specific Resistance	Specific against some races and not to others	Usually not durable
Complete or immune	No visible symptoms	Usually not durable
Slow rusting or non-HR	Susceptible Infection type with low aggressiveness and severity (i.e., rust develops slowly)	…….
Qualitative or monogenic	Controlled by one gene and expresses in two distinct classes within a segregating population	Usually, non-durable
Quantitative or polygenic	May be controlled by more than a single gene and expressed variation within a segregating population	Usually, durable

Non-race specific resistance is the second form of resistance, and it consists of minor genes that provide resistance to mature plants. Minor genes work together and do not provide complete resistance. These low rusting genes are considered polygenic and have an additive impact (Kumar et al., 2015). Such resistance is long-latent, have smaller uredinia, less infection and spore production (Parlevliet, 1975). Slow corrosion or minor genes are shown as a sluggish illness progression curve (Ravi Prakash Singh, Huerta-Espino, & William, 2005). Wheat lines have recently been created that are close to immunity because of the accumulation of four resistance genes. These genes are quantitatively inherited and, when combined, give a high level of resistance.

1.1.4 Mapping of Rust Resistance:

Many genes influence agriculturally essential characteristics such as production, quality, and some types of disease resistance. It is not feasible to identify by phenotypic assessment (Collard, Jahufer, Brouwer, & Pang, 2005). Molecular markers provide a solution to difficulties encountered during traditional breeding procedures and can increase the effectiveness of breeding programmes (Todorovska, Christov, Slavov, Christova, & Vassilev, 2009). Through mapping and flanking markers, more than 150 QTLs for stripe rust have been discovered.

A variety of markers, including Simple Sequence Repeat (SSRs), Random Amplified Polymorphic DNA (RAPDs), Amplified Fragment Length Polymorphism has been researched for stripe rust pathogen (AFLPs). Microsatellites, also referred as simple sequence repeats (SSR), are highly polymorphic, chromosomal specific, robust, and dependable among them (Collard et al., 2005). After DNA markers linked with stripe rust resistance QTLs or gene, they may be utilized as an indirect method to select them. These markers can also be beneficial for genes p*yr*amiding.

These are di- (CA)n, tri- (AAT)n, and tetra-nucleotide (GATA)n tandem repetitions of short nucleotides with 1-6 base pairs. They may be found throughout the genomes of both plants and animals (Xu, 2010). Individual PCR amplification of DNA fragments is performed using distinct oligonucleotide primer pairs based on complementarity to DNA sequences, found in the flanking regions of the SSR sequence (Durand et al., 2010).

SSR markers are PCR-based and exhibit a high degree of polymorphism, which allows for the differentiation of closely related breeding materials. SSR markers are often used to identify a single locus and are quite useful when working with allopolyploid species. SSRs have rapidly become the preferred markers for the development of wheat genetic maps. Hundreds of SSR primer pairs have been produced for each of wheat's three genomes. Additionally, SSR markers are used as anchor markers to detect QTLs.

After providing genetic foundation of rust resistance by Biffen (1905), Physiological specialization by Stakman and Lavine (1922), and Gene for gene theory by Flor (1956), it became possible to enhance wheat by finding resistant genes from wheat as well as foreign sources. These ideas and theories paved the way for wheat

development. Fortunately by advancing in molecular genetics, researchers may now include different commercially significant characteristics, after finding them in various food and fiber crops (Begum et al., 2014). Around 70 resistance genes against stripe rust have been found, either completely or partially. Numerous DNA markers for identifying Yellow/Stripe rust resistance genes are now available, and multiple stripe rust resistant genes have been identified using these markers. The purpose of this study is to learn more about the existence of these genes in advanced Lines and how they respond in the field.

Objectives of the study:

1. Determination of morphological characterization of exotic germplasm in field against stripe rust.
2. Molecular characterization of 210 exotic wheat germplasm for yellow rusts resistance genes by using linked DNA markers.

2 Material and Methods:

2.1 Plant material:

200 advanced wheat lines as plant material were acquired from China. As a control, we utilized four commercial wheat cultivars (Table 2.1) that were supplied by the National Agriculture Research Center in Islamabad, Pakistan.

2.2 Field experiment:

The lines were planted on the 7th of December 2020. The late seeding was intended to offer the best possible conditions for establishment of a rust epidemic in the wheat crop. The randomized complete block design was used for this experiment. Number of replications were two. The ground was adequately prepared by ploughing it with a normal cultivator and then planking it. Each single Line was 2.5 meters long and 30 centimeters apart. Location for this experiment was National Institute for Genomics and Advanced Biotechnology (NIGAB), NARC, Islamabad, for the assessment of yellow rust. In addition, rows of Morocco were planted surrounding the nursery to act as a spreader.

2.3 Growth related traits:

Growth related traits observed during the study were: germination data, days to tillering, days to heading, days to flowering, plant height (cm), flag leaf (cm) and number of nodes.

2.3.1 Germination Data (GD):

Data of successfully germinated plants were being collected twice. 1st data collection was performed after 10 days of sowing and 2nd after 30 days of sowing. Data of 2nd interval was used as a final growth data.

2.3.2 Days to tillering (DTT):

Data were recorded when first tiller appeared within Line.

2.3.3 Days to heading (DTH):

Data were recorded when the 50% of spike (also called the head or ear) is emerged within the flag leaf.

2.3.4 Days to flowering (DTF):

Data were recorded when pollen was being released and the individual grains were being fertilized.

2.3.5 Plant height (PH; measured in cm):

The plant height was recorded based on mean height of three plants that were selected randomly from each Line. Plant height was measured from ground surface to the edge of main stem when pods changed their color from green to lemon yellow. Plant height was measured in centimeter through measuring scale.

2.3.6 Flag leaf (FL; measured in cm):

Emergence of final leaf is termed as flag leaf. Its length is measured from base of that leaf to tip, for the same plants that were selected to measure plant height.

2.3.7 Number of nodes (NN):

A prominent knot, swelling tissue termed the Node; is noticeable above the soil surface on reproductive tillers. It was carefully counted manually.

2.4 Yield Related Traits:

Yield related traits were spike/kernel length (cm), awn length (cm), no. of flower per spike, no. of spikelet's pairs, no. of flowers per spikelet, no. of tillers, Yield in gram per hectare.

2.4.1 Spike length (SL; measured in cm):

The mature spikes were selected randomly from three plants per Line and spike length was measured separately from each plant.

2.4.2 Awn length (AL; measured in cm):

Length of hair- or bristle-like appendage (Awn), extend from the lemmas of the florets, was recorded by measuring with meter rod.

2.4.3 Number of flowers per spike (F/S):

Total number of flowers were counted to estimate the expected seed production by that single spike.

2.4.4 Number of spikelet's pairs (SP):

Spikelets were counted from selected spike to take into consideration the actual number of sets. It has a direct relation with yield. Because increase in spikelet pairs cause increase in seed number.

2.4.5 Number of flowers per spikelet (f/s):

Without discrimination of fertile or infertile flower, the data were recorded of each single flowers in spikelets of selected plants.

2.4.6 Number of tillers (NT):

Tillers/branches per plant were manually counted on three randomly chosen plants from each Line in each replication, and then the mean of all the plants were calculated.

2.4.7 Seed weight in gram per Hectare (Wt):

The spikes were threshed and weighed from Line by using digital electronic balance. After this, weight was calculated by converting Line area into hectare.

The crop was fertilized with 120, 85, and 65 kg of nitrogen, phosphorous and potassium per hectare of land. Four irrigations were applied during tillering, booting, anthesis, and grain production stages of the crop. Manual weed management and hoeing were put into practice.

Table 2. 1 List of 200 segregating Lines along 4 Checks

LINES	Genotype	LINES	Genotype	LINES	Genotype	LINES	Genotype
1	k 456× 82.2118	**26**	K-78 × Y2-154	**51**	DF-8	**76**	Y2-18
2	Y1 495 × C 244	**27**	K-456 × Y2-218	**52**	DF-7	**77**	C-T 248
3	Y1-559×C 1003	**28**	K-456 × Y2-43	**53**	Y2-111	**78**	E1-456
4	Y1-514 × C 223	**29**	K78 × C-Y-	**54**	Y2-118	**79**	Y2-409
5	K456 × Y2-154	**30**	K-78 × Y2-196	**55**	K456 × 248	**80**	F1-78
6	K456 × Y2-139	**31**	K-456 × Y2-196	**56**	Y2-91	**81**	Y2-321
7	K456 × Y2-193	**32**	K-78 × Y2-136	**57**	K-78 × Y2-409	**82**	Y1-389
8	K456 × Y2-382	**33**	Y1-389 × C-233	**58**	Y1-613 × C-T 247	**83**	Y1-431
9	Y2-18 × C-247	**34**	Y1-360 × C-T 211	**59**	K-456 × Y2-197	**84**	Y1-478
10	Y1-514×CT244	**35**	K-78 × Y2-274	**60**	Y1-303 × CT-225	**85**	Y1-495
11	Y1303×C-T245	**36**	K-456 × Y2-71	**61**	K456 × C-280	**86**	Y1-514
12	Y19 × C-T 245	**37**	K-78 × Y2-321	**62**	Y1-7 × Y1-2148	**87**	C-T 244
13	K78 × Y2-130	**38**	K-78 × Y2-31	**63**	K-456 × Y2 × 91	**88**	Y1-236
14	K78 × Y2-406	**39**	K-78 × Y2-361	**64**	K-456 × Y2-415	**89**	Y1-613
15	K78 × Y2-187	**40**	K-456 × Y2-232	**65**	K-456 × Y2-63	**90**	Y1-9
16	K78 × Y2-232	**41**	Y2-58 × C-1102	**66**	K-456 × Y2-357	**91**	Y2-361
17	K1-78 × 42-246	**42**	K-78 × Y2-164	**67**	K-456 × Y2-122	**92**	Y2-382
18	Y2-58 × C-1181	**43**	K-78 × Y2-248	**68**	K-78 × Y2-386	**93**	Y2-386
19	K-456 × Y2-4	**44**	K-456 × Y2-137	**69**	K-456 × Y2-278	**94**	Y2-408
20	K-456 × Y2-477	**45**	Y2-122	**70**	Y2-37	**95**	Y2-406
21	Y2-37 × C-1181	**46**	Y2-63	**71**	C-T 888	**96**	P-78
22	Y2-37 × C-1102	**47**	Y2-287	**72**	C-T 290	**97**	D-F 11
23	K-78 × Y2-357	**48**	Y2-66	**73**	Y2-32	**98**	Y1-559
24	K-456 × Y2-386	**49**	Y2-278	**74**	C-T 232	**99**	C-T 247
25	K-456 × Y2-31	**50**	DF-13	**75**	Y2-58	**100**	C-T 103
101	Y2-259	**126**	K-456 × Y2-164	**151**	Y2-164	**176**	Y1-360 × C-233

102	Y2-232	**127**	K-78 × Y2-223	**152**	Y2-154	**177**	K456 × Y2-204
103	Y2-71	**128**	K-456 × Y2-187	**153**	Y2-139	**178**	K-78 × Y2-477
104	Y2-222	**129**	K-78 × Y2-63	**154**	Y2-137	**179**	K-78 × Y2-66
105	Y2-223	**130**	K-78 × Y2-217	**155**	Y2-288	**180**	Y1-550 × C-T 247
106	Y2-256	**131**	Y1-9 × C-T225	**156**	Y2-136	**181**	K-78 × Y2-272
107	Y1-303	**132**	K-78 × Y2-204	**157**	Y2-357	**182**	K-456 × Y2-288
108	C-T 245	**133**	K-78 × Y2-287	**158**	Y2-306	**183**	K-456 × Y2-361
109	C-T 223	**134**	K-78 × Y2-382	**159**	Y2-218	**184**	K-456 × Y2-406
110	Y2-246	**135**	K-456 × Y2-117	**160**	Y2-217	**185**	K-78 × Y2-197
111	Y2-248	**136**	K-78 × C-T 888	**161**	Y2-204	**186**	K456 × Y2-246
112	Y2-490	**137**	K-456 × Y2-118	**162**	Y2-197	**187**	K-456 × Y2-274
113	Y2-499	**138**	K-78 × Y2-259	**163**	Y2-31	**188**	Y1-613 × C-T 1003
114	C-T 225	**139**	K-456 × Y2-321	**164**	Y2-4	**189**	K456 × Y2-136
115	C-T 211	**140**	K-456 × Y2-408	**165**	K456	**190**	K-78 × Y2-4
116	C-T 233	**141**	K-78 × Y2-111	**166**	K456 × Y2-222	**191**	K-456 × Y-223
117	F1-456	**142**	K-78 × C-T 232	**167**	Y2-32 × C-1102	**192**	K-78 × Y2-122
118	Y2-415	**143**	K-456 × Y2-66	**168**	Y2-32 × C-1181	**193**	J-78 × 137
119	Y2-477	**144**	YY3-118 × DF-13	**169**	K456 × C-232	**194**	K-78 × Y2-278
120	C-T 1181	**145**	C-T 280	**170**	K-456 × C-290	**195**	K-465 × C888
121	Y2-274	**146**	Y2-272	**171**	Y1-478 × C-244	**196**	Y-431 × CI- 233
122	K-456 × Y-306	**147**	Y2-43	**172**	Y1-236 × C-245	**197**	K78 × Y2-154
123	K-78 × Y2-306	**148**	Y2-72	**173**	YY3-114 × DF-11	**198**	Y2-18 × C1003
124	K-78 × Y2-288	**149**	Y2-196	**174**	Y1-236 × C-225	**199**	YY3116 × DF8
125	K-78 × Y2-408	**150**	Y2-193	**175**	Y1-478 × C-223	**200**	CHINESE CROSS
CHECK1	**PAKISTAN-13**	**CHECK2**	**BORLAUG-16**	**CHECK3**	**ZINCOL-16**	**CHECK4**	**MARKAZ-19**

2.5 Rust disease observation:

Stripe rust evaluations were performed at weekly intervals. Disease evaluations started at the flag leaf stage of wheat and ended before the leaves yellowed. For the purpose of evaluating rust infection, two criteria's were kept in mind: the host response and the severity of the rust infection (Aamir Iqbal et al., 2020). The modified Cobb scale (0-100 scale) had been used to assess the severity and the proportion of the rust attack (Peterson, Campbell, & Hannah, 1948). The infection types were assessed in order to rate the host's reaction to infection (Ravi P Singh, 1993). AUDPC was used to determine the area under the disease progress curve (a computer-based programmed developed at CIMMYT). The relative area under the disease progress curve (RAUDPC) was computed by adjusting the AUDPC of Morocco to 100%.

Data were noted after visually observing rust pustules on check Morocco. The stripe rust data were recorded by type of infection (IT) using a scale of 0-9 (Afridi et al., 2019). Lines were graded 0, where there was no evident infection. Material will be classified Resistant (R) when no necrotic region was observed. The Lines were rated Moderately Resistant (MR) when little uredia were surrounded by necrotic areas with a minute chlorosis, Moderately Susceptible (MS) when medium level of uredia was observed by chlorosis, and Susceptible (S) when considerable uredia was detected with maximum chlorosis. The severity of the disease was determined by the percentage of diseased leaf area on the plants.

Table 2. 2 Types of Rust Infection

Symbol	Type of Infection
O	No visible infection
R	Resistant. Necrotic areas may be with or without minute uredia
MR	Moderately resistant. Small uredia may be present and surrounded by necrotic areas
MR-MS	Moderately resistant to moderately susceptible. Small to moderate sized uredinia with some choruses
MS	Moderately susceptible. Medium uredia and no necrosis while possibly some distinct chlorosis may be observed.
MS–S	Moderately Susceptible to susceptible. Medium-large size uredinia with some choruses
S	Susceptible Large uredia, Little or no chlorosis

Source: (Afridi et al., 2019)

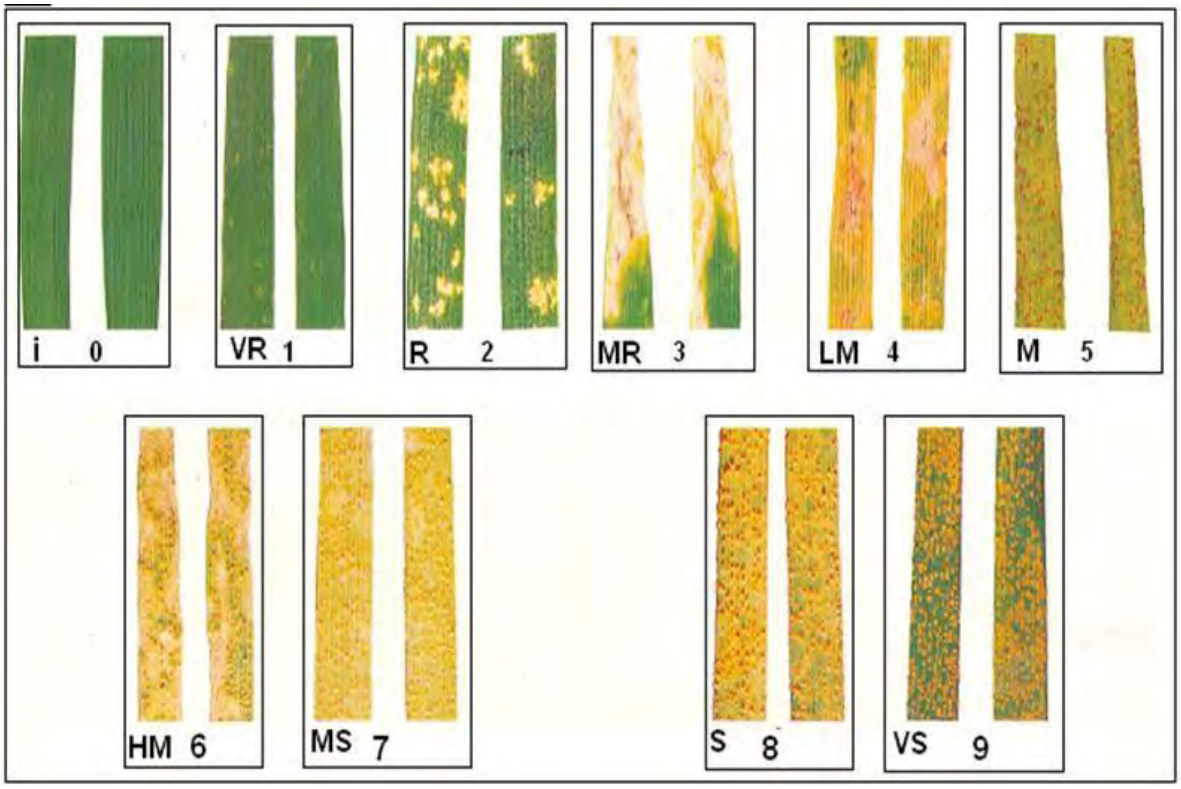

Figure 2. 1 Disease Rating Scale from 0 to 9

2.5.1 Calculation for Average Coefficient of Infection (ACI) and Relative Resistance Index (RRI):

As shown in figure 2.1, the Coefficient of Infection (CI) for stripe rust has been computed according to (Akhtar et al., 2002). By multiplying the response value with the Disease reaction (DR), the Coefficient of Infection was obtained. The average coefficient of infection (ACI) was calculated for each Line by taking average of all replications.

The Relative Resistance Index (RRI) was computed on a range of zero to nine, with 0 representing the most vulnerable and 9 denoting the most resistant. (Akhtar et al., 2002). The RRI was calculated according to the following formula:

$$RRI = \frac{(100 - ACI)}{100} \times 9$$

2.5.2 Area Under Disease Progress Curve (AUDPC):

The area under the disease progress curve (AUDPC) is calculated using fractions of diseased leaf area. Specified time intervals between measurements are not required. Readings should be taken often, if the disease is progressing rapidly (after every 7 days in cold areas, or every 3 to 4 days in warm and humid areas). The time between measurements may be greater if the disease is progressing slowly (after every 10 to 14 days). The goal is to collect disease readings at low, medium, and high levels in all genotypes.

Table 2. 3 Date-wise brief review of whole field trial

Field Operations	Trial Year 2020-21
Planting date	7-Dec-20
Germination Data	17-Dec-20
1st Irrigation	4-Jan-21
Rust severity data 1st	15 March at milking stage
Rust severity data 2nd	21 March at soft dough stage
Rust severity data 3rd	27 March at hard dough stage
Rust severity data 4th	4 April (Final assessment before harvesting)
Harvesting	18-Apr-21

2.6 Molecular genotyping:

2.6.1 Sampling:

Yellow rust leaves with primarily single lesions were selected for molecular genotyping. Leaves were collected and wrapped around themselves and packed in labelled glycine bags, along with information about the date, disease score, and variety with a unique code. The unique code was entered into an Excel sheet along with all other related information.

2.6.2 DNA extraction:

Genomic DNA was isolated using the modified Doyle and Doyle Protocol from fresh wheat lines. Nearly 1.5 g (fresh) leaves of the wheat Line have been harvested and cut down into tiny parts. These parts were then forcefully crushed in pestle and mortar by adding liquid nitrogen. After pouring this powder in 15 ml falcon tube, add 5 ml of 2 percent CTAB buffer solution with 1% Mercaptoethanol was added. It was incubated for 30 minutes in a water bath at about 65°C. This tube was filled with around 5 ml of Chloroform: Isoamyl Alcohol (24:1) and centrifuged for 10 minutes at 14,000 rpm at 10ºC. The supernatant was poured into fresh falcon tubes. After addition of 480µL chilled isopropanol, was placed the tube in a 4°C refrigerator for 20 minutes. The material was discarded after centrifuging the tube for 10 minutes at 14,000 rpm. The DNA pellets were then washed in a 70% ethanol solution. The tube was centrifuged for 10 minutes at 14,000 rpm at room temperature, ejecting 70% ethanol. After air drying for 20-30 minutes, the DNA pellet was resuspended in 200µL TE buffer. (Doyle, 1991).

2.6.3 Gel Electrophoresis for DNA Quantification:

The isolated DNA was quantified using 1% agarose gel. For a 1% gel, 1 gram of agarose was heated for 2 minutes in 100 ml of 1% TBE buffer. Allow the solution to cool. Three microliters of ethidium bromide were added to the solution before pouring it onto the gel tray using combs. After solidification, the gel tray was placed over a gel tank containing 1X TBE gel running buffer.

To quantify DNA, equal quantities of Lambda DNA standards (25 and 50ng/ul) and DNA samples (5ul) were put onto the gel along with 3ul dye. The gel was left to run at 100 volts for almost 30 minutes. Using the Gel Documentation System, DNA samples were analyzed or seen under UV light. By comparing the intensity of the bands with Lambda DNA, the concentrations of DNA samples were estimated. Following measurement, DNA samples were diluted to a concentration of 25ng/L. (Ejaz et al., 2012)

Table 2. 4 List of 15 *Yr* genes with their origin and detail

Sr No.	Genes	Chromosome location	Original source	Tester source	Resistance type	Linked genes
1	*Yr5*	2BL	*Triticum aestivum spelta*	Triticum spelta album	All-stage resistance	
2	*Yr15*	1BS	*Triticum dicoccoides*	G-25	All-stage resistance	
3	*Yr17*	2AS	*Triticum ventricosum*	VPM1	All-stage resistance	*Lr37, Sr38*
4	*Yr18*	7DS	*Triticum aestivum*	Frontana	Adult plant resistance	*Pm38, Lr34, Sr57*
5	*Yr26*	1BL	*Triticum turgidum*	V26/CM42 and V26/Gui22	All-stage resistance	
6	*Yr36*	6BS	*Triticum dicoccoides*	TR.DS: FA-15	Adult plant resistance	
7	*Yr46*	4DL	*Triticum aestivum*	RL6077	Adult plant resistance	*Pm46, Lr67, Sr55*
8	*Yr48*	5AL	*Triticum aestivum*	Synthetic wheat 205	Adult plant resistance	
9	*Yr54*	2DL	*Triticum aestivum*	Quaiu 3	Adult plant resistance	
10	*Yr59*	7BL	*Triticum aestivum*	PI 178759	Adult plant resistance	
11	*Yr60*	4AL	*Triticum aestivum*	Lal Bahadur	All-stage resistance	
12	*Yr61*	7AS	*Triticum aestivum*	Pindong 34	All-stage resistance	
13	*Yr62*	4BL	*Triticum aestivum*	PI 192252	Adult plant resistance	
14	*Yr64*	1BS	*Triticum turgidum* ssp. *durum*	PI 331260	All-stage resistance	
15	*Yr65*	1BS	*Triticum turgidum* ssp. *durum*	PI 480016	All-stage resistance	

This dilution has been done through the following equation:

$C1V1 = C2V2$

Whereas,

C1= Stock solution concentration (ng/ul)

V1 = Volume of stock to be measured

C2 = denotes the minimum necessary working concentration.

V2 = is the total volume of necessary working stock.

2.6.4 Polymerase chain reaction

The polymerase chain reaction took place in a volume of 10 ul. The 1X PCR buffer was included with $(NH4)_2.SO4$, a 0.2mM dNTP mix, a 3mM $MgCl_2$, a 5/10 picomole of each forward and reverse primer, a *Taq* DNA polymerase unit and a 25ng DNA template. The initial stage was the denaturation of DNA at 94 °C for 1 minute, followed by a 45-second annealing (each marker has a distinct temperature for this step) and a 72 °C extension step. The last stage was an extension of 10 minutes at 72 °C.

Table 2. 5 List of primers for genotypic evaluation

SR No.	NAMES	SEQUENCES	MARKER	BAND SIZE	GENES
1	*Yr*5_insertion_F	CTCACGCATTTGACCATATACAAC	INSERTION	507 BP	*Yr*5
	*Yr*5_insertion_R	TATTGCATAACATGGCCTCCAGT			*Yr*5
2	STS-7	GTACAATTCACCTAGAGT	STS	478 BP	*Yr*5
	STS-8	GCAAGTTTTCTCCCTATT			*Yr*5
3	STS-9	AAAGAATACTTTAATGAA	STS	439 BP	*Yr*5
	STS-10	CAAACTTATCAGGATTAC			*Yr*5
4	XGWM-413_F	TGCTTGTCTAGATTGCTTGGG	SSR	95 and 120 BP	*Yr*15
	XGWM-413_R	GATCGTCTCGTCCTTGGCA			*Yr*15
5	XGWM-273_F	ATTGGACGGACAGATGCTTT	SSR	225, 250 BP	*Yr*15
	XGWM-273_R	AGCAGTGAGGAAGGGGATC			*Yr*15
6	XGWM-11_F	GGATAGTCAGACAATTCTTGTG	SSR	203 BP	*Yr*15
	XGWM-11_R	GTGAATTGTGTCTTGTATGCTTCC			*Yr*15
7	XGWM-18_F	TGGCGCCATGATTGCATTATCTTC	SSR	186 BP	*Yr*15
	XGWM-18_R	GGTTGCTGAAGAACCTTATTTAGG			*Yr*15
8	XBARC-8_F	GCGGGAATCATGCATAGGAAAACAGAA	SSR	190, 240, 400 BP	*Yr*15
	XBARC-8_R	GCGGGGGCGAAACATACACATAAAAACA			*Yr*15
9	*URIC/LN2_F*	GGTCGCCCTGGCTTGCACCT	SSR	175, 290, 340 BP	*Yr*17
	URIC/LN2_R	TGCAGCTACAGCAGTATGTACACAAAA			*Yr*17
10	*SC-385_F*	CTGAATACAAACAGCAAACCAG	SCAR	400, 1000 BP	*Yr*17
	SC-385_R	ACAGAAAGTGATCATTTCCATC			*Yr*17
11	csLV34_F	GTTGGTTAAGACTGGTGATGG	STS	229 BP	*Yr*18
	csLV34_R	TGCTTGCTATTGCTGAATAGT			*Yr*18
12	Xgwm295_F	GTGAAGCAGACCCACAACAC	SSR	250, 270 BP	*Yr*18
	Xgwm295_R	GACGGCTGCGACGTAGAG			*Yr*18
13	Xbarc352_F	CCCTTTCTCGCTCGCCTATCCC	SSR/STS	275 BP	*Yr*18

	Xbarc352_R	CTGTTTCGCCCAATCTCGGTGTG			*Yr*18
14	CON-4-F	GTGCTGTACCTGACGACGGA	EST	495, 650 BP	*Yr*26
	CON-4-R	GTGGAGATGTTGGGCTTGG			*Yr*26
15	CON-6-F	GCCGATGGGGAACTGAAT	EST	295, 320 BP	*Yr*26
	CON-6-R	GTTGAACCGCTTGAACACC			*Yr*26
16	STS-BQ74-F	TGGATGAACCAACGATAGT	STS	295 BP	*Yr*26
	STS-BQ74-R	TGGGAAACACTTGACTGC			*Yr*26
17	*we173_F*	GGGACAAGGGGAGTTGAAGC	STS	500, 700 BP	*Yr*26
	we173_R	GAGAGTTCCAAGCAGAACAC			*Yr*26
18	UHW89_F	TCTCCAAGAGGGGAGAGACA	STS	195 BP	*Yr*36
	UHW89_R	TTCCTCTACCCATGAATCTAGCA			*Yr*36
19	UCW71_5UTR_F	CTTGCACCCGTGGATCAG	SNP	710 BP	*Yr*36
	UCW71_5UTR_R	CGATGCAATAATTTATCACACGTA			*Yr*36
20	UCW71_INT6_F	TGGACTTTCTATTTCTCCGTACC	SNP	930 BP	*Yr*36
	UCW71_INT6_R	TCAACCCTTTTAAGCAATTTGAA			*Yr*36
21	UCW79-dCAPSF	AGATAACGACCGATGCGATCTTAGTA	SNP*	190 BP	*Yr*36
	UCW79-dCAPSR	TCCTTTTTCCGATTTTCTTTGTGT			*Yr*36
22	CFD71-F	CAATAAGTAGGCCGGGACAA	SSR	175, 214 BP	*Yr*46
	CFD71-R	TGTGCCAGTTGAGTTTGCTC			*Yr*46
23	CFD23-F	TAGCAGTAGCAGCAGCAGGA	SSR	80, 205, 600 BP	*Yr*46
	CFD23-R	GCAAGGAAGAGTGTTCAGCC			*Yr*46
24	SNF-A2-F	TCCGTCTCCATCATTCAACA	STS	150 BP	*Yr*48
	SNF-A2-R	GTGTTGCGCAAGTTTGTGAC			*Yr*48
25	BE495011-F	TGATTACTGTAGCTACCTCCTCCT	SSR	236 BP	*Yr*48
	BE495011-R	GGTGCAAGATGTGCCTGTAA			*Yr*48
26	cfa2149-F	CTTGGAGCTCGGGTAGTAGC	SSR	225 BP	*Yr*48
	cfa2149-R	AAGGCAGCTCAATCGGAGTA			*Yr*48

27	WMS301-F	GAGGAGTAAGACACATGCCC	SSR	210 BP	*Yr*54
	WMS301-R	GTGGCTGGAGATTCAGGTTC			*Yr*54
28	BARC32-F	GCGTGAATCCGGAAACCCAATCTGTG	SSR	200, 210 BP	*Yr*59
	BARC32-R	TGGAGAACCTTCGCATTGTGTCATTA			*Yr*59
29	WMC557-F	GGTGCTTGTTCATACGGGCT	SSR	300, 320 BP	*Yr*59
	WMC557-R	AGGTCCTCGATCCGCTCAT			*Yr*59
30	WMC776-F	CCATGACGTGACAACGCAG	SSR	200, 300 BP	*Yr*60
	WMC776-R	ATTGCAGGCGCGTTGGTA			*Yr*60
31	WMC313-F	GCAGTCTAATTATCTGCTGGCG	SSR	200, 300 BP	*Yr*60
	WMC313-R	GGGTCCTTGTCTACTCATGTCT			*Yr*60
32	WMC219-F	TGCTAGTTTGTCATCCGGGCGA	SSR	120 BP	*Yr*60
	WMC219-R	CAATCCCGTTCTACAAGTTCCA			*Yr*60
33	FORWARD	CTAATTGCAACAGGTCATGGG	SSR	225 BP	*Yr*61
	REVERSE	TACTTGTGTTCTGGGACAATGG			*Yr*61
34	WMS359-F	AGCCGCGAAATCTACTTTGA	SSR	310 BP	*Yr*61
	WMS359-R	TTAAACGGACAGAGCACACG			*Yr*61
35	*gwm192_F*	GGTTTTCTTTCAGATTGCGC	SSR	185 BP	*Yr*62
	gwm192_R	CGTTGTCTAATCTTGCCTTGC			*Yr*62
36	*gwm251_F*	CAACTGGTTGCTACACAAGCA	SSR	120 BP	*Yr*62
	gwm251_R	GGGATGTCTGTTCCATCTTAG			*Yr*62
37	*gwm413_F*	TGCTTGTCTAGATTGCTTGGG	SSR	105 BP	*Yr*64
	gwm413_R	GATCGTCTCGTCCTTGGCA			*Yr*64
38	*gwm11_F*	GGATAGTCAGACAATTCTTGTG	SSR	200 BP	*Yr*65
	gwm11_R	GTGAATTGTGTCTTGTATGCTTCC			*Yr*65

2.6.5 Dissolution and Dilution of Primers:

The primer sequences for 38 SSRs/STSs/CAPS markers (Table 2.5) that have been previously reported to be associated with *Yr* genes were retrieved from research publications and the Grain genes database, respectively. The primers were made in China and imported. Tris-EDTA (TE) buffer was used to dissolve the primers at the start of the experiment. Initially, a primer stock of 100 pmol/ul was synthesized, and then the primers were diluted to a working concentration of 20 pmol/ul.

2.6.6 Gel electrophoresis

On a 1.5-3 percent agarose gel stained with ethidium bromide, the PCR amplified products were resolved. A UV transilluminator was used to view the bands, and the picture was recorded using a camera in the gel documentation system. By comparing the absence and presence of bands linked with *Yr* genes to a DNA ladder, base pair size of band is obtained

2.6.7 DATA SCORING:

Each band associated with a particular allele/local in genetic diversity analysis and was rated as presence or absence. For molecular marker data and a binary data matrix, the presence or absence of each variation has been coded by 1 or 0, accordingly.

2.7 Analysis on genotypic data:

Genomic data scoring in Power Marker software was used to calculate Polymorphic Information Content (PIC) value. Tassel was used to assess the association mapping of traits with markers. The nei values were extracted for whole population and cluster analysis was performed using R software.

3 Results:

3.1 Growth Related Traits:

3.1.1 Germination data (GD):

Analysis of variance revealed that significant differences among genotypes for germination (p-value 0.00) with a standard deviation of 13.0 were observed (Table 3.1). GD was ranged from 20 to 100 percent germination with the average 95.5%. 174 Lines out of 204 showed 100% germination while our check Pak-13 showed minimum germination (only 20 percent) followed by Line-09 with 30% germination.

3.1.2 Days to Tillering (DTT):

The analysis of variance showed that amount of variability (S.D.=1.633) among genotypes studied was highly significant (Table 3.1). Data on DTT provided the range within 36 to 46 days with a mean value of 42 days. Line-120 reached at tiller stage only in 36 days after sowing. Line-142 took 37 days, Line-18 took 38.5 days to produce its first tiller. However, Line-4 and 16 took maximum duration to produce its first tiller in 46 days. After them, Line-24 and 127 ranked 2nd in most time taking Lines for tiller development (45 days).

3.1.3 Days to Heading (DTH):

With standard deviation of 2.612 and p-value 0.00, significant differences were observed for days to heading among genotypes. According to data, whole Lines were fall between a range of 94 to 105 days. Ten Lines reached at heading stage on 94th day after sowing and so on. However, Line-93 took maximum time to initiation its heading stage (105 days), followed by Line-67 with 104 days.

3.1.4 Days to Flowering (DTF):

P-value for this trait was 0.000 which is much lower than standard value 0.01. It means all genotypes are highly dispersed from each other. Standard deviation value 2.885 is also supporting this dispersion. All Lines were dispersed between a range of 98 to 111 days to produce their first flower. Line-79 showed minimum days for 50% flowering (98 days) followed by Line-115, 50 and 07 (99 days) while the Line-168 took maximum days (111 days) followed by Line-78 (110 days) and Line-93 (109.5 days) for 50% flowering.

3.1.5 Plant Height (PH, in centimeters):

The analysis of variance showed that amount of variability among genotypes studied was non-significant. p-value observed 0.505, which is much higher than standard value. Plant height ranged from 66 cm to 105 cm with mean value of 88.23 cm. The Line-182 showed maximum plant height (105.3 cm) followed by Line-130 (105 cm) and Line-176 (104 cm). While the Line-151 showed minimum plant height (66 cm) followed by Line-61 (69.8 cm) and Line-67 (71.8 cm).

3.1.6 Flag Leaf (FL, in centimeters):

Flag leaf length also showed non-significant differences among genotypes with a P-value of 0.057 which is nearly crossed the standard 0.05 value. It ranged from 9 cm to 24.9 cm with average value of 16.44 cm. The Line-79 showed maximum length (24.9 cm) followed by Line-118 (24.6 cm) and Line-20 (23.4 cm). Reciprocally, the Line-145 showed minimum flag leaf length (9.4 cm) followed by Line-120 (9.8 cm) and Line-36 (11.0 cm).

3.1.7 Number of nodes (NN):

With standard deviation of 0.487 and p-value 0.032, significant differences were observed among genotypes. However, it is non-significant by comparing with 0.01 standard but significant with 0.05 (95%) standard value (Table 3.1). From 2.7 to 5.5, number of nodes are dispersed in whole experiment. Their average value 3.75. Line-156 and Line-184 gave 2.7 nodes, followed by Line-120 and 144 (2.9 nodes). 24 Lines produced 3 nodes. However, Line-85 had maximum nodes among all (5.5 nodes).

3.2 Yield Related Traits:

3.2.1 Spike Length (SL, in centimeter):

For spike length, the analysis of variance showed significant differences among genotypes. P-value was observed significant at 0.05 standard (Table 3.1). It ranged from 9.1 cm to 20.2 cm with average value of 15.7 cm. The Line-168 showed maximum length (20.2 cm) followed by Line-194 (20 cm) and Line-113 (19.7 cm) while the Line-101 showed minimum flag leaf length (9.1 cm) followed by Line-155 (9.5 cm) and Line-165 (10.0 cm).

3.2.2 Awn length (AL, in centimeters):

ANOVA for awn length showed highly significant differences among genotypes with a P-value of 0.01 which is exact equal to standard value. From awn-less to 11.9 cm awn, the Lines are highly dispersed with an average value of 6.13 cm. Ten Lines including 101, 55 ,65 ,97 etc. were awn-less. Line-124 had 0.6cm awn. In contrast, Line-177 had longest awn of 11.9 cm length, followed by Line-163 with 10.4 awn length.

3.2.3 Number of Flowers per Spike (F/S):

High standard deviation value (14.562) ultimately means high variability among genotypes. ANOVA gave p-value obtained 0.02, which is significant at 0.05 (95%) standard value. Within 39 to 120, number of nodes were distributed in whole experiment with a mean value of 88.5. With respect to yield, It's a highly appealing factor for a researcher. Line-202 produced 120 flowers, followed by Line 140 (118 flowers). Antagonistically, Line-64 had minimum flowers per spike among all (39 flowers), and Line-115 is second lowest with 46.7 flowers.

3.2.4 Number of Spikelet's Pair (SP):

P-value for this trait was 0.017 which is lower from standard alpha value 0.05. It means all genotypes are highly dispersed from each other. Standard deviation value 1.29 was also supporting this dispersion. Spikelet's pairs ranged from 6.7 to 14 in number with an average mean of 10.8 pairs. Lowest pairs among all, were produced by Line-64 (6.7 spikelet pairs) and Line-165 was 2nd lowest with 7.5 spikelet's pair. On the other hand, the Line-131 and 161 produced maximum pairs (14 spikelet's pair).

3.2.5 Number of flowers per spikelet (f/s):

The p-values (0.01) obtained due to analysis of variance for flowers per spike showed highly significant differences among genotypes. It ranged from 2.4 to 5 in number with an average value of 4.20. 33 Lines produced maximum flowers per spikelet (5 flowers). Contrastingly, Line-115 showed minimum flowers/spikelet (2.4 flowers) followed by Line-64 (2.5 flowers) and Line-144 (2.7 flowers)

3.2.6 Number of tillers (NT):

ANOVA for number of tillers showed non-significant differences among genotypes. P-value observed 0.505, which is higher than standard value. Tillers number ranged from 2.2 to 6.2 in number with an average value of 4.6 tillers. 6 Lines like Line-13, 11, 107, 133, 106 and 109 produced maximum number of tillers (6 tillers). Contrastingly, Line-101 showed minimum tillers (2.2 tillers) followed by Line-156 (2.7 tillers) and Line-163 (3 tillers).

3.2.7 Seed weight (Wt, in gram per hectare):

Significant variability among wheat Line was observed for seed weight with highly significant value 0.000. This trait produced maximum dispersion than all other traits with a standard deviation 332.452. Its genotypes were ranged from 386.7 g to 2163.5 g with average of 1098.2 g (Table 3.1). Maximum fresh seed weight (2163.5g) was observed by Line-100 followed by Line-111 (2010.02 g) and Line-170 (1927.1 g) while minimum fresh seed weight (386.7 g) observed by Line-105 followed by Line-181 (396 g) and Line-106 (403.2 g).

Table 3. 1 Analysis of Variance of all growth and yield relevant parameters

Variable	Min	Max	Mean	S.D.	F. Value	P. Value

GD	20.00	100.00	95.49	13.01	2.52	0.00**
DTT	36.20	46.50	41.84	1.63	1.96	0.00**
DTH	94.00	104.50	97.70	2.61	3.60	0.00**
DTF	98.40	111.00	103.39	2.86	3.47	0.00**
PH	66.00	105.30	88.24	7.29	1.00	0.51
FL	9.40	24.90	16.44	2.72	1.25	0.06
NN	2.70	5.50	3.75	0.49	1.30	0.03*
SL	9.10	20.20	15.70	1.96	1.05	0.37
AL	0.00	11.90	6.13	1.81	1.57	0.00**
F/S	39.00	120.00	88.47	14.56	1.50	0.00**
SP	6.70	14.00	10.84	1.21	1.35	0.02*
f/s	2.40	5.00	4.21	0.54	1.57	0.00**
NT	2.20	6.20	4.55	0.65	1.26	0.05*
Wt	386.70	2163.50	1097.92	322.45	2.12	0.00**

****:** Symbol representing highly significant level ***:** Significance level **GD:** Growth data, **DTT**: Days to tillering**, DTH**: Days to heading, **DTF**: Days to flowering, **PH**: Plant height, **FL**: Flag leaf length, **NN**: Number of Nodes, **SL**: Spike length, **AL**: Awn length, **F/S**: Flowers per spike, **S.P**: Spikelet's Pair, **f/s**: Flowers per spikelet, **NT**: Number of tillers**, Wt**: Seed Weight in g per hectare.

3.3 Heritability:

Table 3.2 shows the genotypic variance (Vg), phenotypic variance (Vp), environmental variance (Ve), genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), and broad sense heritability (H2) for growth and yield contributing characteristics of wheat advanced Lines. The PCV was larger than the GCV in terms of estimated variance components for the tested characteristics, indicating that environmental effects impacted on trait expression. The magnitudes of GCV and PCV for the characteristics studied in this research ranged from 1.9 and 2.2 for DTH to 30.9 and 55.6 for SL, respectively. It implies that there is a lot of genetic diversity across the board. Minimum GCV: PCV is obtained for days to heading, followed by days to flowering, tillering. In contrast, maximum ratio is obtained for stem length, awn length and weight. Rest factors fall between these extremes. The greatest broad sense heritability (72.3) was recorded for DTH, while the lowest (20.0) was reported for FL (Table 3.2). Very high heritability (Heritability >50%) was assigned to DTF (71.2), G.D. (60.3), and WT (57.6). Similarly, DTT, f/s, AL, F/S, SL had moderate heritability (Heritability =30 to 50%). Rest factors including PH, SP, NN, NT, FL had lowest heritability.

Table 3. 2 Heritability with GCV, PCV values

Factors	Vg	Ve	Vp	Heritability	GCV	PCV
GD	68.1	44.9	112.9	60.3	8.7	11.2
DTT	0.9	1	1.8	48.9	2.3	3.2
DTH	3.3	1.3	4.6	72.3	1.9	2.2
DTF	3.9	1.6	5.5	71.2	2	2.3
PH	145	354.4	499.4	29.1	13.7	25.4
FL	2.4	9.3	11.6	20	9.3	20.7
NN	0.1	0.2	0.2	23.1	5.1	10.6
SL	23.5	52.6	76.1	30.9	30.9	55.6
AL	1.6	2.8	4.4	36.2	20.6	34.2
F/S	54.1	108.4	162.4	33.3	8.4	14.5
SP	0.3	0.9	1.2	25.8	5	9.8
f/s	0.1	0.2	0.3	36.4	6.5	10.8
NT	0.1	0.3	0.3	20.5	5.4	11.8
WT	39894.7	29423	69317.7	57.6	18.2	24

****:** Symbol representing highly significant level ***:** Significance level **Vg**: genotypic variance **Vp:** phenotypic variance **Ve:** environmental variance **GCV:** genotypic coefficient of variation **PCV**: phenotypic coefficient of variation **H2**: and broad sense heritability

3.4 Correlation among agronomic traits:

Correlation among all agronomical traits were studied by Pearson's correlation coefficient for pairwise alignment. It showed the relatedness of one variable with another, hence revealing the traits association more efficiently. Proximity/ correlation coefficient matrix of fourteen agronomic traits showed in (Table 3.3). GD was negatively correlated with DTT, DTH, DTF, PH, FL, NN, SL, AL, F/S and SP. DTT positively correlated with f/s and negatively correlate with PH, FL, NN. PH, W.T. DTF was significantly correlated with PH, FL, f/s, NT, WT. PH had strongly negative relation with flower per spike and spikelets. Similarly, nodes numbers (NN) have highly significant relation with spike length (SL), awn length (AL), and a little bit relation with seed weight too. SL relation is significant for seed weight.

Table 3. 3 Correlation among all morphological traits with significance value

	G.D. 1	G.D. 2	D.T.T.	D.T.H.	D.T.F.	P.H.	F.L.	N.N.	S.L.	A.L.	F/S	S.P.	f/s	N.T.	WT
G.D. 1	**1**	0.48	-0.229**	-0.280**	-0.257**	-0.024**	0.114	0.013*	-0.154**	-0.103**	-0.086**	-0.171**	0.028*	0.136	0.158
G.D. 2		**1**	-0.182**	-0.268**	-0.264**	-0.016**	0.026*	-0.055**	-0.171**	-0.044**	0.127	-0.008**	0.16	0.067	0.259
D.T.T.			**1**	0.268	0.293	-0.095**	-0.059**	0.036*	0.131	0.084	0.068	0.126	0.011*	0.071	-0.068**
D.T.H.				**1**	0.955	-0.172**	-0.270**	0.035*	0.159	0.139	0.015*	0.141	-0.085**	-0.109**	-0.158**
D.T.F.					**1**	-0.153**	-0.277**	0.107	0.186	0.153	0.018*	0.145	-0.077**	-0.082**	-0.152**
P.H.						**1**	0.08	0.143	0.082	0.059	-0.106**	-0.053**	-0.137**	0.13	0.217
F.L.							**1**	-0.071**	0.25	-0.055**	0.201	0.11	0.176	0.14	0.116
N.N.								**1**	-0.020**	-0.013**	0.143	0.05	0.151	-0.071**	0.043*
S.L.									**1**	0.576	0.279	0.312	0.116	0.217	0.046*
A.L.										**1**	-0.008**	-0.008**	-0.016**	0.105	-0.049**
F/S											**1**	0.64	0.783	0.053	0.098
S.P.												**1**	0.062	-0.035**	0.024*
f/s													**1**	0.096	0.081
N.T.														**1**	0.111
WT															**1**

****:** Symbol representing highly significant level ***:** Significance level **G.D:** Growth data, **DTT**: Days to tillering**, DTH**: Days to heading, **DTF**: Days to flowering, **PH**: Plant height, **FL**: Flag leaf length, **N.N**: Number of Nodes, **SL**: Spike length, **AL**: Awn length, **F/S**: Flowers per spike, **S.P**: Spikelet's Pair, **f/s**: Flowers per spikelet, **N.T**: Number of tillers**, WT**: Weight in g per hectare.

3.5 Principle Component Analysis (PCA):

3.5.1 Estimation of cumulative variability based on eigenvalue:

PCA analysis based on correlation used to identify the data pattern classifies on relationship of traits. Presently, data divided into 15 principles components (PCs), or factors (Fs) based on eigenvalue and variability (Table 3.4). First six PCs contribute more variability having eigenvalue >1, represented in bold. Those six factors contribute total 68.94% variability (Table 3.4). Eigenvalue one was used as cutoff value for selecting PCs for further analysis. First two factor F1 (19.0%), F2 (15.9%) contribute higher variability revealing their importance in constricting biplot.

DTF (0.50%), DTH (0.49%) and SL (0.22%) contributes majorly to variability of principle factor F1 (Table 3.5) while minimum variability 0.339% contributed by GD and 0.22% by Wt. whereas for F2 highest variability contributed by variables F/S, SP, and f/s as 0.59%, 0.46% and 0.38% respectively. The contribution of traits in factor variability revealing their importance and implication in further breeding program.

Table 3. 4 Description of eigenvalue, variability, and cumulative variability of principle factors

	Eigenvalue	Variability (%)	Cumulative %
F1	**2.861**	19.076	19.076
F2	**2.386**	15.905	34.982
F3	**1.633**	10.886	45.867
F4	**1.255**	8.369	54.236
F5	**1.204**	8.029	62.265
F6	**1.002**	6.678	**68.943**
F7	0.984	6.557	75.500
F8	0.812	5.411	80.911
F9	0.779	5.190	86.101
F10	0.745	4.970	91.071
F11	0.589	3.929	95.000
F12	0.426	2.842	97.842
F13	0.265	1.767	99.609
F14	0.041	0.274	99.884
F15	0.017	0.116	100.000

F: Principle factors

Scree plot based on eigenvalue and cumulative variability, reveal the fifteen principal components and their contributions (Figure 4.1; Figure 4.2). The first three principal component touching the curve Line showed their highest contribution in distributing

the genotypes based on agronomic traits. The remaining nine factors with eigenvalue <1 had small contribution in variability that accounts 31% cumulative variability.

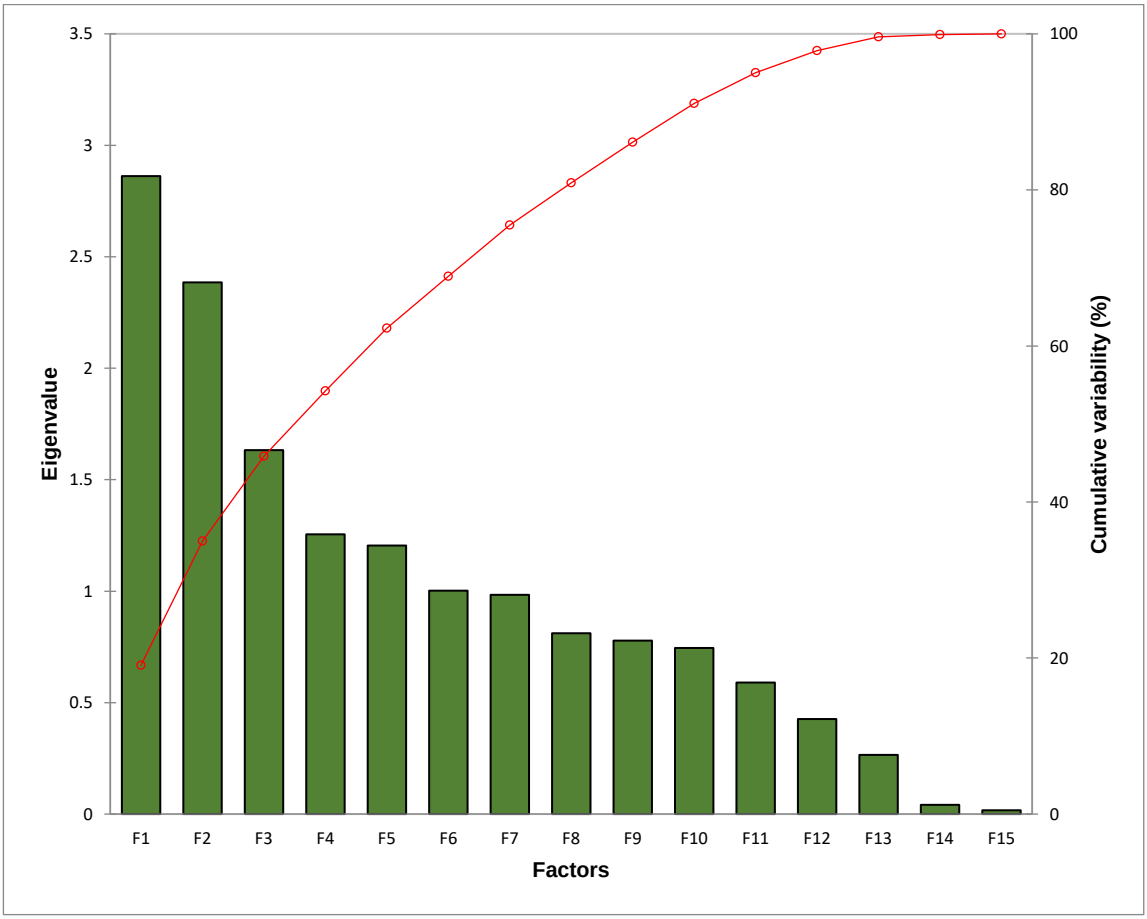

Figure 3. 1 Scree Plot showing principle factors based on eigenvalue and cumulative variability of agronomical traits wheat.

3.5.2 Variable analysis based on principle components:

Total variability depicted by first two principal factors F1 (19.0%) and F2 (15.9 used to construct the biplot (Figure 3.5) based on correlation of variable/traits with principle factors (Table 3.6**).** Positive and negative factor loading explains the trends of correlation among the variables and factors. Biplot divided data into four groups based on factor loading values**.** The vectors that were close to origin including PH, NN, and NT showed less variability as compared to F/S, FL, GD and DTH (Figure 3.3).

Biplot revealed that group 1 consist of F/S, S.P., S.L., A.L., D.T.T. and N.N. that had positive value for both factors F1 and F2 as showed in (Table 3.4) hence, these vectors also positively correlated with each other. Similarly group 2 had GD and DTT. The remaining variables falls in group 3 (Figure 3.3).

Table 3. 5 Percentage variability contribution for principle factors by agronomical traits

	F1	F2	F3	F1 + F2
G.D.	-0.313	0.124	-0.143	-0.190
D.T.T.	0.278	0.049	0.018	0.327
D.T.H.	0.499	-0.082	-0.108	0.416
D.T.F.	0.501	-0.070	-0.094	0.431
P.H.	-0.116	-0.001	0.379	-0.117
F.L.	-0.166	0.287	0.196	0.121
N.N.	0.050	0.090	-0.137	0.140
S.L.	0.224	0.337	0.473	0.562
A.L.	0.187	0.107	0.486	0.294
F/S	0.072	0.585	-0.267	0.658
S.P.	0.182	0.379	-0.121	0.561
f/s	-0.040	0.465	-0.272	0.425
N.T.	-0.077	0.163	0.351	0.086
WT	-0.188	0.161	0.112	-0.027

G.D: Growth data, **DTT**: Days to tillering**, DTH**: Days to heading, **DTF**: Days to flowering, **PH**: Plant height, **FL**: Flag leaf length, **N.N**: Number of Nodes, **SL**: Spike length, **AL**: Awn length, **F/S**: Flowers per spike, **S.P**: Spikelet's Pair, **f/s**: Flowers per spikelet, **N.T**: Number of tillers**, WT**: Weight in g per hectare.

Angles between the two vectors represent their correlation among them. As the angle gradually decrease from 90° revealed stronger correlation among traits while gradually increase from 90° showed negative correlation among traits. Angle equal to 90° (right angle) deputed no correlation or independent behavior (Figure 3.3**).**

Table 3. 6 Correlation between agronomic traits and principle factors in wheat

	F1	**F2**
G.D. 2	-0.53	0.191
D.T.T.	0.47	0.075
D.T.H.	0.843	-0.127
D.T.F.	0.847	-0.108
P.H.	-0.196	-0.002
F.L.	-0.281	0.443
N.N.	0.085	0.139
S.L.	0.38	0.521
A.L.	0.317	0.165
F/S	0.122	0.904
S.P.	0.308	0.585
f/s	-0.068	0.719
N.T.	-0.13	0.251
WT	-0.318	0.248

G.D: Growth data, **DTT**: Days to tillering**, DTH**: Days to heading, **DTF**: Days to flowering, **PH**: Plant height, **FL**: Flag leaf length, **N.N**: Number of Nodes, **SL**: Spike length, **AL**: Awn length, **F/S**: Flowers per spike, **S.P**: Spikelet's Pair, **f/s**: Flowers per spikelet, **N.T**: Number of tillers**, WT**: Weight in g per hectare.

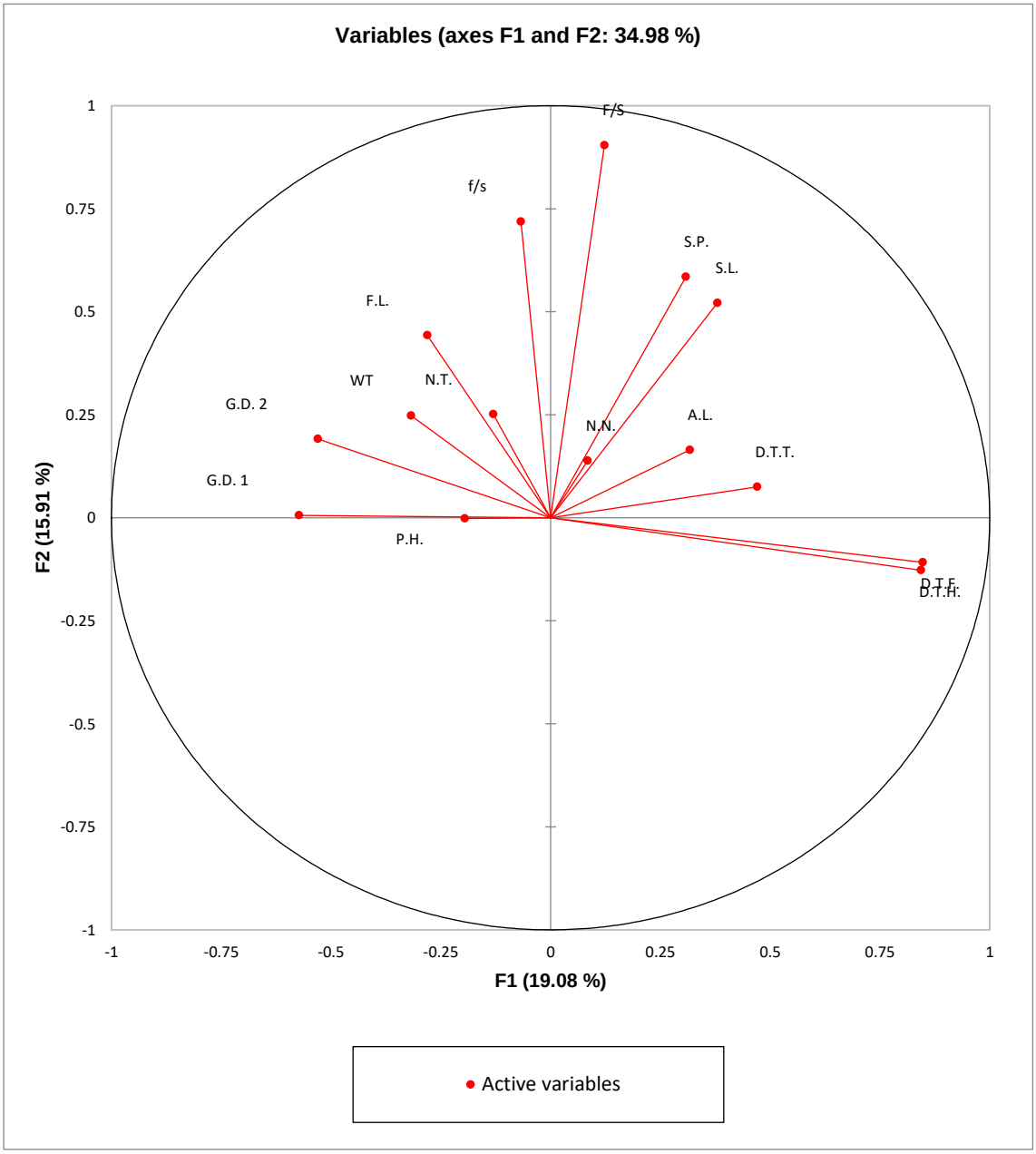

Figure 3. 2 Variable analysis based on principle components, variability, and correlation of ten agronomical traits of wheat

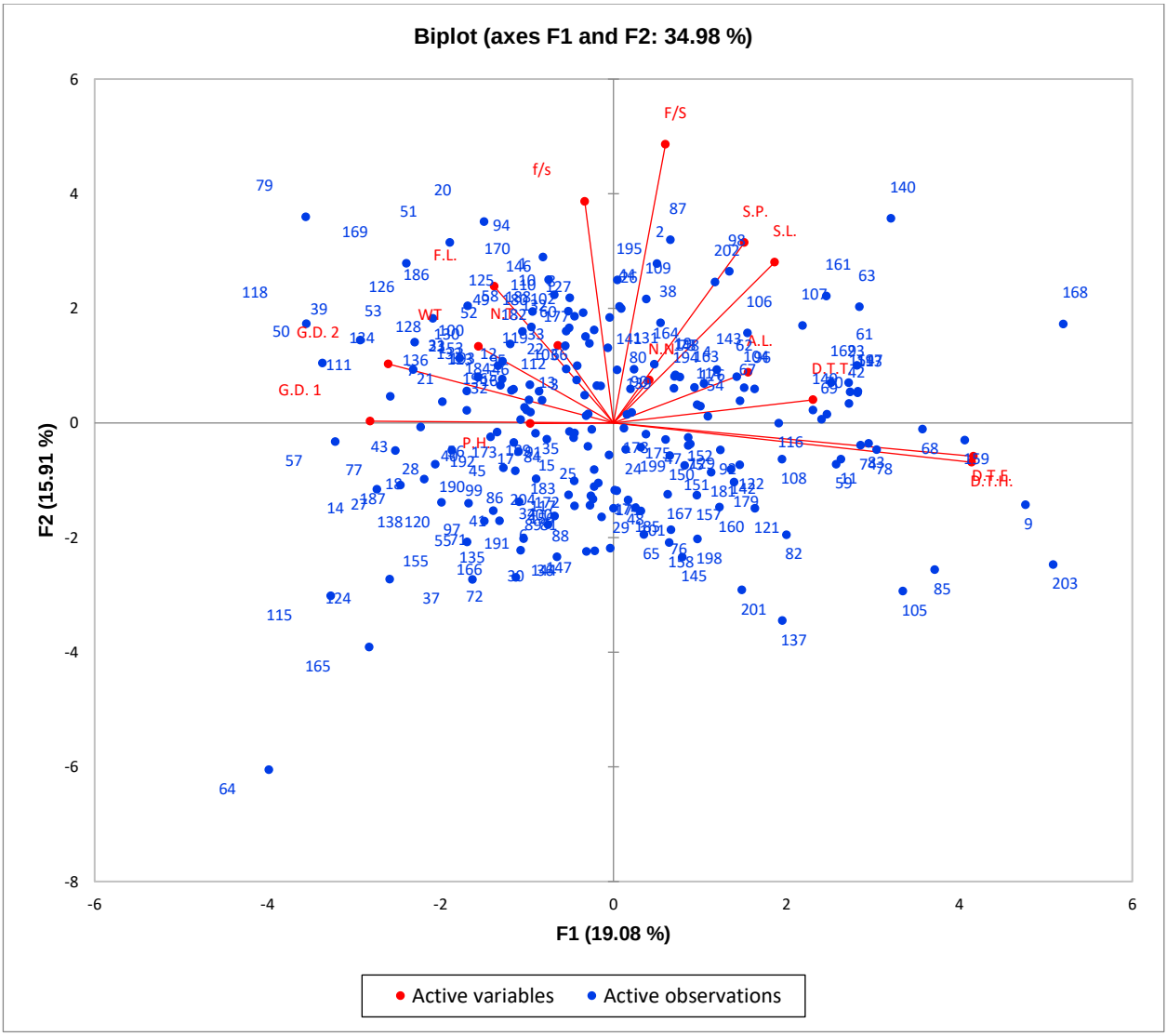

Figure 3. 3 Biplot representing correlation of Fifteen agronomical traits with 204 wheat genotypes

3.6 Disease:

In the present study, a total number of 204 Lines including 4 checks i.e., Pak-13, Bor-16, Zin-16, Markaz-19 were evaluated for yellow rusting resistance.

The following parameters were used to assess yellow rusting: Disease Reaction (DR), Average Coefficient of Infection (ACI), Relative Resistance Index (RRI), Area under disease progress curve (AUDPC), Relative area under disease progress curve (RAUDPC).

3.6.1 Disease Reaction (DR):

Of 204 Lines, no Line showed Susceptible (S) symptoms (Average Coefficient of Infection 90-100%), 3 (1.5%) Lines (i.e. Line-23, 126, 202) showed Moderately

Susceptible (MS) symptoms (ACI 80-<90%), 14 (6.9%) Lines (i.e. Line-12, 21, 28, 40, 45, 49, 52, 80, 124, 179, 180, 185, 187, 193) showed Moderately Susceptible to Susceptible (MSS) symptoms (ACI: 60-<80%), 19 (9.3%) Lines showed Moderately Resistant to Moderately Susceptible (MRMS) symptoms (ACI: 40-<60%), 12 (5.9%) Lines showed Resistant to Moderately Resistant (RMR) symptoms (ACI: 30-<40%), 18 (8.8%) Lines showed Moderately Resistant (MR) symptoms (ACI: 20-<30%) and 47 (23.0%) Lines were Resistant (R) and rest 91 Lines are immune with ACI value of 0%. Moreover, all 4 checks were also showed highly immune phenotype.

Table 3. 7 Division of Lines on the base of Disease Reaction

DR	T.A.	%age	Names of Lines
S	0	0.0	
MS	3	1.5	23, 126, 202
MSS	14	6.9	12, 21, 28, 40, 45, 49, 52, 80, 124, 179, 180, 185, 187, 193
MRMS	19	9.3	6, 17, 26, 30, 31, 42, 88, 98, 121, 133, 135, 136, 138, 143, 167, 177, 178, 192, 199
RMR	12	5.9	44, 48, 53, 56, 57, 65, 114, 122, 127, 139, 183, 194
MR	18	8.8	1, 5, 14, 16, 20, 27, 32, 94, 117, 123, 128, 130, 132, 141, 157, 170, 186, 198
R	47	23.0	3, 7, 9, 13, 15, 18, 22, 24, 25, 29, 35, 36, 38, 39, 41, 47, 50, 54, 61, 62, 66, 70, 71, 72, 73, 75, 77, 87, 100, 106, 108, 109, 125, 129, 140, 156, 169, 171, 172, 175, 181, 182, 184, 191, 195, 196, 197,
I	91	44.6	Rest Lines

DR: Disease Reaction **T.A:** Total Lines **%age:** Percentage **R:** Resistant **MR**: Moderately Resistant **RMR**: Resistant to Moderately Resistant **MRMS**: Moderately Resistant to Moderately Susceptible **MSS**: Moderately Susceptible to Susceptible **MS**: Moderately Susceptible **S**: Susceptible **I:** Immune

3.6.2 RRI:

Frequency distribution of RRI values of trial for 204 RILs is presented in **Fig**. The distribution was continuous and approached normality, indicating a quantitative type of inheritance, and thereby RRI is under the control of multiple genes. Based on the RRI values (Table 3.9), among the 204 tested Lines, 91 Lines had expressed resistant (R) type of reaction. These genotypes were having highest relative resistance index (RRI = 9) of yellow rust resistance, including the resistant checks Pak-13, Bor-16, Zin-16, Markaz-19. No Line had 0 RRI value, means no Line is severely susceptible to rust. Lowest RRI value of whole data was 1.8.

Table 3. 8 Groups of Lines by using RRI value

RRI Range	Lines

RRI (0)	0
RRI (1 to 1.9)	3
RRI (2 to 2.9)	7
RRI (3 to 3.9)	8
RRI (4 to 4.9)	10
RRI (5 to 5.9)	17
RRI (6 to 6.9)	19
RRI (7 to 7.9)	20
RRI (8 to 8.9)	29
RRI (9)	91

The RRI for LINE-23, LINE-126, LINE-202, LINE-12, LINE-21, LINE-52, LINE-28: were 1.8, 1.8, 1.8, 2.52, 2.52, 2.7, 3.33 respectively Contrastingly, minimum stripe rust severity was recorded in C-187, C-198, C-71, BORL-16, MARK-19, PAK-13, ZIN-16 with RRI of 8.775, 8.775, 8.82, 9, 9, 9

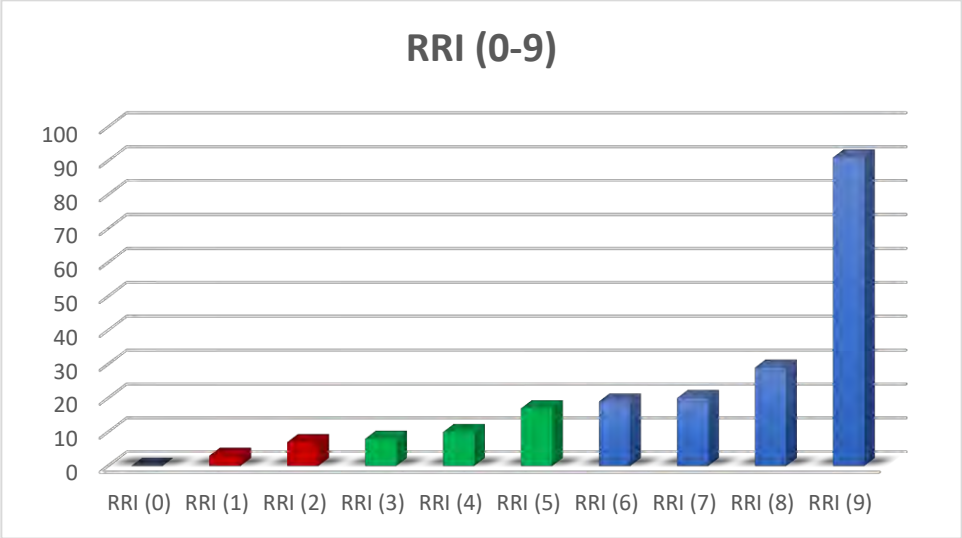

Figure 3. 4 Population distribution based on RRI value

3.6.3 Area Under Disease Progress Curve (AUDPC):

The impact of changes in both the duration and intensity of disease outbreaks are factored into the AUDPC. Typically, disease begins at a low level and increases frequently over time. During crop cycle, disease presence was detected on plants four times. Extent of disease is assessed visually at each observation using scales. Plant disease incidence (PDI value) in first observation was 18.8, followed by 21.0 in 2nd, 21.6 in 3rd and 21.4 in last observation (Table 3.10). Van der Plank suggested

computing the area under the disease progress curve to integrate this multiple time observed data into a single figure (AUDPC).

Table 3. 9 AUDPC of overall population by using PDI values

Time (Symbol)	Time in Days	PDI	Audpc
T_0	0	0.0	0.0
T_1	7	18.8	65.7
T_2	14	21.0	139.3
T_3	21	21.6	149.3
T_4	28	21.4	150.6
		Total	**504.9**

Decline in graphical representation of disease severity is clearly supporting the fact that genotypic material strongly resists the disease without affecting their yield.

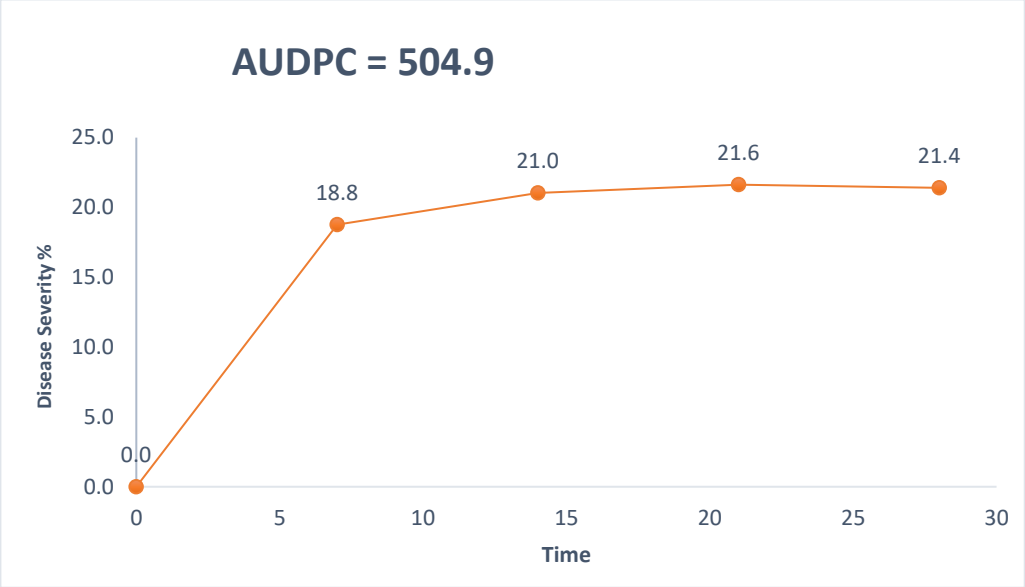

Figure 3. 5 Graphical representation of AUDPC

3.6.4 Relative Area Under Disease Progress Curve (RAUDPC):

Relative Area Under Disease Progress Curve obtained with ratio between actual AUDPC and maximum potential AUDPC (Annexure). Maximum RAUDPC was observed for Line-180 and 202. Contrastingly, majority of Lines produce 0.00 value which is highly significant factor in researcher point of view.

3.6.5 Lines selection by yield/disease data comparison:

Based on yield (Appendix 2), 68 lines performed better than high performing check Zincol-16. And 114 lines gave higher yield than rest of 3 checks. Similarly, 87 lines showed no rust symptom along checks (Table 3.8, Appendix 3).

By analyzing above both parameters simultaneously, 34 immune lines were marked that gave higher yield and resistance than Zincol-16 (Best performing Check). And 51 lines results were above than the rest of 3 Checks. Below is the list of those 34 lines; C-121, C-88, C-59, C-176, C-158, C-99, C-164, C-203, C-54, C-104, C-114, C-155, C-159, C-174, C-188, C-204, C-109, C-12, C-102, C-157, C-165, C-76, C-122, C-42, C-144, C-101, C-93, C-49, C-105, C-98, C-112, C-64, C-78 and C-106.

3.7 DNA markers-based gene postulation for rust resistance:

As I have discussed earlier in material and method, I had imported 38 markers against 15 *Yr*-genes. I had applied these primers on my Lines with their respective positive controls.

Except annealing temperature (Tm), PCR conditions for all primers are same.

Denaturing step: 94°C, 10 min

35 cycles of: [94°C for 60 sec, **(Tm)** for 45 sec, 72°C for 45 sec]

Extension step: 72°C for 10 min

Here is the gene wise explanation of all primers.

3.7.1 *Yr*5:

Microsatellite marker *Yr5_insertion* was used to detect *Yr5* gene. Marker *Yr5_insertion* amplifies 507bp fragment in *Yr5* gene positive genotypes. Out of 204 wheat Lines, 104 wheat Lines and positive control showed a 507bp fragment associated with the presence of *Yr5* gene. Rest wheat Lines and negative control did not produce any band, suggesting the absence of *Yr5*.

Furthermore, there are two STS markers available for detecting the presence of *Yr*5: *STS-7/STS-8* and *STS-9/STS-10*. Out of 204, *STS-7/STS-8* (Figure 3.6) amplified fragment of 478 bp in167 wheat Lines, confirming *Yr*5 gene in maximum population. Similarly, result of *STS-9/STS-10* indicates the presence of band in 78 Lines, while 120 wheat Lines did not produce any band. Band size of respective primer is 439 bp.

Annealing temperature for *Yr5_insertion* is 59°C, for *STS-7/STS-8* Tm is 57°C and for *STS-9/STS-10* is 55°C.

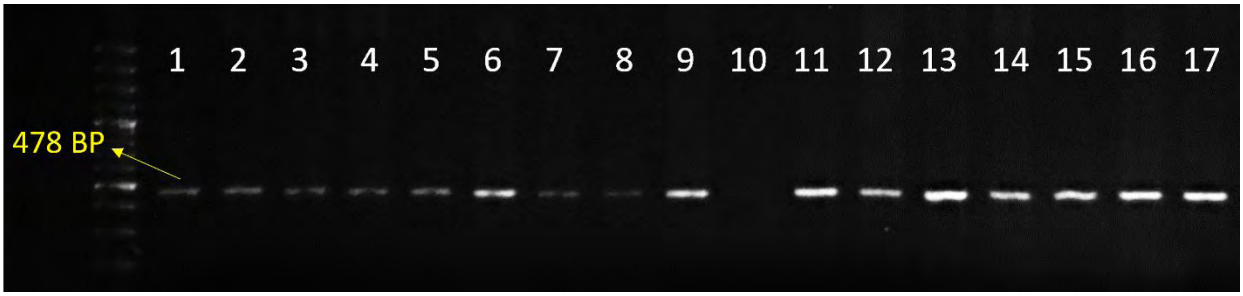

Figure 3. 6 STS-7/STS-8 marker for confirmation *YR*5 genes

3.7.2 *Yr*15:

Five different molecular markers available for *Yr*15. After obtaining results, I have found that one out of 5 markers are polymorphic for this populations. The Table (3.10) below shows some of the closer markers with the relative distances to *Yr*15.

Gene specific marker *Xgwm413* amplified two fragments of 95 and 120bp. 162 wheat Lines and positive control showed 95bp fragment, suggesting the presence of *Yr15* gene. Comparatively, only sixty-three wheat Lines amplified 120bp fragment. And rest Lines with empty wells were indicating that these lines don't carry *Yr15* gene.

The primers *Xgwm273* were used to detect *Yr15* gene that is associated with amplification of a 225bp DNA fragment. 200 wheat Lines and positive control produced band which showed the presence of *Yr17* gene, whereas only 8 wheat Lines and the negative control did not show anything, indicating the likely absence of *Yr15* gene.

In present study, *Xgwm11* was also used to determine the presence of *Yr15* and produce 273bp fragment in 172 Lines out of 204 Lines. Similarly, *Xgwm18* is expressing a sharp band in 182 Lines under UV lights. The band size of this marker is 186 bp (Figure 3.7).

Last SSR marker that I had used was *Xbarc8* to detect the presence of *Yr15* gene. This marker amplified three different types of bands: 190, 240 and 400 bp fragment. Among 204 Lines, 100 Pakistani wheat Lines and the positive control produced 190bp fragment, suggesting the likely presence of this gene. 67 Lines are showing 240 bp fragment. While 400bp fragment is expressed in only 17 Lines. Eleven Lines produce double bands and four Lines all above-described bands (Triple band).

Table 3. 10 *Yr*15 primers with their location and PCR Protocol

Microsatellites	Position relative to *Yr*15	Annealing temperature (Tm)
Xgwm413	4.4 cM	57°C
Xgwm273	5.7 cM	55°C
Xgwm11	6.2 cM	60°C
Xgwm18	6.9 cM	58°C
Xbarc8	9 cM	57°C

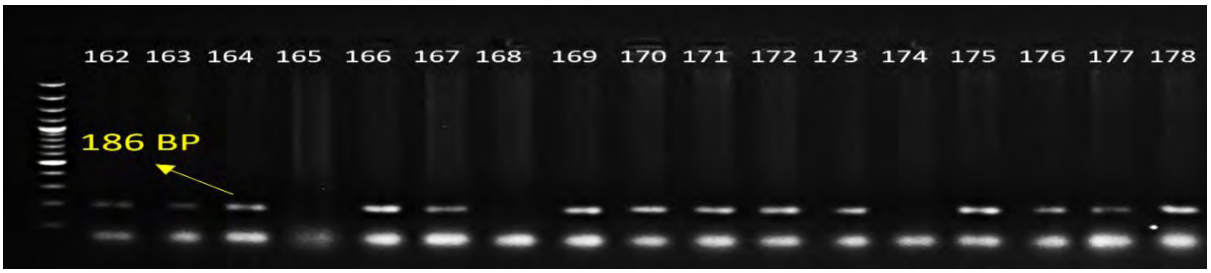

Figure 3. 7 XGWM-18 marker for confirmation of *YR*15 gene

3.7.3 *Yr*17:

Primer pairs *URIC/LN2 and SC-385* were selected to identify *Yr*17. Primers *URIC/LN2* amplify fragments of (+) 285 bp (from the N genome) indicating the presence of the resistance genes while (−) 285 bp (A genome) indicate their absence. However, two other band sizes are also noticed by increasing gel concentration up to 2 percent (2 gram in 100 ml). This primer gave band in each single Line which ultimately support 100% presence of *Yr*17 gene in entire population.

Primer *SC-385* amplifies a 400 bp fragment (Figure 3.8) which is already reported by researchers in past. But my PCR conditions assist it to amplify a second band of 1000 bp in few Lines. One hundred seventeen entries tested positive for 400 bp fragment, including 30 Lines with and extra band of 1kb.

Annealing temperature for both primer is same, which is 55°C.

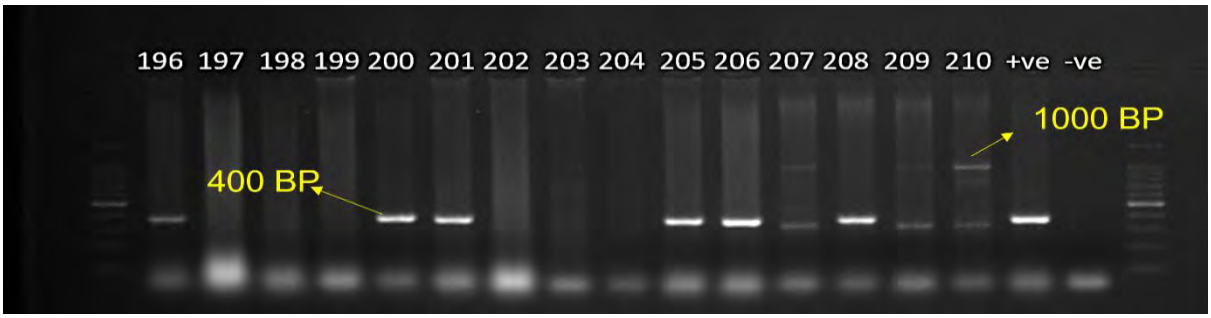

Figure 3. 8 SC-385 marker for confirmation of *YR*17

3.7.4 *Yr*18:

*Yr*18 is located on the short arm of chromosome 7D, close to locus *Xgwm295*. Below we provide protocols for using these 3 markers.

CSLV34 results in amplification of band with 229 bp, which indicate the presence of*Yr*18. This primer produced bands in only 27 Lines, which is approximately 10% of overall population. Comparatively, *Xgwm295* produce a sharp band in 101 out of 204 Lines, indicating the likely presence of *Yr18* gene. Band size for this primer is 250bp (Figure 3.9). *Xbarc352* amplify 275 bp under ultraviolet radiations, which indicate the presence of *Yr*18. Of the 204 tested entries, 111 carried *Yr*18.

Annealing temperature for *Xgwm295 and Xbarc352* primers are 57°C. However, *CSLV34* contradict with above 2 primers in annealing temperature, which is 59°C.

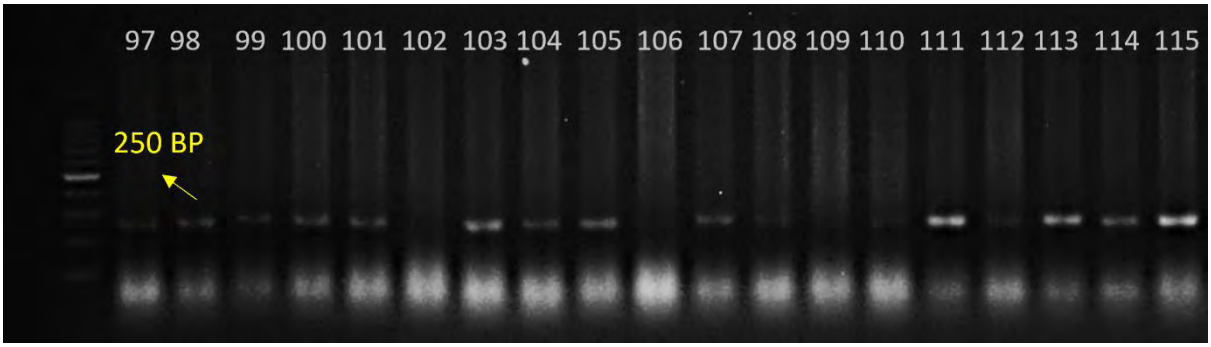

Figure 3. 9 XGWM-295 marker for confirmation of *YR*18

3.7.5 *Yr*26:

CON-4 Primer amplified two fragments of 495 and 650 bp. Result of this primer is 100% monomorphic. All Lines have double band, exhibiting the presence of *Yr*26 gene. *CON-6* also produced two types of bands in end results but here story is entirely different. Two band sizes for this primer are 295 and 320 bp. No Line had both

bands simultaneously. Of these 2, only one amplified. Although, 31 Lines didn't produce any band, indicating the absence of *Yr*26 in them.

STS-BQ74 was also used to determine the presence of *Yr26*. All 204 wheat Lines and the positive control produced 295bp fragment, suggesting the likely presence of this gene.

In my research, *we173* was a last primer against *Yr*26. Based on visualization, it has 4 scenarios. 105 Lines amplified 500bp fragment. 131 Lines Line produced 700bp fragment. Among them, 48 Lines have both bands while 39 Lines didn't have any sort of fragment, proceeding the absence of this gene (Figure 3.10).

Table 3. 11 Primer list for *YR*26 confirmation

Microsatellites	Position relative to *Yr*26	PCR protocol
CON-4	0.48 cM	63°C
CON-6	Linked	60°C
STS-BQ74	0.43 cM	59°C
we173	1.48 cM	60°C

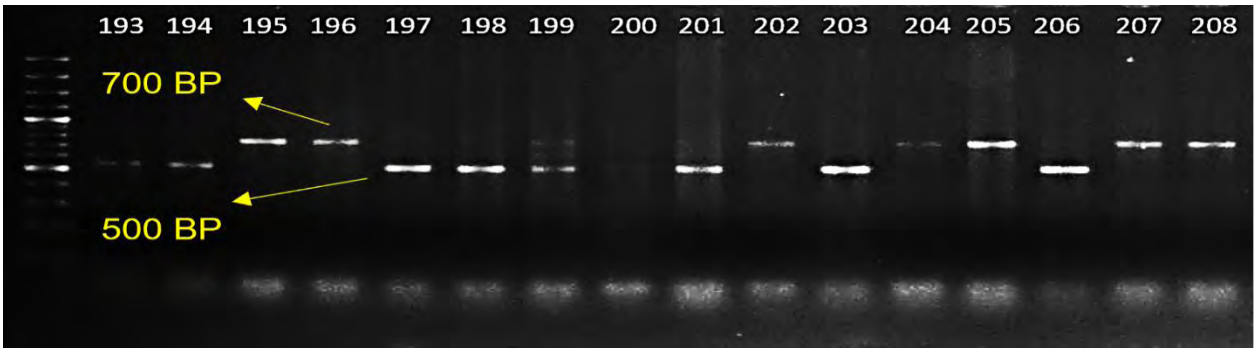

Figure 3. 10 WE-173 marker for *Yr*26 confirmation

3.7.6 *Yr*36:

Four gene-specific primer sets to detect the locus were *UHW89, UCW71_5UTR, UCW71-INT6 and UCW79-dCAPS.* In the case of STS marker *UHW89,* all entries tested positive. It means these Lines are resistant to those stripe rust strains that are specifically linked with *Yr*36 gene. 2nd primer is *UCW71_5UTR* amplified a 710 bp band. Of 204, 73 Lines produced the band, with a clear-cut indication of *Yr*36 gene presence. Other 138 Lines with no band are supporting the absence of gene in them (Figure 3.11).

3rd primer against this gene is *UCW71-INT6*, this marker amplified a 930bp fragment in 112 wheat Lines and the positive control. 98 Lines including negative control did not amplify the 930bp, suggesting the likely absence of *Yr*36 gene. Last primer *UCW79-dCAPS* amplified in 126 Lines with a band of 190bp. It means these 126 Lines tested positive against *Yr*36 gene.

Annealing temperature of *UHW89* is 57°C, *for UCW71_5UTR* and *UCW71-INT6* is 57°C, and for *UCW79-dCAPS* is 59°C.

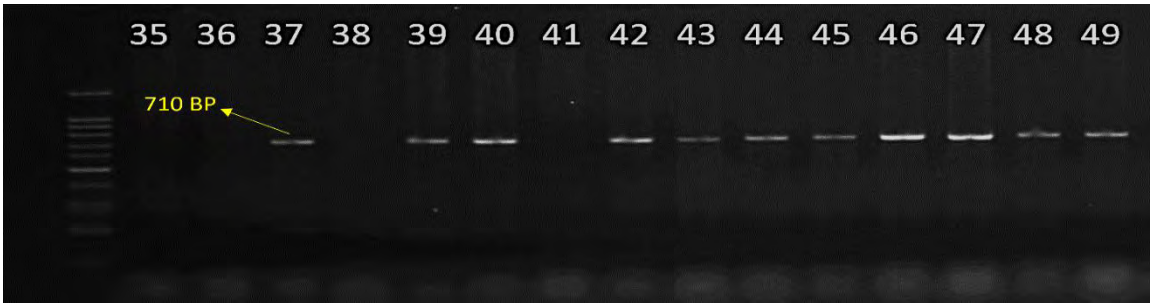

Figure 3. 11 UCW71_5UTR marker for *YR*36 confirmation

3.7.7 *Yr*46:

A genome-wide association study revealed a strong association between Xcfd71-4D and *Yr*46 (p 0.001). Additional testing of nearby markers reported on 4D in various recombinant populations, as well as a screening of wheat Lines from varied origins, showed that Xcfd71-4D and Xcfd23-4D are viable candidates for breeding molecular markers. So, we imported both markers to check the presence or absence of *Yr*46 gene in Lines. And yes, the results were clearly supporting the findings of past researchers.

Primer set *Xcfd71-4D* amplified 175 or 214 bp fragment and primer set *Xcfd23-4D* START amplified 3 different band size, 80, 205, 600 bp fragments. Results were completely monomorphic. Twelve entries were found to have *Yr*36. All 204 wheat Lines and positive control showed the presence of both band fragments for 1st primer, associated with the presence of *Yr46* gene.

However, in the case of 2nd primer, 89 and 205 bp fragment were present in whole population but 600bp fragment was absent in 32 Lines. Overall, results of both primers were clearly indicating the domination of *Yr*46 gene in my population (Figure 3.12).

Annealing temperature for *Xcfd71-4D* is 54°C and for *Xcfd23-4D*, keep same protocol with 55°C annealing temperature.

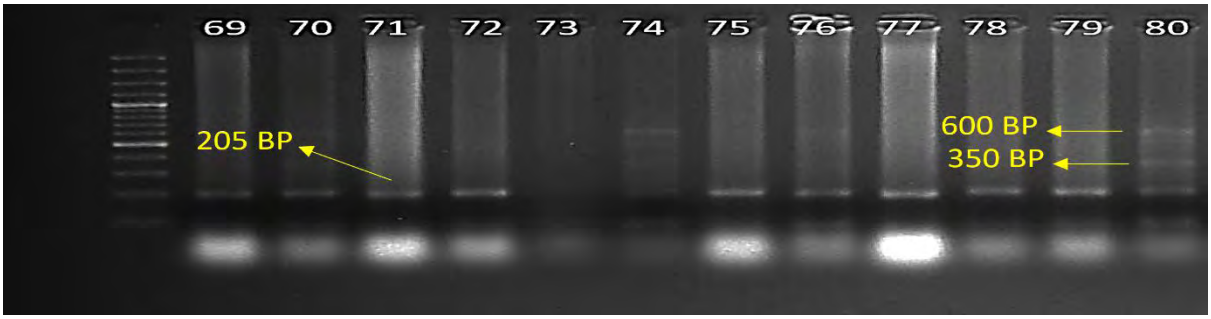

Figure 3. 12 XCFD-23-4D marker for *YR*46 confirmation

3.7.8 *Yr*48:

SNF-A2 marker amplified a 150bp fragment in 92 wheat Lines and the positive control. 116 Lines including negative control did not amplify the 185bp, suggesting the likely absence of *Yr*48 gene. The primers *BE495011* was also used to detect *Yr48* gene that is associated with amplification of a 236bp DNA fragment. 166 out of 204 wheat Lines and positive control produced a 259bp fragment which showed the presence of *Yr48* gene. In the case of marker *cfa2149*, all 204 wheat Lines and the positive control produced 225bp fragment, suggesting the likely presence of this gene (Figure 3.13).

Table 3. 12 Primers relevant to *Yr*48 with their relative position and PCR protocol

Microsatellites	Position relative to *Yr*48	PCR protocol
SNF-A2	0.18 cM	58°C
BE495011	0.09 cM	56°C
cfa2149	0.06 Cm	60°C

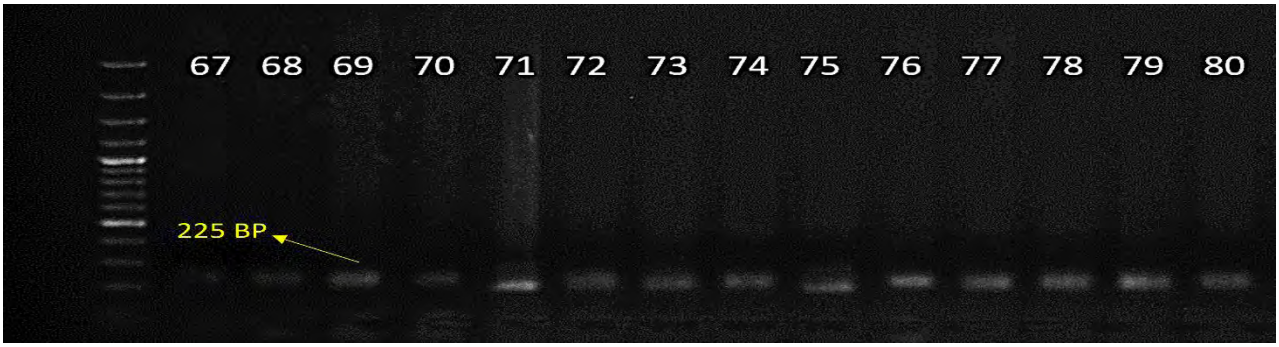

Figure 3. 13 CFA-2149 marker for confirmation of *YR*48 gene

3.7.9 *Yr*54:

Microsatellite marker *Xgwm301* was found at 0.5 cM and could be useful for breeding. 160 wheat Lines and positive control showed amplification of 210bp fragment, exhibiting the presence of *Yr54* gene, whereas 48 wheat Lines and negative control did not show the 210bp fragment, indicating the absence of *Yr54* gene (Fig 3.14).

Annealing temperature for *Xgwm301*is 62°C.

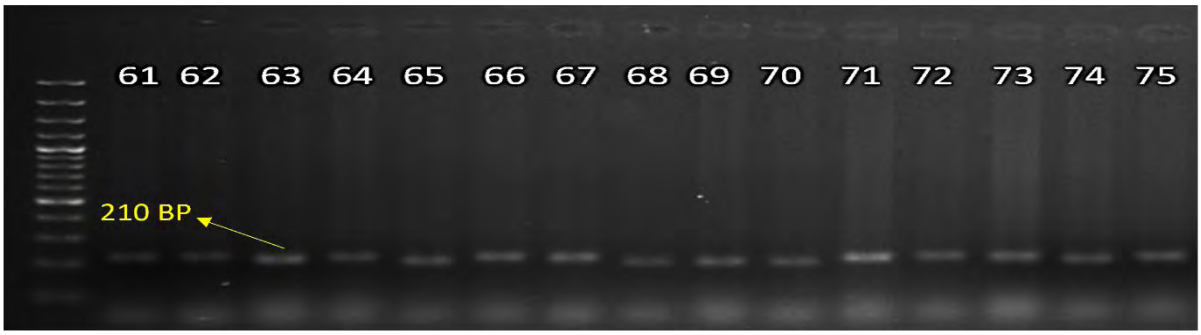

Figure 3. 14 XGWM-301 marker for confirmation of *YR*54

3.7.10 *Yr*59:

SSR locus *Xbarc32* is located on the proximal side of *Yr*59 at less than 2.1 cM. It amplified 200 or 220 bp diagnostic fragment. 170 Lines produced 200bp fragment while 134 Lines gave 220bp fragment. Among them, 48 Lines are those which has both bands. There is no single Line which didn't produced any sort of band (Figure 3.15).

Also, on the distal side, *Xwmc557* amplifies a 300-bp or 320bp products in agarose gel. 88 Lines gave 300bp band size, while 61 produced 320 bp size. Among them, 48 Lines

were those which gave both bands. However, 39 Lines entries tested negative for *YR*59 gene with no band.

PCR conditions for both primers are exact similar with 59°C annealing temperature.

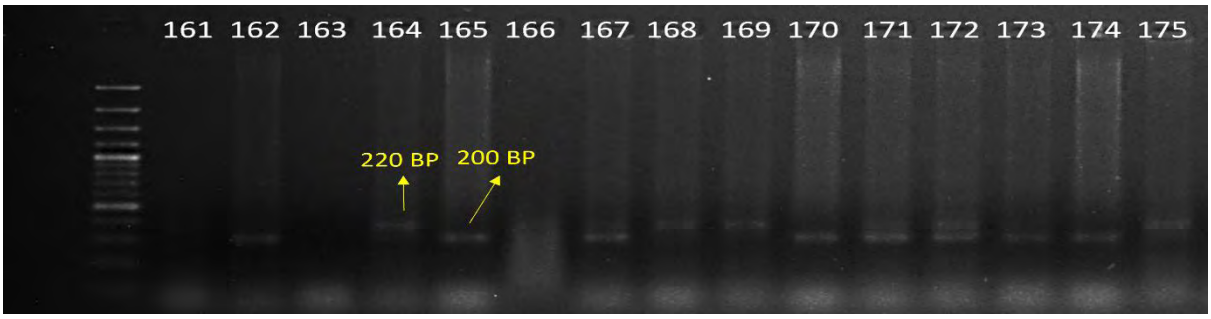

Figure 3. 15 Xwmc-557 marker for confirmation of *YR*59 gene

3.7.11 *Yr*60:

*Yr*60 is co-segregating with WMC776 on the distal end of chromosomal arm 4AL. WMC313 and WMC219, which are situated 0.6 cM distal to *Yr*60, are additional markers that may be helpful in marker assisted selection.

Marker *WMC776*, tested positive in my all Lines by producing double bands. Fragment sizes of bands 200bp and 300bp (Figure 3.16). Second marker *WMC313*, also gave same band sizes under UV light; 200bp as well as 300bp fragment. Only 7 Lines didn't produce double band indicating the absence of *Yr*60 gene in those Lines. Codes of those seven Lines are 13, 49, 55, 57, 130, 139, 180.

Last primer against *Yr*60 is *WMC219*, amplifies a 120 bp diagnostic product. 180 entries were showing fragment, indicating the presence of respected gene.

Annealing temperature for *WMC313* and *WMC219* primers are 59°C. For *WMC776*, annealing temperature is 57°C and rest protocol is same.

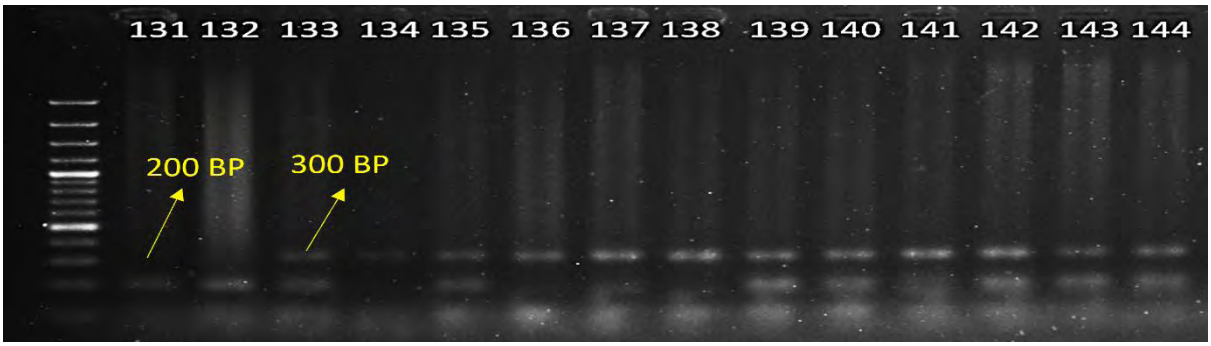

Figure 3. 16 Xwmc-776 marker for confirmation of *YR*60 gene

3.7.12 *Yr*61:

Two SSR markers were mapped close to *Yr*61. These markers *WMS359* and *BE518379* amplified 225 bp and 310 bp diagnostic bands in the present material, respectively. Result were 100 percent monomorphic. All entries were identified as likely carrying *Yr*61.

PCR conditions for both primers are exact similar. Both had 59°C annealing temperature.

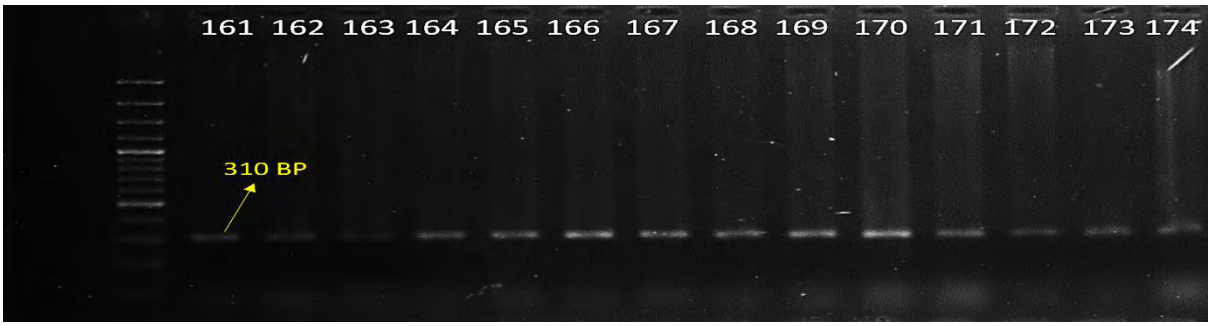

Figure 3. 17 BE518379 marker for confirmation of *Yr*61 gene

3.7.13 *Yr*62:

Microsatellite marker *gwm192 and gwm251* were used to determine the presence/absence of *Yr61* gene whose presence is indicated by the amplification of specific fragment. Band size for *gwm192* is 185bp (Figure 3.18) and for *gwm251* is 120bp. End results under UV light are clearly interpreting the presence of both marker in every single Line of experiment.

Annealing temperature for *gwm192* is 59°C and for *gwm251 is* 55°C.

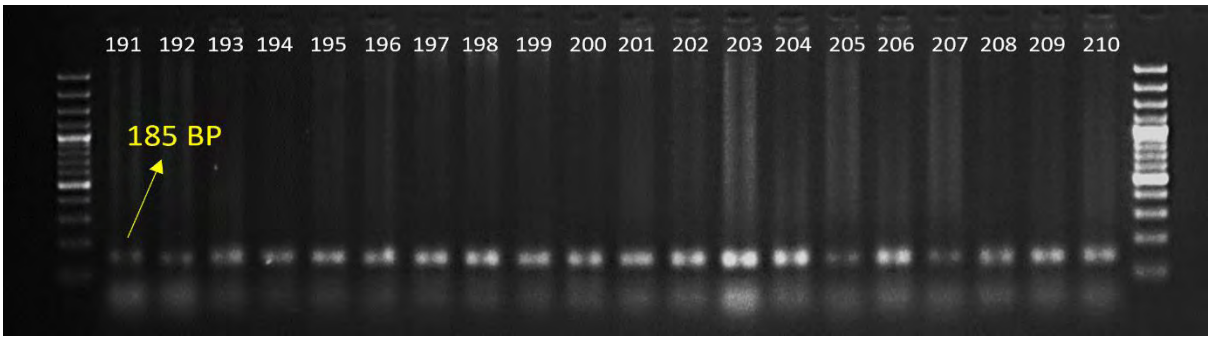

Figure 3. 18 GWM-192 marker for *YR*62 confirmation

3.7.14 *Yr*64:

GWM413 was used to detect *Yr64* gene that is associated with amplification of a 105bp DNA fragment. At 60°C annealing temperature, all Lines produce band under UV lights, indicating the likely presence of *Yr64* gene.

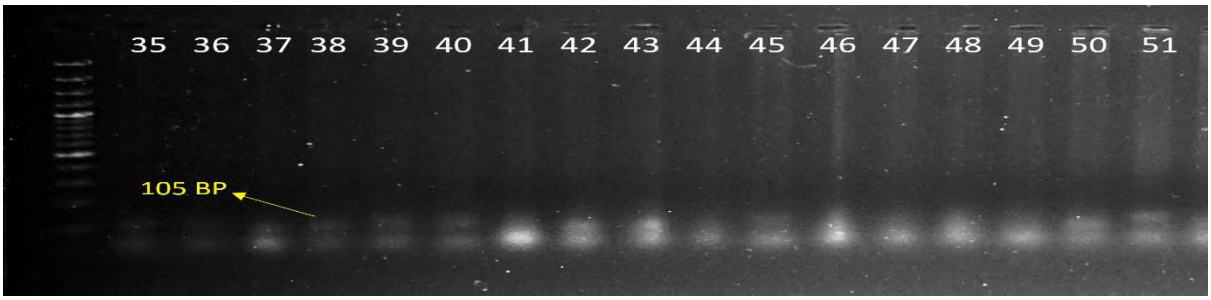

Figure 3. 19 GWM-413 marker for confirmation of *Yr*64 genes

3.7.15 *Yr*65:

Xgwm11 was used to detect the presence of *Yr*65. In many previous paper, same primer is also reported to check *Yr15* and *Yr26* genes. This marker amplified 200bp fragment in 187 wheat Lines and the positive control, which showed the presence of *Yr65* gene. Twenty-one wheat Lines and negative control did not amplify the 200bp fragment, suggesting the absence of *Yr65* gene. Annealing temperature for X*gwm11* is 58°C

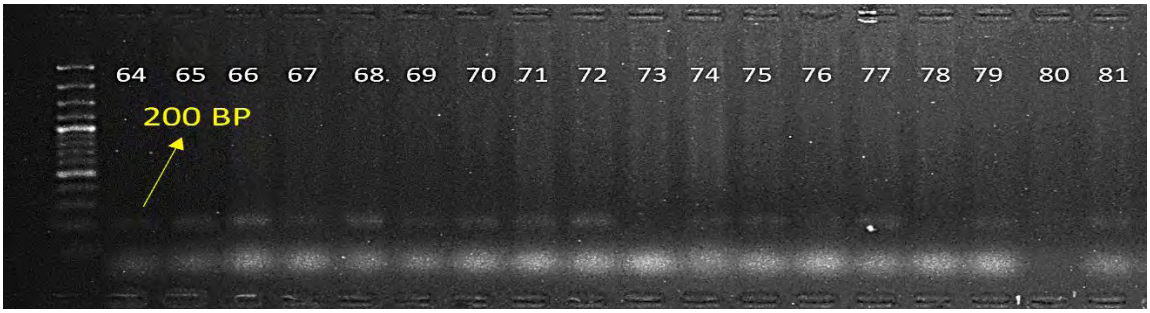

Figure 3. 20 GWM-11 marker for confirmation of *YR*65 gene

3.8 DNA Markers analysis

All wheat Lines were subjected to a genetic diversity study utilizing 38 widely used DNA markers. Seven of the 38 markers were monomorphic, whereas the remaining 31 were polymorphic. 38 polymorphic markers identified a total of 56 alleles. Each marker, except for 11 primers, produces a single allele. Two allele were found in Xgwm-413, Xgwm-273, Xgwm-295, CON-4, CFD-71, wmc-557, wmc776, wmc313, we173, and sc385. Cfd-23 and URIC/LN2, on the other hand, only generate three

alleles. The size of the alleles ranged from 95 bp (Xgwm-413) to 1000 bp (SC-385). The major allele frequency varied from 50 (*STS7/8*) to 100% (*Xgwm-413, Xgwm-11, Xgwm-251*). Gene diversity on the other end, varied from 0.00 (*Xgwm-413, Xgwm-11*) to 0.50 (*we173, Xbarc-8*). The polymorphic information content (PIC), which quantifies each SSR locus's capacity to discriminate between wheat, varied from 0.00 to 0.38 with an average of 0.18 (Table 3.13).

Table 3. 13 Polymorphic information content (PIC) value with genetic diversity

Marker	No. of obs.	Allele No	Allele Size Range	Major Allele Frequency	Gene Diversity	PIC
***Yr*5_insertion**	204	2	507	0.5	0.5	0.38
STS-7/STS-8	204	2	478	0.8	0.32	0.27
STS-9/STS-10	204	2	439	0.63	0.47	0.36
XGWM-413 (1)	204	2	95	0.54	0.5	0.37
XGWM-413 (2)	204	2	120	0.7	0.42	0.33
XGWM-273 (1)	204	2	225	0.96	0.07	0.07
XGWM-273 (2)	204	2	250	0.99	0.02	0.02
XGWM-11	204	2	203	0.83	0.29	0.25
XGWM-18	204	2	186	0.88	0.22	0.19
XBARC-8 (1)	204	2	190	0.52	0.5	0.37
XBARC-8 (2)	204	2	240	0.68	0.44	0.34
XBARC-8 (3)	204	2	400	0.93	0.13	0.12
CSLV34	204	2	229	0.87	0.23	0.2
XGWM-295 (1)	204	2	250	0.96	0.08	0.08
XGWM-295 (2)	204	2	270	0.56	0.49	0.37
XBARC-352	204	2	275	0.53	0.5	0.37
CON-4 (1)	204	2	95	0.96	0.07	0.07
CON-4 (2)	204	2	120	0.96	0.07	0.07
STS-BQ74	204	2	295	0.98	0.05	0.05
UHW-89B	204	1	195	1	0	0
UCW71-5UTR	204	2	710	0.65	0.46	0.35
UCW-INT6	204	2	930	0.54	0.5	0.37
UCW79-Dcaps	204	2	190	0.63	0.46	0.36
CFD-71 (1)	204	1	175	1	0	0
CFD-71 (2)	204	1	214	1	0	0
CFD-23 (1)	204	1	205	1	0	0
CFD-23 (2)	204	1	350	1	0	0
CFD-23 (3)	204	2	600	0.83	0.28	0.24
SNF-A2	204	2	210	0.56	0.49	0.37
BE495011	204	2	236	0.8	0.32	0.27
CFA-2149	204	2	225	0.99	0.03	0.03
WMS-301	204	2	210	0.77	0.36	0.29
BARC-32	204	2	200	0.82	0.3	0.25
BARC-32	204	2	210	0.64	0.46	0.35
WMC-557 (1)	204	2	300	0.58	0.49	0.37

WMC-557 (2)	204	2	320	0.71	0.41	0.33
WMC-776 (1)	204	2	200	0.98	0.04	0.04
WMC-776 (2)	204	2	300	0.96	0.08	0.08
WMC-313 (1)	204	2	200	0.96	0.07	0.07
WMC-313 (2)	204	2	300	0.96	0.07	0.07
WMC-219	204	2	120	0.87	0.23	0.21
WMS-359	204	1	225	1	0	0
BE-518379	204	2	310	0.99	0.03	0.03
URIC/LN2 (1)	204	2	175	0.98	0.04	0.04
URIC/LN2 (2)	204	2	285	0.98	0.04	0.04
URIC/LN2 (3)	204	2	340	0.98	0.04	0.04
SC-385 (1)	204	2	385	0.85	0.25	0.22
SC-385 (2)	204	2	1000	0.86	0.25	0.22
WE-173 (1)	204	2	500	0.5	0.5	0.37
WE-173 (2)	204	2	700	0.63	0.47	0.36
GWM192	204	1	185	1	0	0
GWM251	204	1	120	1	0	0
GWM413	204	1	105	1	0	0
GWM11	204	2	200	0.9	0.18	0.17

3.9 Genetic relationship and cluster analysis:

The data obtained from the DNA analysis was used to generate a similarity matrix using the Nei (1983) method with data from 38 DNA markers. The resulting matrix showed a mean genetic similarity among the 204 genotypes, revealing a high level of genetic relatedness. About the pairwise combinations, the genetic similarities between the genotypes varied from 0.02 to 0.57.

Using the clustering based on the UPGMA analysis, a dendrogram was constructed for the 204 genotypes with 38 microsatellite primers, as presented in (**Figure**). Dendogram analysis separated wheat Lines in 4 major groups: Group A, B, C, D. On the base of DNA markers, the maximum genetic distance observed in group D. This group contained 21 Lines. It is further divided in 2 sub-clusters. Line 85 and 285 were most diverged Lines in whole experiment. Group C contained 25 Lines with nei value of 0.42. Furthermore, 4 sub-cluster were observed under this group. Line 61 and 159 are 99.9 percent similar within this cluster. But within population these Lines were also counted as diverse Lines. Maximum Lines fall in cluster B (92 Lines) with 51 % similarity among other clusters. More than 7 sub-clusters fell under this group. Cluster A with lowest diversity contained rest 66 Lines. Line 195, 64, 94 were most alike Lines within population.

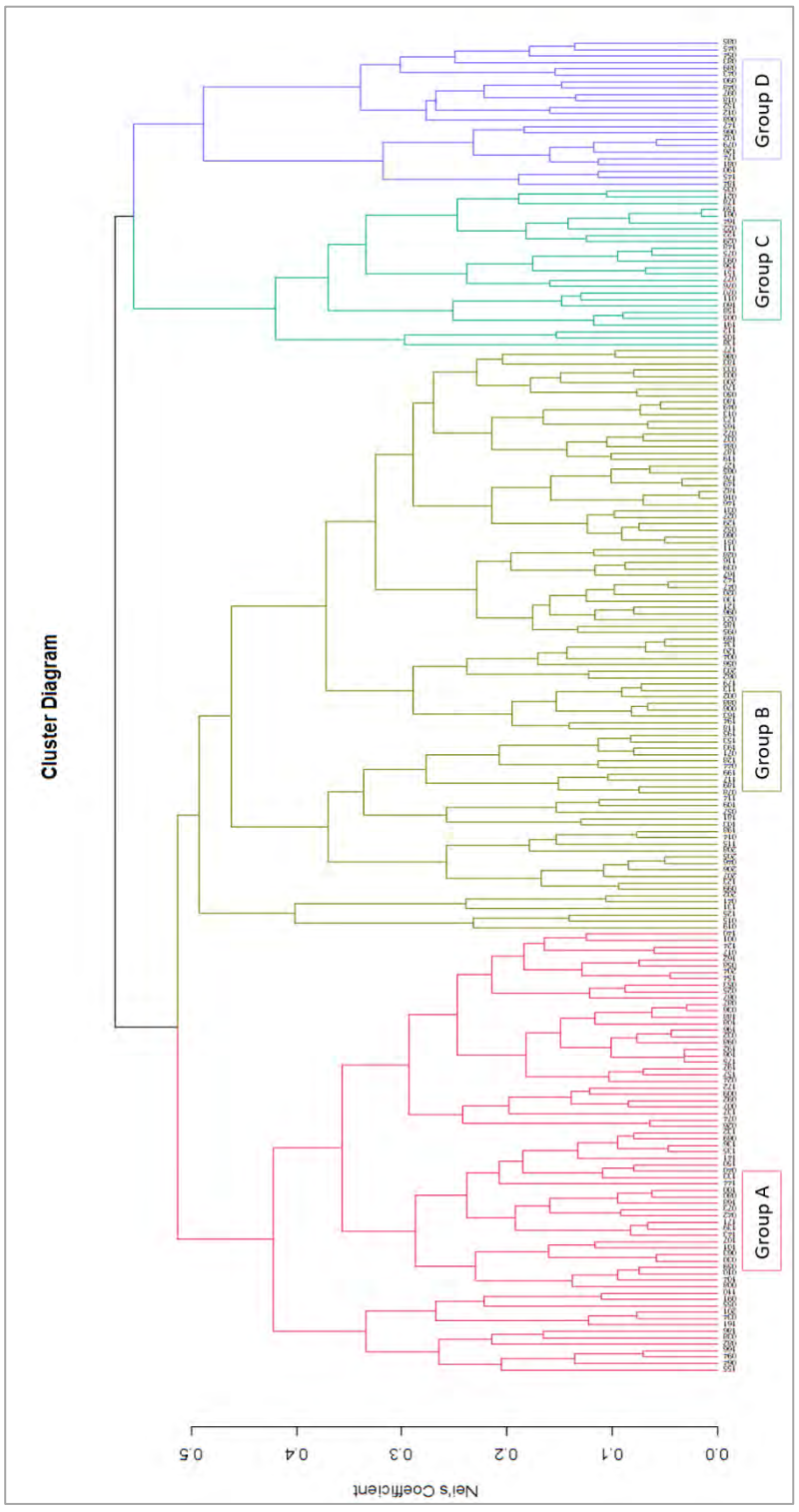

Figure 3. 21 Diversion of whole population shown in Cluster Analysis

3.10 Association mapping of growth, yield, and disease traits:

For the estimate of marker-trait relationships, the phenotypic data were entered into the TASSEL software programme. The association analysis and detection of DNA markers linked with characteristics in the wheat population were conducted using a general Linear model (GLM).

23 markers were found to be associated ($P < 0.05$) with the growth and yield related traits (Growth data, Days to tillering, Days to heading, Days to flowering, Plant height, Flag leaf length, Number of Nodes, Spike length, Awn length, Flowers per spike, Spikelet's Pair, Flowers per spikelet, Number of tillers, yield) (Table 3.15). Remaining 15 markers which were not found to be associated with any trait.

Marker XGWM-273, SNF-A2, BARC-32 and WMC-557 associated with the maximum number of traits (4 traits). Marker STS-9/STS-10, XGWM-273, SNF-A2, WMC-776 and URIC/LN2 gave probability below than 0.05 percent for seed weight. So, they probably had a relation with yield. Marker GWM-295, BARC-352, BARC-32, WMC-219, GWM-413 were associated with AUDPC, ultimately enhancing connection rust disease. Similarly, most primers showed a connection with several traits, making themselves very significant in the eyes of the researcher.

Table 3. 14 Association mapping of markers with traits using GLM. Values are p values for significance of association

Primers	G.D.	DTT	DTH	DTF	PH	FL	NN	SL	AL	F/S	SP	f/s	NT	WT	AUDPC
STS-7/STS-8					0.013										
STS-9/STS-10														0.034	
XGWM-413											0.042				
XGWM-273					0.027								0.004	0.043	
XGWM-11			0.046	0.036											
XGWM-18	0.037														
csLV34										0.002		0.006			
Xgwm295															0.016
Xbarc352															0.016
UCW71_5UTR											0.006		0.018		
UCW79-Dcaps							0.042								
SNF-A2	0.007	0.017												0.004	
BE495011											0.042				
cfa2149									0.036			0.041			
WMS301								0.036	0.025						
BARC32			0.049	0.006											0.001
WMC557 (1)						0.017				0.032		0.011			
WMC557 (2)	0.001					0.050		0.028							
WMC776 (1)						0.035								0.015	
WMC776 (2)								0.035							
WMC219			0.037	0.021											0.039
URIC/LN2 (1)		0.023												0.038	
URIC/LN2 (2)		0.023												0.038	
URIC/LN2 (3)		0.023												0.038	
we173 (1)										0.029					
we173 (2)										0.029					
gwm413															0.004
gwm11					0.038		0.021								

4 Discussion:

Morphological characterization of germplasm is basic footstep to disclose the genetic diversity privileged by genotypes (Rakshit et al., 2012; Shrestha, 2013). Any crop's breeding programme is largely determined by the degree of genetic diversity (Smith, Al-Doss, & Warburton, 1991). Current research work aims to genetically characterize the spring wheat genotypes using multivariate analysis. Total of 204 wheat genotypes were collected and evaluated for 15 morphological traits. Mean value of morphological traits explains that wide variation exhibited by genotyped for Germination data, Days to tillering, Days to heading, Days to flowering, Plant height, Flag leaf length, Number of Nodes, Spike length, Awn length, Flowers per spike, Spikelet's Pair, Flowers per spikelet, Number of tillers and Seed Weight. Table 3.1 shows the basic statistics for grain production and quality characteristics, such as mean, standard deviation, and variance. It was observed that there was a highly significant variation in Analysis of variance for days to germination data, days to tillering, days to heading, days to flowering, awn length, flowers per spike, flowers per spikelet and seed weight at ($P < 0.01$) significance level. While number of nodes and tillers are significant at 0.05 level of significance. Several reports available that studied the descriptive statistics of wheat morphological traits (Al-Maskri, Sajjad, & Khan, 2012; Habib, Awan, Sadia, & Zia, 2020; Arshad Iqbal, Khalil, Shah, & Kakar, 2017; Shah et al., 2007).

The correlation coefficient is a statistical measure of how closely two variables differ or how strong a connection exists (Feiyu, Xueqin, Wangcheng, Wang, & Wenjun, 2012). In plant breeding, the investigation of correlations between various morphological characteristics is critical for cultivar development. Genetic improvement could not be accurately handled without a comprehensive understanding of character associations in complex biological entities. Positive and highly significant (P 0.01) correlation coefficients between the relative values of majority of the characteristics were also detected. The relationship between number of tillers and grain production suggests that the number of tillers should be utilized as a selection criterion for improving a species' grain output. The existence of a positive significant connection between grain yield and flowers per spike (F/S) indicates that a plant with many flowers will generate a high grain yield, and therefore, selecting on the base of number of flowers per spike at an earlier stage would increase a plant's grain output (Habtamu

and Million, 2013). Previous research has shown that WT (Seed weight) is strongly correlated with DTF, DTT, DTH, AL, SL, and NN (Dogan, 2009). Our research found a positive connection between F/S and SL (Maric et al., 1998; Shah et al., 2007; Khodadadi et al., 2011; Eivazi et al., 2007; Ali et al., 2008; Ajmal et al., 2013).

GCV:PCV ratio for the characteristics investigated in this research suggests that there is a lot of genetic variability across the spectrum. Heritability value for DTF (71.2), G.D. (60.3), WT (57.6) were highest among all (Deshmukh et al., 1986). The analysis indicates that if these characteristics are evaluated during screening as well as hybridization, the examined genotypes seemed to have a larger and much more effective chance of crop improvement. According to the researchers, (Johnson et al., 1955) as well as (Udeh & Ogbu, 2011) "strong broad sense heritability alone may not necessarily offer high prediction of genetic gain to enable effective selection for progress; rather, greater heredity combined with better estimates of GCV and GAM." Similarly, DTT, f/s, AL, F/S, SL showed a moderate heritability of 30 to 50%, signifying that selection of the abovementioned characteristics might result in acceptable improvement. While rest had recorded low in heritability. This means that they transmit a small amount of heritable genetic (additive) characteristics on to next generation, emphasizing that there is no need to focus in improving these qualities. (Ranjith et al., 2017), Chavan et al., 2011)

Principle component analysis (PCA) more precisely indicates the differences in wheat genotypes (Bhanupriya, Satyanarayana, Mukherjee, & Sarkar, 2014; Mecha, Alamerew, Assefa, Assefa, & Dutamo, 2017). PCA widely used for the evaluation of genotypes based on morphological traits and for their grouping (Khodadadi, Fotokian, & Miransari, 2011; Ranjbar, Naghavi, Zali, & Aghaei, 2007). Eigenvalue helps in selection of factor that has highest impact in variation. In present study first four factors (F1, F2, F3, F4, F5 and F6) had eigenvalue >1 from which first two factor retains the highest information. F1 accounts 19.0 values for original variable while F2 retain 15.9. First principle factor explained the maximum variability then succeeded factors (Tadesse, Mekbib, Wakjira, & Tadele, 2018). Days to Flowering was the major contributing vector in F1 accounts for diversity while in F2 flowers per spike was major contributing vector (Syafii, Cartika, & Ruswandi, 2015; Westerlund, Andersson, Hämäläinen, & Åman, 1991). Based on PCA genotypes with highest score and desirable characters can be selected for further breeding programs.

Rust evaluation in field dispersed the whole data in 8 groups (from Immunity to susceptibility). 91 Lines were observed which had minimum ACI value and maximum RRI value. Both these factors are directly linked with immune Lines, which is highly desirable for researcher. Results suggesting that resistant genotypes are expected to possess diverse resistance genes and could be efficiently used as parents to improve resistance to yellow rust in breeding programs. Decline in AUDPC curve based on physiological data analysis proves that even in the presence of susceptible environment, the resistance level in population is too high.

It has been determined that the serious yellow rust issue in wheat cultivars is caused by extra dependency on the resistance genes *Yr*2, *Yr*9 and the APR genes like *YR*27, and the non - availability of major avirulent genes (which include *Yr*10, *Yr*15, *Yr*24/*Yr*26). After a period, the rapid evolution and aggressiveness of *Pst* races had resulted in the loss of resistance to the mono genetic varieties. This resistance outbreak has worried wheat breeders, who believe that future wheat types would need to expand their genetic basis by adding further yellow rust resistant alleles. The discovery of new genes for yellow rust resistance may aid breeders in the fast and precise incorporation of such genes into breeding material via MAS, reducing disease occurrences. Because single genes are vulnerable to genetic changes in the pathogen, employing gene combinations offers longer lasting and better resistance than using single genes. A total of 38 tightly linked markers specific for 15 *Yr* genes were used to determine the likely presence of *Yr* genes throughout wheat genotypes and examine overall contribution to the *Pst* resistance profile.

To improve the accuracy of the findings, most of the genes were amplified using several gene related markers. The existence of genes was confirmed using the combined findings of all the markers. Gene-based markers are much more precise and would be used to assess resistance genes in wheat breeding. The results of our molecular screening for expected PCR product size matched those of earlier studies, giving useful information for choosing specific resistance genes against Pst pathotypes. Stripe rust caused severe economic damage in wheat all over the world when the conditions are favorable for its growth. Stripe rust may be prevented by finding and integrating resistance genes into modified cultivars. The current investigation was done to discover stripe rust resistance genes, as mentioned before in the above section, we utilized 38 markers against fifteen stripe rust resistance genes;

Yr5, Yr15, Yr17, Yr18, Yr26, Yr36, Yr46, Yr48, Yr54, Yr59, Yr60, Yr61, Yr62, Yr64 and *Yr65* genes in Pakistani adapted 204 wheat Lines.

Pst resistance genes have not been identified in a wide range of germplasms and breeding Lines, particularly recently reported ones (Dracatos, Zhang, Park, McIntosh, & Wellings, 2016). Additionally, several *Yr* genes' effectiveness against recently emerged Pst races notably PST-V26 is unclear (Tian et al., 2016). The resistance gene's function is also impacted by an organism's genetic heritage (Ellis, Lagudah, Spielmeyer, & Dodds, 2014). As a result, testing the effectiveness of *Yr* genes across a diversity of germplasms, not only near-isogenic Lines, is essential (NILs).

Macer initially reported the seedling expressed gene *Yr*5 in the *Triticum spelta* album in 1966 (Macer & Van den Driessche, 1966). This gene gives resistance to all the races that are known to exist in the United States. In the human genome, *Yr*5 is found on chromosomal arm 2BL, 21 centimeters from the centromere (Macer & Van den Driessche, 1966). Kema introduced this gene into certain crop species, and it has proven to be effective against a diverse variety of Pst strains throughout the world (Kema, 1992). The *Yr*5 gene was discovered using the microsatellite marker *Yr*5 insertion. A 507 bp fragment linked with the existence of the *Yr*5 gene was found in 104 wheat Lines and a positive control out of 204 wheat Lines. The remaining wheat Lines and negative control produced no band, indicating that *Yr*5 is not present. Furthermore, there are two STS markers available for detecting the presence of *Yr*5: *STS-7/STS-8* and *STS-9/STS-10*. Out of 204, *STS-7/STS-8* amplified fragment of 478 bp in167 wheat Lines, confirming *Yr*5 gene in maximum population. Similarly, result of *STS-9/STS-10* indicates the presence of band in 78 Lines, while 120 wheat Lines did not produce any band. Band size of respective primer is 439 bp. According to (Begum et al., 2014) The expression of the *Yr*5 gene in the examined Lines was different for the two STS markers. S19M93, may be favored over S23M41 in *Yr*5 gene selection since it is more closely related to this gene. These markers against *Yr5* gene results supported this research.

(Gerechter-Amitai & Stubbs, 1970) discovered that *Triticum dicoccoides* corn Line G-25 was resistant to a variety of *Puccinia striiformis* races from diverse geographical bases. Later research revealed that the dominant gene *Yr*15 provided stripe rust resistance. (Gerechter-Amitai, Van Silfhout, Grama, & Kleitman, 1989). According to (Friebe, Jiang, Raupp, McIntosh, & Gill, 1996) findings, gene located on chromosome

1B. Xianming Chen stated that no virulent isolates on *Yr*15 have been discovered after several years of greenhouse and outdoor testing. Five different molecular markers available for *Yr*15. In present study, after obtaining results we have found that 1 out of 5 markers were polymorphic for this population. Gene specific marker *Xgwm413* amplified two fragments of 95 and 120 bp. The wheat Lines (162) and positive control showed 95 bp fragments, suggesting the presence of *Yr15* gene. Comparatively, only sixty-three wheat Lines amplified 120 bp fragments. And rest Lines with empty wells were indicating that these do not carry *Yr15* gene. The primers *Xgwm273*were used to detect *Yr15* gene that is associated with amplification of a 225bp DNA fragment. Only 8 wheat Lines and the negative control revealed no bands. Contrastingly, 200 wheat Lines and indeed the positive control generated bands, showing the existence of the *Yr*15 gene.

In present study, *Xgwm11* was also used to determine the presence of *Yr15/Yr26* and produce 273bp fragment in 172 Lines out of 204 Lines. Xgwm11, 1.9 cM distal to *Yr*15/*Yr*26, was utilized to amplify a 215-bp fragment in 71 wheat Lines and the positive control, demonstrating the presence of the *Yr*15/*Yr*26 gene. The other 29 Lines and the negative control did not generate it, confirming that the *Yr*15/*Yr*26 gene was not present (Begum et al., 2014). Three markers including *Xbarc181, Xwmc419* and STS marker *CYS-5* gene produced fragments of 185, 141, and 348 bp across all 100 Lines and the positive control "Avocet *Yr*26," demonstrating the existence of this gene. DNA marker that we have used was *Xbarc8* to confirm the presence of *Yr15* gene. Marker *Xgwm18,* expressing a sharp band in 182 Lines under UV lights. The band size of this marker is 186 bp. This marker amplified three different types of bands: 190, 240 and 400 bp fragment. Among 204 Lines, 100 advanced Lines and the positive control produced 190bp fragment, suggesting the likely presence of this gene. 67 Lines are showing 240 bp fragments, while 400bp fragment is expressed in only 17 Lines. Eleven Lines produce double bands and four Lines all above-described triple bands.

In present study, we used two markers including STS marker URIC/*LN2 and SC385* to detect adult plant resistance gene *Yr17/Lr37 and Sr38* in 204 wheat Lines Races of rust that are virulent against the Lr37 and *Yr*17 resistance genes have previously been discovered. However, these genes remain resistant to a broad variety of races by combining with other resistance genes. (McIntosh, Wellings, & Park, 1995). Begum et al., 2014 detected *YR*17 genes in Pakistani advanced wheat Lines using LN2 and

VENTRIUP. Thirty-seven wheat Lines and a positive control generated a 259-bp fragment linked with the presence of the *Yr*17 gene, according to the findings. Qamar *et al*. (2008) described the presence of *Yr17* in all Australian spring wheat cultivars tested and in positive control Avocet + *Yr17* NIL. They also confirmed the results from rust screening data. Expression of *Yr17* is affected by genetic background of the cultivar as well as environment. Because of the connection of *Yr*17 to Sr38 and Lr37 the frequency of this gene cluster in future wheat varieties in Pakistan must be raised to give resistance to the three rusts. Primer pairs *URIC/LN2 and SC-385* were selected to identify *Yr*17. URIC/LN2 primers amplify segments of (+) 285 bp (from the N genome) showing the existence of resistant strains, whereas (-) 285 bp indicating their lack. However, two other band sizes are also noticed. This primer gave band in each single Line which ultimately supports 100% presence of *Yr*17 gene in entire population. These results almost like Begum et al., 2014. Primer *SC-385* amplifies a 400 bp fragment which is already reported by researchers in past. But our PCR conditions assist it to amplify a second band of 1000 bp in few Lines. One hundred seventeen entries tested positive for 400 bp fragment, including 30 Lines with and extra band of 1kb.

(Begum et al., 2014) used two markers including STS marker *csLV34* and a gene specific marker c*ssfr-5* to detect adult plant resistance gene *Yr18* in 100 wheat Lines. The STS marker csLV34 produced a 150-bp fragment in 22 genotypes and a positive control, showing the presence of the *Yr*18 gene, whereas a 229-bp fragment in 78 Lines and a negative control, indicating the lack of the Lr34/*Yr*18 gene. Two fragments of 523 and 751 bp were amplified using the gene specific marker cssfr-5. Only seventeen wheat Lines and a positive control had a 751-bp fragment, indicating that the *Yr*18 gene was present. A 523-bp fragment was amplified from 83 wheat Lines and a negative control, confirming the lack of the *Yr*18 gene. Wheat Lines under study were also screened with gene specific marker *cssfr* amplified 751bp fragment associated with the presence of *Yr18*, whereas 523-bp fragment was produced by 83% Pakistani wheat Lines. We compared the results of both markers used to detect *Yr18* which did not show much difference. This means both markers reliably identify the *Yr*18 gene and its usage in MAS. Both markers are co-dominant, making them ideal for early separating generations. Both markers showed low *Yr*18 gene frequency in Pakistani wheat Lines, which must be raised to widen race-specific resistance to stripe rust. *Yr*18

is found on the short arm of chromosome 7D, near the Xgwm295 gene. This gene has a resistance phenotype that includes a prolonged latency period. Below we provide protocols for using these 3 markers. *CSLV34*results in amplification of band with 229 bp, which indicate the presence of*Yr*18. This primer produced bands in only 27 Lines, which is approximately 10% of overall population. Comparatively, *Xgwm295* produce a sharp band in 101 out of 204 Lines, indicating the likely presence of *Yr18* gene. Band size for this primer is 250bp. *Xbarc352*amplify 275 bp under ultraviolet radiations, which indicate the presence of*Yr*18.Of the 204 tested entries, 111 carried *Yr*18.

Because of its efficacy against the dominant races, C*YR*32 and C*YR*33, *Yr*26 is a key resistance gene that has been widely used in China, particularly in the Sichuan Basin, since the 1990s. Furthermore, it has been proposed that the genes *Yr*26 and *Yr*24 are the same. (Schnurbusch, Bossolini, Messmer, & Keller, 2004). During research study *CON-4* Primer amplified two fragments of 495 and 650 bp. Result of this primer is 100% monomorphic. All Lines have double band, exhibiting the presence of *Yr*26 gene. *CON-6*also produced two types of bands in end results but here story is entirely different. Two band sizes for this primer are 295 and 320 bp. No Line had both bands simultaneously. Of these 2, only one amplified. Although, 31 Lines didn't produce any band, indicating the absence of *Yr*26 in them. *STS-BQ74*was also used to determine the presence of *Yr26*. All 204 wheat Lines and the positive control produced 295bp fragment, suggesting the likely presence of this gene. In my research, *we173*was a last primer against *Yr*26. Based on visualization, it has 4 scenarios. 105 Lines amplified 500bp fragment. 131 Lines produced 700bp fragment. Among them, 48 Lines have both bands while 39 Lines did not give any sort of fragment, preceding the absence of this gene. Begum et al., 2014 used *Yr26,* has been translocated from *Triticum aestivum Haynaldia villosa* and mapped on chromosome 1B. Microsatellite markers including *Xbarc181, Xgwm11, Xgwm413, Xwmc419,* and STS marker *CYS-5* were used to detect the presence/absence of *Yr26* genes in Pakistani wheat Lines.

Four gene-specific primer sets to detect the locus were *UHW89, UCW71_5UTR, UCW71-INT6 and UCW79-dCAPS.* In the case of STS marker*UHW89,* all entries tested positive. It means these Lines are resistant to those stripe rust strains that are specifically linked with *Yr*36 gene. 2nd primer is *UCW71_5UTR* amplified a 710 bp band Out of 204, 73 Lines produced the band, with a clear-cut indication of *Yr*36 gene presence. Other 138 Lines with no band are supporting the absence of gene in them.

3rd primer against this gene is *UCW71-INT6,* this marker amplified a 930 bp fragment in 112 wheat Lines and the positive control. 98 Lines including negative control did not amplify the 930bp, suggesting the likely absence of *Yr*36 gene. Last primer *UCW79-dCAPS* amplified in 126 Lines with a band of 190bp it means these 126 Lines tested positive against *Yr*36 gene.

Xcfd71-4D was found to be strongly linked with *Yr*46 (p 0.001) in a genome-wide scan. Xcfd71-4D and Xcfd23-4D were found to be suitable options as molecular markers for breeding after further testing of nearby markers reported on 4D in distinct recombinant populations (Kovacs, Howes, Clarke, & Leisle, 1998)**.** So, we used both markers to check the presence or absence of *Yr*46 gene in Pakistani Lines. And the results were clearly supporting the findings of past researchers. Primer set *Xcfd71-4D*amplified 175 or 214 bp fragment and primer set *Xcfd23-4D* START amplified 3 different band sizes, 80, 205, 600 bp fragments. Results were completely monomorphic. Twelve entries were found to have *Yr*36. All 204 wheat Lines and positive control showed the presence of both band fragments for 1st primer, associated with the presence of *Yr46* gene. However, in the case of 2nd primer, 89 and 205 bp fragment were present in whole population but 600bp fragment was absent in 32 Lines. Overall, results of both primers were clearly indicating the domination of *Yr*46 gene in population.

Initial high-density mapping studies localized *Yr*48 to a 5.3 cM area on the distal end of 5AL (Lowe et al., 2011). SNF-A2 (0.18 cM proximal of *Yr*48), BE495011 (0.09 cM proximal of *Yr*48), and cfa2149 are three closely related markers that are suggested for use in marker-assisted breeding projects (0.06 cM distal of *Yr*48). In 92 wheat Lines and the positive control, the SNF-A2 marker amplified a 150bp fragment. 116 Lines, including the negative control, did not amplify the 185bp, indicating that the *Yr*48 gene is most likely absent. The primers *BE495011*was also used to detect *Yr48* gene that is associated with amplification of a 236 bp DNA fragment. 166 out of 204 wheat Lines and positive control produced a 259bp fragment which showed the presence of *Yr48* gene. In the case of marker *cfa2149*, all 204 wheat Lines and the positive control produced 225bp fragment, suggesting the likely presence of this gene.

The *Yr*59 gene is located on chromosome 7BL and was identified in an Iraqi spring wheat designated PI 178759. The SSR locus Xbarc32, which is located 2.1 cM downstream of *Yr*59, yields a 165 bp in resistant Line and a 175 bp susceptible Line.

During screening with this primer, four alleles were amplified at 165 bp (+), 175, 190 bp, and 250 bp, respectively. Xwmc557 (> 2.2 cM) is situated on the distal side. This primer amplified two alleles, 315 bp and 500 bp, during screening. This gene was found in thirteen genotypes, suggesting a polymorphism rate of 19.2 percent. Overall, 17 genotypes (25%) amplified one of the primer pairs, whereas four genotypes amplified both primer pairs. Our results are similar with their findings. Xbarc32 is an SSR locus which is situated on the proximal side of *Yr*59 with distance < 2.1 cM. It amplified 200 or 210 bp diagnostic fragment.170 Lines produced 200bp fragment while 134 Lines gave 210bp fragment. Among them, 48 Lines are those which has both bands. There is no single Line which didn't produce any sort of band. Also, *Xwmc557* amplifies a 300-bp or 320bp products in agarose gel. 88 Lines gave 300bp band size, while 61 produced 320 bp size. Among them, 48 Lines were those which gave both bands. However, 39 Lines entries tested negative for *YR*59 gene with no band.

The *Yr*60 (*Yr*Lalb) resistance gene is found on the distal end of chromosomal arm 4AL (Wiliam et al., 2003). At both the seedling and adult plant stages, this gene provides modest resistance. SSR locus wmc776 amplified three alleles of 150, 160, and 170 bp, respectively, at 0.6 cM. This gene was amplified by 14 genotypes (20.6%) with 150, 160, and 170 bp, respectively. Wmc313 is 0.6 cM away from *Yr*60 and amplified 180 and 200 bp alleles in 20 genotypes (29.3 percent), whereas wmc219, which is also 0.6 cM away from *Yr*60, amplified 200 and 220 bp alleles in 16 genotypes (29.3 percent). Wmc313 is 0.6 cM away from *Yr*60 and amplified 180 and 200 (23.6 percent). The remaining genotypes were unable to amplify any product, indicating that this gene is absent from the population. Using all the indicators, HI 8774 was estimated to be 60 years old (d). Similarly, *WMC313* and *WMC219* are also useful for this study. Our results supported to the previous research findings. The results of these 3 Markers *WMC776,* tested positive in all Lines by producing double bands. Fragment sizes of bands 200bp and 300bp. Second marker *WMC313,* also gave same band sizes under UV light; 200bp as well as 300bp fragment. Only 7 Lines didn't produce double band indicating the absence of *Yr*60 gene in those Lines. Codes of those seven Lines are 13, 49, 55, 57, 130, 139 and 180. Last primer against *Yr*60 is *WMC219,* amplifies a 120 bp diagnostic product. 180 entries were showing fragment, indicating the presence of respected gene.

Two SSR markers were found near *Yr*61. In the current material, these markers (WMS359and BE518379) amplified 225 bp and 310 bp diagnostic bands, respectively. The outcome was completely monomorphic. All the entries were found to be *Yr*61 positive. The data presented in this research, which is based on genetic diversity and the existence of *Yr* genes in synthetic hexaploid wheat Lines, will be helpful in future breeding efforts.

Lu et al. discovered two SSR markers near *Yr*62 (2014). These markers (Gwm192 and Gwm2511) amplified diagnostic bands in the current sample that were 185 bp and 120 bp in length, respectively.

For analyzing Lines and allocating them to corresponding gene pools, morphological characteristics and suitable statistical techniques such as cluster analysis and principal component analysis (PCA) are helpful tools (Jurowski & Reich, 2000). The phenotypic data of Morpho-physiological characteristics was utilized in a subsequent association mapping research. The discovery of DNA markers related to phenotypic characteristics involved in rust resistance was done via association mapping using 204 Lines.

The PIC values are used to calculate the diversity of a locus by taking into account the total number of alleles and their relative frequencies (Raghami et al., 2014). The PIC value ranged from 0.00 to 0.38, with an average of 0.18, depending on the 38 SSR markers. (Haghnazari, Samimifard, Najafi, & Mardi, 2005; Loridon et al., 2005; Smýkal, Horáček, Dostálová, & Hýbl, 2008) found that the average PIC values in other crops were 0.62, 0.53, and 0.52 correspondingly. In 16 genotypes of sunflower, 170 SSRs showed 3.5 alleles per locus with an average heterozygosity of 0.55. All the polymorphic SSR primer pairs employed in this research had a moderate value of number of alleles, gene diversity, and polymorphic information content. but still the diversity of SSRs markers is enough for cultivar discrimination and genetic diversity analysis

Cluster analysis is an effective technique for finding homogenous plant groupings. Based on nei value, the current research divided entire Lines into four clusters, with nei values ranging from 0.02 to 0.57. The genetic connection of different agricultural characteristics offers the possibility of improving economic qualities (Aasim, 2012; Akhtar, Pervez, & Nasim, 2011). Understanding the genetic linkage between various

characteristics is critical for the creation of varieties with a genetic attribute, that ultimately enhance crop acceptance.

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Appendix

Appendix 1: Protocols

DNA extraction by CTAB method

- Crush 0.2mg leaf tissues in mortar and pestle with liquid nitrogen (-196 ^{0}C).
- Add 2-3 ml of pre-warmed 2X CTAB having 1% B-marcepto-ethanol.
- Transfer 750 µl of sample extract to eppendorf tube (1.5 ml)
- Incubate the eppendorf tubes at 65 ºC for 30 minutes.
- Add 600 µl volume of Chloroform: Iso-amyl alcohol (24:1) solution to the eppendorf tubes and mix gently.
- Centrifuge the samples at 14000 rpm for 10 minutes.
- Transfer the supernatant to a new tube and add 480 µl volume of pre-chilled Iso-propanol and mix gently by inverting the tubes. Also add 80 µl of 3M sodium acetate to adjust ion balance.
- Incubate the sample at 20 ºC for 20 minutes.
- Again, centrifuge the samples at 14000 rpm for 10 minutes.
- Discard the supernatant and wash the pellet with 70% ethanol.
- Air dry the pellet for 30-40 min or overnight.
- Dissolved the pellet in 50/100 µl ddH2O or T.E buffer and store at -20 ºC for further use.

Preparation of stock solutions

Preparation of 2X CTAB solution (1 L)

- 1M TrisHCl (PH 8.0) = 100 ml
- 0.5M EDTA (PH 8.0) = 40 ml
- CTAB Salt = 20 g
- PVP = 10 g
- NACL =280 ml
- Adjust volume up to 1000 ml with distilled water. Then autoclave and store at room temperature.

Preparation of 1 M TrisHCl pH 8.0 (1L)

- Weigh 121.1 g of Tris-base
- Dissolve in about 700 ml of distilled water
- Bring pH to 8.0.
- Adjust total volume to 1 L with distilled water
- Autoclave and store at room temperature.

Preparation of 0.5 M EDTA pH 8.0 (1L) (MW= 372.24)

- Weigh 186.12g of EDTA (Ethylene diamine tetra acetic acid)
- Dissolve in about 700 ml of distilled water
- Add about 20 g of NaOH pellets.

- Slowly add more NaOH until pH is 8.0. EDTA will not dissolve until the pH is near 8.0
- Autoclave and store at room temperature

Preparation of 5 M NaCl (1L)

- Weigh 292.2 g of NaCl
- Dissolve in about 700 ml of distilled water
- Bring volume to 1L with distilled water

Preparation of 3M sodium acetate (1L)

- Weigh 246.1 g of sodium acetate
- Dissolve in 600ml distilled water
- Bring volume to 1000ml with distilled water
- Autoclave and store at room temperature

Preparation of 1X TE (Tris - EDTA) buffer (100 ml)

- Weigh 0.121g Tris base
- Dissolve in 50 ml autoclave distilled water
- Add 0.02ml (20µl) of) 0.5M EDTA PH 8
- Adjust to PH 7.5
- Bring volume to 100ml with autoclaved distilled water
- Autoclave and store at room temperature

Preparation of 70 percent ethanol (100ml)

- Add 30 ml distilled water in 70 ml absolute ethanol.

Preparation of 50X TAE tris acetate EDTA Buffer (1L)

- Weigh 242g of Tris-base
- Dissolve in 600ml of distilled water
- Add 57.1 ml of glacial acetic acid
- Add 100ml of 0.5M EDTA (PH 8)
- Adjust PH 8.0
- Bring volume to 1000ml with distilled water

Preparation of 1.5 % Agarose gel (100 ml)

- Wear gloves
- Weigh 1.5g Agarose
- Add 1.5 g Agarose in 100 ml 1X TAE buffer
- Boil the solution in microwave to dissolve Agarose
- Let it cool to 50-60 ˚C on the bench top for few minutes
- Add 5µl Ethidium bromide to solution and mix well

Appendix 2: Morphological data of Growth Parameters of entire population

Sr No.	LINE	G.D.	DTT	DTH	DTF	PH	FL	NN
1	k 456 × 82.2118	95	42	96	101	81.5	23	3.5
2	Y1 495 × CIMMYT 244	100	41	96.5	102	86.3	17	4.5
3	Y1-559 ×CIMMYT 1003	100	40.5	97.5	103	94.9	14.1	4
4	Y1-514 × CIMMYT 223	80	46.5	96	101	81.7	15	4
5	K456 × Y2-154	100	43	96	102.5	95.9	19	4
6	K456 × Y2-139	70	43.5	95	99.5	93.8	18	3.5
7	K456 × Y2-193	100	40	94	99	86.2	18.7	3.5
8	K456 × Y2-382	100	44	95.5	101	85.6	18.8	4
9	Y2-18 × CIMMYT 247	30	42.4	102.9	107.5	87.4	13	3.5
10	Y1-514 × C-T 244	100	43.5	96.5	101.5	97.6	15.6	4
11	Y1303 × C-T 245	65	43.2	100.5	107.9	96.7	19.2	4
12	Y19 × C-T 245	100	40.5	96.5	101	94.2	16.4	4
13	K78 × Y2-130	100	41.4	97.9	105	100.1	16	5.2
14	K78 × Y2-406	100	40	95	100.5	98.7	20.2	3
15	K78 × Y2-187	90	41.5	97.5	104	99.6	15.4	3
16	K78 × Y2-232	100	46	94.5	100.5	83.8	17.4	4
17	K1-78 × 42-246	100	41	97	103	91.8	12.6	4.5
18	Y2-58 × C-1181	100	38.5	95	101	93.3	13.3	3
19	K-456 × Y2-4	100	43	98.5	104.5	85.1	12.3	4.5
20	K-456 × Y2-477	100	40.5	96.5	101.5	94.3	23.4	3.5
21	Y2-37 × C-1181	100	41	96.5	101.5	95.8	18.1	3.5
22	Y2-37 × C-1102	100	42	97.5	103	96.4	17	4
23	K-78 × Y2-357	100	42.5	95	99.5	85.9	14.8	4.5
24	K-456 × Y2-386	100	45	96.5	102.5	86.6	15.8	3.5
25	K-456 × Y2-31	100	41.5	97.5	104.5	91.2	20.6	4
26	K-78 × Y2-154	100	40.5	98	103.5	84.2	16	4
27	K-456 × Y2-218	100	40.5	95	100	99.1	16.9	3.5
28	K-456 × Y2-43	100	41	96	101	84.7	19.4	4
29	K78 × C-Y-	85	42	95.5	101	84.3	12.7	3.5
30	K-78 × Y2-196	100	42	96.5	103.5	97.2	15.3	3.5
31	K-456 × Y2-196	100	42.5	94.5	100	84.1	17.1	4
32	K-78 × Y2-136	100	43	95	100.5	90.3	14.5	3.5
33	Y1-389 × C-233	100	42.5	96	101	86.6	14.3	4
34	Y1-360 × C-T 211	100	40.5	98	103	89.8	15.6	4
35	K-78 × Y2-274	100	43	96.5	102	87.9	17.6	3
36	K-456 × Y2-71	100	42.5	96	101.5	78	11	4
37	K-78 × Y2-321	100	40.5	95.5	101	96	13.2	3.5
38	K-78 × Y2-31	100	42.5	98.5	104.5	92.8	18.4	4

39	K-78 × Y2-361	100	43	94.5	99.5	92.5	22.8	4
40	K-456 × Y2-232	100	40	97	102.5	101.8	18	4
41	Y2-58 × C-1102	100	40.5	95	100.5	85.5	14.4	3.5
42	K-78 × Y2-164	100	43.7	100.4	106.7	85.9	12.4	4.4
43	K-78 × Y2-248	100	40	95	100	85	17	4
44	K-456 × Y2-137	100	40.5	97.5	103	88.5	19.1	4.5
45	Y2-122	100	42	96	101.5	98.3	13.8	3.5
46	Y2-63	100	42	97.5	103	86.1	17.1	4
47	Y2-287	55	42.5	95.5	100.5	80.6	18.2	4.5
48	Y2-66	100	41.5	97.5	103.5	81.4	15.5	3.5
49	Y2-278	100	39	96	102	82.3	20.9	3.5
50	DF-13	100	40	94	99	89.1	21.2	4
51	DF-8	100	42	94	99.5	84.7	21.1	3.5
52	DF-7	100	41	95	100.5	103	17.4	3.5
53	Y2-111	100	40.5	94.5	99.5	88.9	21.5	4
54	Y2-118	100	43.5	97.5	103.5	83.2	16.9	4
55	K456 × 248	100	40	96	101	91.6	14.6	3
56	Y2-91	100	40	95	100	90.1	15.5	4.5
57	K-78 × Y2-409	100	39.5	94.5	99.5	88.9	18.6	3.5
58	Y1-613 × C-T 247	100	40	95	101.5	79.6	18.8	3.5
59	K-456 × Y2-197	100	43	103	108.5	97.9	15.8	4.5
60	Y1-303 × CT-225	100	40	96.5	102	88.4	17.4	4
61	K456 × C-280	100	44.5	101.5	108.5	69.8	19	4.5
62	Y1-7 × Y1-2148	100	42	103	108.5	98.3	18.3	4
63	K-456 × Y2 × 91	100	44	101	107	73.7	16.6	3
64	K-456 × Y2-415	100	38.9	94.9	99.7	93.4	13.6	3
65	K-456 × Y2-63	100	43	97	103	89	17.4	4
66	K-456 × Y2-357	90	42	96	101	87.4	16.3	3.5
67	K-456 × Y2-122	100	40.5	104	108.5	71.8	17.8	3.5
68	K-78 × Y2-386	75	43	102.5	108	84.3	15.5	3.5
69	K-456 × Y2-278	100	44	103.5	109.5	77.4	15.5	4
70	Y2-37	90	41.5	103.5	109	95.8	17.8	4
71	C-T 888	100	40.5	96	102	86	15.1	3.5
72	C-T 290	100	41	98	103.5	86.2	20	3.5
73	Y2-32	100	42	99.5	105.5	83.1	17.5	4
74	C-T 232	75	42.5	101	107	84.5	14.4	3
75	Y2-58	100	43.5	99	104.5	86.7	15.3	4
76	Y2-18	100	42.5	101	105.5	84.4	14.1	3
77	C-T 248	100	38.5	95	101.5	92.2	19.3	4.5
78	E1-456	100	44.5	104	110	85.9	14.3	3.5
79	Y2-409	100	39	94	98.4	90.8	24.9	3.2
80	F1-78	100	43.5	98	104.5	75.5	14	3
81	Y2-321	100	41.5	96	101	78.1	12.3	4
82	Y1-389	100	42.5	103.5	109	82.8	16.1	4

83	Y1-431	100	41.5	104	108.5	75.3	11.7	3.5
84	Y1-478	100	41.5	97.5	102.5	96.1	14.4	3.5
85	Y1-495	41.7	41.9	101.7	109	97	11.6	5.5
86	Y1-514	100	41.5	97	102	93	16.8	3.5
87	C-T 244	100	41.5	99.5	105	87.3	17.4	4.5
88	Y1-236	100	39.5	100.5	106.5	92.3	16.5	3.5
89	Y1-613	100	41.5	97	102.5	90.9	13.2	4.5
90	Y1-9	100	43.5	97.5	103	82.6	19.9	3.5
91	Y2-361	100	41.5	97.5	104	97	12.6	4
92	Y2-382	100	41	100.5	106	81.3	12.4	3
93	Y2-386	100	44.5	104.5	109.5	92.1	17.7	3
94	Y2-408	100	42.5	94	99	87.6	18.8	3
95	Y2-406	100	42	97.5	103	85.8	17.1	3
96	P-78	100	44	100.5	107	83.5	13.5	4
97	D-F 11	100	41	98.5	103.5	86.7	13.3	3.5
98	Y1-559	100	42.5	98	104.5	87.9	21.5	4
99	C-T 247	100	41	97	102.5	92.8	17.3	3.5
100	C-T 103	100	43	96	101.5	92.5	15.3	4
101	Y2-259	100	39.5	100.7	108.9	73.8	11.5	5.2
102	Y2-232	100	39.5	97	102.5	93.2	16.9	4
103	Y2-71	100	40.5	100	105.5	85.5	17	3.5
104	Y2-222	100	43.5	98.5	106	86.1	14.7	4
105	Y2-223	60	40.5	102	108	75.1	13.8	3.5
106	Y2-256	100	40.5	101.5	107.5	76	17	3
107	Y1-303	75	42.5	98.5	105.5	86.5	15.1	3.5
108	C-T 245	75	42	100.5	106	93.5	16.2	4
109	C-T 223	100	40	99	105.5	81.6	17	4.5
110	Y2-246	100	40	95.5	101.5	73	16.7	3.5
111	Y2-248	100	39.5	95	100	94.3	17.6	4
112	Y2-490	100	43	97.5	103.5	84.3	15.1	4
113	Y2-499	100	42.5	104	109.5	80.7	18.3	3
114	C-T 225	100	43	98	103	85.6	16.1	3
115	C-T 211	100	39.7	94.5	99	83.9	21	3
116	C-T 233	100	42.5	100	106	95.2	14.2	4
117	F1-456	100	42	98	103.5	75.4	17.2	4
118	Y2-415	100	41.5	94	99	96.8	24.6	3.5
119	Y2-477	100	44.5	95	100	79.2	17.8	4
120	C-T 1181	100	36.2	95	100	74.2	9.8	2.9
121	Y2-274	100	44	101.5	106	89.9	14.5	3.5
122	K-456 × Y-306	100	44	99.5	105.5	94.3	13.6	4
123	K-78 × Y2-306	100	43	96	101.5	94.4	20.6	4
124	K-78 × Y2-288	100	40.5	95	100.5	95.3	19	5
125	K-78 × Y2-408	100	44	94	99.5	84	20	4
126	K-456 × Y2-164	100	42	94.5	100	77.4	20.6	4

127	K-78 × Y2-223	100	45	95	100.5	83.9	18.3	3.5
128	K-456 × Y2-187	100	40.5	95.5	100.5	84.4	18.9	4
129	K-78 × Y2-63	100	43.5	97.5	103	77.9	16.8	3.5
130	K-78 × Y2-217	100	42	94	99.5	105	16.1	4.5
131	Y1-9 × C-T225	100	41.5	96.5	102.5	86.9	19.3	3
132	K-78 × Y2-204	100	42.5	97	103.5	87.5	17	4
133	K-78 × Y2-287	100	43	95	101	97.7	13.9	3.5
134	K-78 × Y2-382	100	40.5	94.5	99.5	90.3	16.5	4.5
135	K-456 × Y2-117	100	41.5	96	102	83.4	13	4
136	K-78 × C-T 888	100	38.5	95.5	101.5	85.8	19.3	4
137	K-456 × Y2-118	45	42	97.5	102.5	84.9	16.1	3.5
138	K-78 × Y2-259	100	41	95.5	101.5	78.3	15.9	4.5
139	K-456 × Y2-321	100	41.5	98	103.5	92.5	16.5	3.5
140	K-456 × Y2-408	75	43.5	101	107	89.9	20.2	3.5
141	K-78 × Y2-111	100	42	98.5	104.5	87	15.8	4.5
142	K-78 × C-T 232	100	36.9	102.9	107.7	83.1	11.6	4.2
143	K-456 × Y2-66	100	43.5	100	108.4	88.6	15.8	4.1
144	YY3-118 × DF-13	100	39.7	97.7	107	90.5	17.1	2.9
145	C-T 280	100	43.4	101.4	108.2	83.9	9.4	4.2
146	Y2-272	100	41	97	103.5	92.9	14.6	4
147	Y2-43	100	43	99	104.5	88.9	12	4
148	Y2-72	100	40.5	99	105	85.2	12.3	4
149	Y2-196	100	44.5	100	106	80.9	14.9	3.5
150	Y2-193	100	40.5	100	106	91.6	15.7	3.5
151	Y2-164	100	42.5	100.5	106	66	19.3	3
152	Y2-154	100	44	100.5	105.5	74.6	13.4	3.5
153	Y2-139	100	42.5	98	104	94.8	21.3	3
154	Y2-137	70	43	100.5	105.5	100.3	15.5	4
155	Y2-288	100	42.5	96	102	86.6	14.9	3.5
156	Y2-136	100	42.9	99.5	103.2	87.5	16.6	2.7
157	Y2-357	100	42.5	99.5	105.5	97.3	12.8	4
158	Y2-306	100	44.7	98	105.9	81.8	14	3.5
159	Y2-218	70	42.5	101	108	77.8	14.6	4
160	Y2-217	80	42	98	104.5	89.5	16.6	3.5
161	Y2-204	100	41	102	107.5	83.8	19.2	4
162	Y2-197	72.5	44	100.5	105.5	96	14.5	4
163	Y2-31	100	42	98	103	78.9	16.2	4
164	Y2-4	100	43	99.5	106.5	81.2	14.7	3.5
165	K456	100	40.5	96.5	101.5	82.8	16.6	3.5
166	K456 × Y2-222	100	41.5	94.5	100.5	90.9	13.4	3.5
167	Y2-32 × C-1102	100	42	101	106	86.7	15.6	3.5
168	Y2-32 × C-1181	41.7	44.2	102	111	86.6	21	4.4
169	K456 × C-232	100	40	94	99	92.2	18	4
170	K-456 × C-290	100	44	95.2	101.4	88.9	12.4	3.4

171	Y1-478 × C-244	100	43.5	97	105.5	96	16.3	3.5
172	Y1-236 × C-245	85	42	96.5	101.5	88.8	16.4	3.5
173	YY3-114 × DF-11	100	42	98	104	91.8	20.3	4
174	Y1-236 × C-225	100	39	98.5	104	91.9	14.8	3.5
175	Y1-478 × C-223	100	43	98.5	104.5	103.2	16.3	4
176	Y1-360 × C-233	100	42	100	105.5	104.1	14.4	4
177	K456 × Y2-204	100	39	96	101.5	86.3	18.2	3.5
178	K-78 × Y2-477	80	42	96.5	102	87.4	17.8	3.5
179	K-78 × Y2-66	100	44.5	99	104	86	13.5	3.5
180	Y1-550 × C-T 247	100	38.5	96.5	102	102.8	19.8	4
181	K-78 × Y2-272	55	41	96	101.5	81.7	12.7	3.5
182	K-456 × Y2-288	100	42	95.5	101	105.3	16	3.5
183	K-456 × Y2-361	90	42.5	96.5	102.5	95.5	15.4	3.5
184	K-456 × Y2-406	100	41.7	96.7	100.4	72.2	19.2	2.7
185	K-78 × Y2-197	100	43.5	97.5	102.5	84.2	15.6	3.5
186	K456 × Y2-246	100	40.5	94	100.5	85.5	17.1	3.5
187	K-456 × Y2-274	100	40.5	95	99.5	88.5	17.3	3.5
188	Y1-613 × C-T 1003	100	40	96	102	91.3	15.5	4
189	K456 × Y2-136	100	41.5	97.5	103.5	88.8	15.6	3.5
190	K-78 × Y2-4	100	41	95.5	102.5	85.9	16.1	3.5
191	K-456 × Y-223	100	43.5	97.5	103.5	88.5	15.8	4.5
192	K-78 × Y2-122	100	42	97	102	82.3	16.7	3
193	J-78 × 137	100	40	96.5	101.5	101.2	15.5	3.5
194	K-78 × Y2-278	100	42.5	98	102	91.5	17.5	3.5
195	K-465 × C888	100	44.5	95	103	98.8	20.1	4
196	Y-431 × CIMMYT 233	100	40.5	95.5	102	84.3	16.4	4.5
197	K78 × Y2-154	100	43.5	102	108	87.6	13	4
198	Y2-18 × C1003	60	42.5	98	103.5	83.7	18.5	3
199	YY3116 × DF8	85	41	96	102.5	91.6	15.7	3.5
200	BORL-16	100	41	97	102.5	95.4	17.3	4
201	MARK-19	100	41	101	106	95.4	12.7	3
202	CHINESE CROSS	100	41	100	106	90.5	17.1	4.5
203	PAK-13	20	44.5	103.5	108.5	97.3	19.6	4
204	ZIN-16	100	42.5	97.5	104.5	95.4	14.5	4
Standard Deviation (S.D.)		**13.01**	**1.63**	**2.61**	**2.86**	**7.29**	**2.72**	**0.49**

G.D: Growth data, **DTT**: Days to tillering**, DTH**: Days to heading, **DTF**: Days to flowering, **PH**: Plant height, **FL**: Flag leaf length, **N.N**: Number of Nodes.

Appendix 3: Morphological data of Yield Parameters of entire population

SR no.	LINE	SL	AL	F/S	SP	f/s	NT	WT
1	k 456 × 82.2118	16.3	5.8	107.5	11	5	4.5	910.1
2	Y1 495 × CIMMYT 244	19.2	7.2	110	11.5	5	5.5	773.4
3	Y1-559 ×CIMMYT 1003	17.8	6	86.5	10	4.5	4.5	1216.8
4	Y1-514 × CIMMYT 223	16.5	6.2	94.5	11	4.5	5	883.4
5	K456 × Y2-154	16.8	6.4	82	10.5	4	4.5	1393.5
6	K456 × Y2-139	11.8	0	84	11	4	3.5	863.4
7	K456 × Y2-193	18	6	80.5	12	3.5	5	1140.1
8	K456 × Y2-382	16.9	6.4	103	11.5	4.5	5	1120.1
9	Y2-18 × CIMMYT 247	18.8	6.8	82	11.2	3.7	4.4	920.1
10	Y1-514 × C-T 244	17	7.3	105	11	5	4.5	1366.8
11	Y1303 × C-T 245	16.5	6.6	75.5	10.5	3.9	6	800.1
12	Y19 × C-T 245	15.8	6.5	89.5	10.5	4.5	5	1480.1
13	K78 × Y2-130	16.2	8.5	78	10.2	3.7	6.2	1840.1
14	K78 × Y2-406	15.5	7.4	66	9.5	3.5	5.5	1273.4
15	K78 × Y2-187	16.3	6	82	10.5	4	4.5	1850.1
16	K78 × Y2-232	15.4	6.8	83.5	9.5	4.5	4.5	1286.8
17	K1-78 × 42-246	14.8	5.6	86.5	10	4.5	5	1293.4
18	Y2-58 × C-1181	13.8	5.7	86.5	10	4.5	5	1086.8
19	K-456 × Y2-4	15.5	5.5	101.5	11.5	4.5	5.5	1023.4
20	K-456 × Y2-477	16.3	6.8	117.5	12	5	5	1626.8
21	Y2-37 × C-1181	16.7	6.8	82	10.5	4	4.5	1753.5
22	Y2-37 × C-1102	18.2	7.2	88	11.5	4	4.5	1263.4
23	K-78 × Y2-357	13.6	4.3	100	10.5	5	5	910.1
24	K-456 × Y2-386	14.2	5.6	86	11	4	5.5	563.4
25	K-456 × Y2-31	16.8	6.3	76.5	11	3.5	4	933.4
26	K-78 × Y2-154	16	5.8	115	12	5	4	1086.8
27	K-456 × Y2-218	14.6	5.5	76.5	9.5	4	5	960.1
28	K-456 × Y2-43	15.8	6	72	9.5	4	4.5	1693.5
29	K78 × C-Y-	16.2	5.7	73.5	11	3.5	4.5	1160.1
30	K-78 × Y2-196	14.2	6.9	63	9	3.5	5	1226.8
31	K-456 × Y2-196	15.5	6.9	92.5	9.5	5	5	880.1
32	K-78 × Y2-136	15.6	6.2	90.5	10.5	4.5	5	590.1
33	Y1-389 × C-233	14	6.3	105	11	5	4	1156.8
34	Y1-360 × C-T 211	17.5	7.2	69.5	10.5	3.5	5	633.4
35	K-78 × Y2-274	14.4	5.9	90	11.5	4	4	1180.1
36	K-456 × Y2-71	13.8	6.3	73	10.5	3.5	4.5	670.1
37	K-78 × Y2-321	13.3	6.4	74	8.5	3.5	3.5	1330.1

38	K-78 × Y2-31	15.5	6	110	11.5	5	4.5	660.1
39	K-78 × Y2-361	13.6	7.7	90	9.5	5	5.5	1410.1
40	K-456 × Y2-232	15.9	7.7	78.5	11.5	3.5	5.5	983.4
41	Y2-58 × C-1102	14.9	6.9	74	10.5	3.5	5	1113.4
42	K-78 × Y2-164	15	5.7	100.4	13.7	3.9	3.9	916.8
43	K-78 × Y2-248	14.9	5.9	85	11	4	4.5	1283.4
44	K-456 × Y2-137	18.2	7.1	106.5	12	4.5	3.5	1146.8
45	Y2-122	14.1	6.2	88	11	4	5	1070.1
46	Y2-63	14.4	6.5	90	10.5	4.5	4.5	1536.8
47	Y2-287	15.7	5.5	85.5	10	4.5	4	936.8
48	Y2-66	13.6	7.1	80	10	4	4.5	833.4
49	Y2-278	15.2	4.9	105	11	5	4	1150.1
50	DF-13	11.2	0	107.5	12	4.5	4.5	1193.4
51	DF-8	16.3	7	110	11.5	5	5.5	1686.8
52	DF-7	17.4	6.8	99.5	11.5	4.5	4.5	953.4
53	Y2-111	15.5	6.5	94.5	11	4.5	4.5	1306.8
54	Y2-118	16.7	7.3	88	11	4	5	536.7
55	K456 × 248	16.1	7.3	68.5	10	3.5	4.5	1226.8
56	Y2-91	13.8	6.5	90	11.5	4	4.5	840.1
57	K-78 × Y2-409	14.3	3.8	81	10.5	4	5	1436.8
58	Y1-613 × C-T 247	17.2	8.7	97.5	10	5	5	1193.4
59	K-456 × Y2-197	13.8	8.1	90.5	11.5	4	3.5	680.1
60	Y1-303 × CT-225	17.2	5.7	100	11.5	4.5	5	930.1
61	K456 × C-280	19	6.6	97.5	11	4.5	3.5	523.4
62	Y1-7 × Y1-2148	17.7	6.1	91	12	4	4.5	1473.5
63	K-456 × Y2 × 91	17.7	9	115.5	13	4.5	4	766.8
64	K-456 × Y2-415	10.2	4.9	23.5	6.7	2.5	4.5	1173.4
65	K-456 × Y2-63	16.6	7.4	60.5	9	3.5	4	1136.8
66	K-456 × Y2-357	17.5	6.7	93	10.5	4.5	4.5	1066.8
67	K-456 × Y2-122	15.6	5.7	97.5	11	4.5	4	1333.4
68	K-78 × Y2-386	16.6	8.5	93.5	10.5	4.5	4.5	780.1
69	K-456 × Y2-278	15.8	4.8	90.5	10.5	4.5	5	1223.4
70	Y2-37	15.8	5.8	94	12	4	4	1260.1
71	C-T 888	15.4	8.1	70	9	4	4.5	763.4
72	C-T 290	13.9	6.4	60	8.5	3.5	3.5	1123.4
73	Y2-32	17.6	6.7	92	12	4	4.5	1140.1
74	C-T 232	17.7	6.5	90.5	10.5	4.5	4.5	616.7
75	Y2-58	15.8	6.5	83	10.5	4	5	1116.8
76	Y2-18	16.2	7	69	10	3.5	5	1126.8
77	C-T 248	15.7	6.8	71.5	8.5	4.5	5	1126.8
78	E1-456	17.1	7.4	84	11	4	5	943.4
79	Y2-409	18.1	0	130.7	12.2	5.7	4.4	1753.5
80	F1-78	14.7	5.7	97.5	11	4.5	5.5	1556.8
81	Y2-321	15.8	6.5	73.5	12.5	3	4	1136.8

82	Y1-389	17.7	6.7	66	9.5	3.5	5	563.4
83	Y1-431	14.4	6.2	96.5	12.5	4	5	950.1
84	Y1-478	15.4	5.9	92	11.5	4	3.5	1293.4
85	Y1-495	14.2	5.2	80	10	4	3.2	790.1
86	Y1-514	17.2	8.3	72	9	4	4.5	1066.8
87	C-T 244	18.7	6.6	149.5	17.5	4.5	5.5	1890.1
88	Y1-236	13.4	5.3	80	10	4	4	1010.1
89	Y1-613	15.2	7.4	70	9	4	5	1260.1
90	Y1-9	15.8	7.6	84	10.5	4	4	1606.8
91	Y2-361	14.3	5.2	95	10	5	4	923.4
92	Y2-382	15.7	6.9	90	11.5	4	4.5	1313.4
93	Y2-386	16.7	6.1	94.5	11	4.5	5	1353.5
94	Y2-408	18	9.3	112.5	13	4.5	4.5	1146.8
95	Y2-406	17.9	3.8	88.5	10	4.5	5	1516.8
96	P-78	15.3	6.2	97.5	11	4.5	5.5	1280.1
97	D-F 11	10.7	0	86.5	10	4.5	5	1213.4
98	Y1-559	19.4	7	108.5	12.5	4.5	4	916.8
99	C-T 247	16.6	6.6	72	9.5	4	4.5	1393.5
100	C-T 103	15.9	5.9	92.5	10.5	4.5	5	2163.5
101	Y2-259	9.1	0	100.9	10.9	4.9	2.2	983.4
102	Y2-232	17.7	8.1	101.5	11.5	4.5	4.5	1253.4
103	Y2-71	15.4	6.7	95.5	11	4.5	5.5	986.8
104	Y2-222	18.4	7.8	90.5	11.5	4	4	1516.8
105	Y2-223	15.6	5.8	68	10	3.5	5	386.7
106	Y2-256	17.9	7.3	105	11	5	6	403.4
107	Y1-303	18.7	6.9	103.5	12	4.5	6	1096.8
108	C-T 245	14.9	5.6	91	11.5	4	4	833.4
109	C-T 223	17.8	6.6	105	11	5	6	930.1
110	Y2-246	16.1	6	110	13	4.5	4.5	946.8
111	Y2-248	14.8	6.2	85	11	4	5	2010.2
112	Y2-490	14.5	4.8	95	10	5	4.5	1333.4
113	Y2-499	19.7	8.5	88.5	10	4.5	4.5	990.1
114	C-T 225	14.3	5.2	101.5	13.5	4	3.5	680.1
115	C-T 211	12.9	6.3	46.7	10.7	2.4	5.5	1010.1
116	C-T 233	16	7.7	92.5	12.5	4	3.5	846.8
117	F1-456	15.3	5.2	75.5	9	4.5	4	760.1
118	Y2-415	13	0	104.5	12	4.5	5.5	1053.4
119	Y2-477	14.8	5.9	97.5	10	5	4.5	826.8
120	C-T 1181	14	7.3	90	8.9	5	3.7	530.1
121	Y2-274	15.1	6.1	76	9.5	4	4	1473.5
122	K-456 × Y-306	14.1	5	86	13	3.5	4.5	916.8
123	K-78 × Y2-306	14.3	5.5	90	11.5	4	5	1046.8
124	K-78 × Y2-288	11	0.6	64.5	9.5	3.5	4	863.4
125	K-78 × Y2-408	16.7	5.6	100.5	11.5	4.5	4.5	1363.5

126	K-456 × Y2-164	16.9	5.5	95	10	5	5	1373.5
127	K-78 × Y2-223	16.1	10.2	105	11	5	4.5	760.1
128	K-456 × Y2-187	14.4	5	102.5	11.5	4.5	5	746.8
129	K-78 × Y2-63	17	7	79	10.5	4	5	720.1
130	K-78 × Y2-217	17.6	7.7	93.5	10.5	4.5	4	1106.8
131	Y1-9 × C-T225	17.8	6.7	91.5	14	3.5	3.5	1170.1
132	K-78 × Y2-204	15.4	5.9	105	11	5	5	840.1
133	K-78 × Y2-287	16	5.8	92.5	10.5	4.5	6	1193.4
134	K-78 × Y2-382	14.3	5.7	96.5	11	4.5	5	1323.4
135	K-456 × Y2-117	14.3	6.5	69.5	10	3.5	4	1433.5
136	K-78 × C-T 888	15	7.9	87.5	10.5	4.5	4	1490.1
137	K-456 × Y2-118	15.8	6.7	56.5	10	3	4	623.4
138	K-78 × Y2-259	12.1	2.2	82	10.5	4	4	946.8
139	K-456 × Y2-321	16.6	6.3	89	11.5	4	4.5	1026.8
140	K-456 × Y2-408	19.5	7.4	127.5	13	5	5.5	1196.8
141	K-78 × Y2-111	16	6.2	100	10.5	5	3.5	1143.4
142	K-78 × C-T 232	11.5	7.3	96.7	10.2	5	3.9	1106.8
143	K-456 × Y2-66	16.5	6.6	95.6	10.1	5	5.2	1033.4
144	YY3-118 × DF-13	17.3	5.4	57.9	11.2	2.7	5.1	1100.1
145	C-T 280	11.9	5.2	76.5	9.9	4.1	3.9	1500.1
146	Y2-272	15.7	5.2	115	12	5	5	1343.5
147	Y2-43	15.9	7.4	62	9	3.5	5	1610.1
148	Y2-72	15.4	5.5	103.5	12	4.5	4.5	1420.1
149	Y2-196	15.7	6.8	92.5	12.5	4	4.5	930.1
150	Y2-193	17.2	5.9	84	9.5	4.5	4.5	706.8
151	Y2-164	14.6	6.8	81.5	9.5	4.5	4	933.4
152	Y2-154	14.2	5.9	87.5	13.5	3.5	4.5	1566.8
153	Y2-139	15.8	6.2	88	10	4.5	5.5	1336.8
154	Y2-137	16.9	8.4	97.5	11	4.5	5	766.8
155	Y2-288	9.5	0	83.5	11	4	3.5	1100.1
156	Y2-136	12.4	4.9	85.4	9.2	4.7	2.7	1400.1
157	Y2-357	14.6	6.8	80	10	4	5	996.8
158	Y2-306	13.1	6.9	71.4	9.5	4	5	560.1
159	Y2-218	17.9	7.3	88.5	13.5	3.5	3.5	853.4
160	Y2-217	17.7	8.1	71.5	9	4	4	813.4
161	Y2-204	19.4	7	110	14	4	3.5	1070.1
162	Y2-197	18.1	6.5	95.5	11	4.5	4.5	1410.1
163	Y2-31	14.2	10.4	100	11.5	4.5	3	1140.1
164	Y2-4	14.5	5.1	105.5	12	4.5	4.5	1660.1
165	K456	10	0	59.5	7.5	4	4	906.8
166	K456 × Y2-222	16	6.9	68.5	10	3.5	3.5	650.1
167	Y2-32 × C-1102	15.8	6.7	74	9.5	4	5	1151.8
168	Y2-32 × C-1181	20.2	6.4	100	13.2	4	5	1026.8
169	K456 × C-232	17.7	5.9	112.5	11.5	5	4.5	1463.5

170	K-456 × C-290	15.1	4.9	116.7	12.4	5	5.4	1926.8
171	Y1-478 × C-244	15.6	5.9	73.5	9.5	4	4.5	1223.4
172	Y1-236 × C-245	16.9	5.5	73.5	11	3.5	5	873.4
173	YY3-114 × DF-11	12.9	0	89	11.5	4	5	1143.4
174	Y1-236 × C-225	14.2	5.3	85.5	11	4	3.5	1000.1
175	Y1-478 × C-223	15	5.5	84	12.5	3.5	5	1216.8
176	Y1-360 × C-233	16.7	7.1	92	11.5	4	4.5	1403.5
177	K456 × Y2-204	17.6	11.9	97	11	4.5	4.5	723.4
178	K-78 × Y2-477	16.8	6.8	82	9	4.5	5	1363.5
179	K-78 × Y2-66	15.3	6.3	82	10.5	4	4.5	856.8
180	Y1-550 × C-T 247	18	7.9	97.5	11	4.5	4.5	1173.4
181	K-78 × Y2-272	15.1	5.8	95.5	12.5	4	3.5	396.7
182	K-456 × Y2-288	16.1	6.4	105	11	5	4.5	696.8
183	K-456 × Y2-361	15.5	5.5	79.5	11.5	3.5	5.5	920.1
184	K-456 × Y2-406	13.1	0	102.4	12.4	4.4	4	883.4
185	K-78 × Y2-197	14.2	6.1	78.5	11	3.5	4.5	633.4
186	K456 × Y2-246	17.2	7	105	11	5	5.5	856.8
187	K-456 × Y2-274	13.9	5.9	78	9	4.5	5	590.1
188	Y1-613 × C-T 1003	19	7.5	99.5	11.5	4.5	4	1540.1
189	K456 × Y2-136	14.9	5.1	88	11.5	4	4.5	1396.8
190	K-78 × Y2-4	15.2	5.9	81	10.5	4	4	1116.8
191	K-456 × Y-223	13.5	6.8	70	9	4	4	1153.4
192	K-78 × Y2-122	15.7	5.7	86.5	10	4.5	4	916.8
193	J-78 × 137	17.8	7.4	90.5	10.5	4.5	4.5	1113.4
194	K-78 × Y2-278	20	7	83	12	3.5	5	1123.4
195	K-465 × C888	18.9	8.4	103.5	12	4.5	4	1243.4
196	Y-431 × CIMMYT 233	16.5	6.6	80	10.5	4	4.5	1486.8
197	K78 × Y2-154	15.6	6.1	104.5	12	4.5	4.5	693.4
198	Y2-18 × C1003	14.4	6.6	68	9	4	5	890.1
199	YY3116 × DF8	15.8	5.7	84.5	12.5	3.5	4.5	1136.8
200	BORL-16	14.9	7.1	71	12.5	3	4	1033.4
201	MARK-19	15.9	5	64.5	11	3	4	896.8
202	CHINESE CROSS	15.4	5.8	120	12.5	5	5	806.8
203	PAK-13	17.4	7.7	66	9.5	3.5	4	680.1
204	ZIN-16	14.9	5.6	77.5	9	4.5	4.5	1210.1
Standard Deviation (S.D.)		**1.96**	**1.81**	**14.56**	**1.21**	**0.54**	**0.65**	**322.45**

SL: Spike length, **AL**: Awn length, **F/S**: Flowers per spike, **S.P**: Spikelet's Pair, **f/s**: Flowers per spikelet, **N.T**: Number of tillers**, WT**: Weight in kg per hectare.

Appendix 4: Disease Assessment, AUDPC, and RAUDPC values of each single line

SR no.	LINE	DISEASE REACTION	R.V.	C.I.	RRI	AUDPC	RAUDPC
1	k 456 × 82.2118	6 MR	0.4	24	6.84	992.5	0.59
2	Y1 495 × CIMMYT 244	ZERO	0	0	9	0	0
3	Y1-559 ×CIMMYT 1003	1 M	0.5	5	8.55	182.5	0.11
4	Y1-514 × CIMMYT 223	ZERO	0	0	9	0	0
5	K456 × Y2-154	3 MSS	0.9	27	6.57	445	0.27
6	K456 × Y2-139	7 MRMS	0.6	42	5.22	1107.5	0.66
7	K456 × Y2-193	3 MRMS	0.6	18	7.38	497.5	0.3
8	K456 × Y2-382	ZERO	0	0	9	0	0
9	Y2-18 × CIMMYT 247	4 MR	0.4	16	7.56	645	0.39
10	Y1-514 × C-T 244	ZERO	0	0	9	0	0
11	Y1303 × C-T 245	ZERO	0	0	9	0	0
12	Y19 × C-T 245	8 MSS	0.9	72	2.52	1222.5	0.73
13	K78 × Y2-130	2 MRMS	0.6	12	7.92	347.5	0.21
14	K78 × Y2-406	4 MRMS	0.6	24	6.84	645	0.39
15	K78 × Y2-187	2 MRMS	0.6	12	7.92	315	0.19
16	K78 × Y2-232	4 MRMS	0.6	24	6.84	645	0.39
17	K1-78 × 42-246	6 MSS	0.9	54	4.14	942.5	0.56
18	Y2-58 × C-1181	1 MR	0.4	4	8.64	182.5	0.11
19	K-456 × Y2-4	ZERO	0	0	9	0	0
20	K-456 × Y2-477	4 MRMS	0.6	24	6.84	645	0.39
21	Y2-37 × C-1181	8 MSS	0.9	72	2.52	1272.5	0.76
22	Y2-37 × C-1102	5 M	0.5	2.5	8.775	100	0.06
23	K-78 × Y2-357	8 SS	1	80	1.8	1290	0.77
24	K-456 × Y2-386	5 MRM	0.5	2.5	8.775	100	0.06
25	K-456 × Y2-31	4 MR	0.4	16	7.56	645	0.39
26	K-78 × Y2-154	7 MRMS	0.6	42	5.22	1175	0.7
27	K-456 × Y2-218	4 MRMS	0.6	24	6.84	645	0.39
28	K-456 × Y2-43	7 MSS	0.9	63	3.33	1107.5	0.66
29	K78 × C-Y-	1 MSS	0.9	9	8.19	182.5	0.11
30	K-78 × Y2-196	7 MRMS	0.6	42	5.22	1107.5	0.66
31	K-456 × Y2-196	5 MSS	0.9	45	4.95	827.5	0.49
32	K-78 × Y2-136	4 MRMS	0.6	24	6.84	645	0.39
33	Y1-389 × C-233	ZERO	0	0	9	0	0
34	Y1-360 × C-T 211	ZERO	0	0	9	0	0
35	K-78 × Y2-274	3 MRMS	0.6	18	7.38	497.5	0.3
36	K-456 × Y2-71	2 MRMS	0.6	12	7.92	347.5	0.21
37	K-78 × Y2-321	ZERO	0	0	9	0	0
38	K-78 × Y2-31	5 MRS	0.5	2.5	8.775	100	0.06

39	K-78 × Y2-361	3 MR	0.4	12	7.92	462.5	0.28
40	K-456 × Y2-232	7 MSS	0.9	63	3.33	1075	0.64
41	Y2-58 × C-1102	3 MR	0.4	12	7.92	462.5	0.28
42	K-78 × Y2-164	7 MRMS	0.6	42	5.22	1090	0.65
43	K-78 × Y2-248	ZERO	0	0	9	0	0
44	K-456 × Y2-137	4 MSS	0.9	36	5.76	645	0.39
45	Y2-122	8 MSS	0.9	72	2.52	1255	0.75
46	Y2-63	ZERO	0	0	9	0	0
47	Y2-287	4 MR	0.4	16	7.56	645	0.39
48	Y2-66	6 MRMS	0.6	36	5.76	942.5	0.56
49	Y2-278	8 MSS	0.9	72	2.52	1257.5	0.75
50	DF-13	2 MRMS	0.6	12	7.92	315	0.19
51	DF-8	ZERO	0	0	9	0	0
52	DF-7	7 SS	1	70	2.7	1110	0.66
53	Y2-111	4 MSS	0.9	36	5.76	612.5	0.37
54	Y2-118	2 MRMS	0.6	12	7.92	315	0.19
55	K456 × 248	ZERO	0	0	9	0	0
56	Y2-91	6 MRMS	0.6	36	5.76	992.5	0.59
57	K-78 × Y2-409	4 MSS	0.9	36	5.76	645	0.39
58	Y1-613 × C-T 247	ZERO	0	0	9	0	0
59	K-456 × Y2-197	ZERO	0	0	9	0	0
60	Y1-303 × CT-225	ZERO	0	0	9	0	0
61	K456 × C-280	1 MR	0.4	4	8.64	182.5	0.11
62	Y1-7 × Y1-2148	5 MR	0.4	2	8.82	100	0.06
63	K-456 × Y2 × 91	ZERO	0	0	9	0	0
64	K-456 × Y2-415	ZERO	0	0	9	0	0
65	K-456 × Y2-63	5 MRMS	0.6	30	6.3	810	0.48
66	K-456 × Y2-357	2 MRM	0.5	10	8.1	347.5	0.21
67	K-456 × Y2-122	ZERO	0	0	9	0	0
68	K-78 × Y2-386	ZERO	0	0	9	0	0
69	K-456 × Y2-278	ZERO	0	0	9	0	0
70	Y2-37	1 MR	0.4	4	8.64	182.5	0.11
71	C-T 888	5 MR	0.4	2	8.82	100	0.06
72	C-T 290	3 MRMS	0.6	18	7.38	462.5	0.28
73	Y2-32	2 M	0.5	10	8.1	315	0.19
74	C-T 232	ZERO	0	0	9	0	0
75	Y2-58	2 MSS	0.9	18	7.38	315	0.19
76	Y2-18	ZERO	0	0	9	0	0
77	C-T 248	5 MR	0.4	2	8.82	100	0.06
78	E1-456	ZERO	0	0	9	0	0
79	Y2-409	ZERO	0	0	9	0	0
80	F1-78	8 MSS	0.9	72	2.52	1240	0.74
81	Y2-321	ZERO	0	0	9	0	0
82	Y1-389	ZERO	0	0	9	0	0
83	Y1-431	ZERO	0	0	9	0	0

84	Y1-478	ZERO	0	0	9	0	0
85	Y1-495	ZERO	0	0	9	0	0
86	Y1-514	ZERO	0	0	9	0	0
87	C-T 244	5 MR	0.4	2	8.82	100	0.06
88	Y1-236	5 SS	1	50	4.5	792.5	0.47
89	Y1-613	ZERO	0	0	9	0	0
90	Y1-9	ZERO	0	0	9	0	0
91	Y2-361	ZERO	0	0	9	0	0
92	Y2-382	ZERO	0	0	9	0	0
93	Y2-386	ZERO	0	0	9	0	0
94	Y2-408	7 MR	0.4	28	6.48	1110	0.66
95	Y2-406	ZERO	0	0	9	0	0
96	P-78	ZERO	0	0	9	0	0
97	D-F 11	ZERO	0	0	9	0	0
98	Y1-559	7 MS	0.8	56	3.96	1140	0.68
99	C-T 247	ZERO	0	0	9	0	0
100	C-T 103	2 MRMS	0.6	12	7.92	347.5	0.21
101	Y2-259	ZERO	0	0	9	0	0
102	Y2-232	ZERO	0	0	9	0	0
103	Y2-71	ZERO	0	0	9	0	0
104	Y2-222	ZERO	0	0	9	0	0
105	Y2-223	ZERO	0	0	9	0	0
106	Y2-256	5 MRMS	0.6	3	8.73	100	0.06
107	Y1-303	ZERO	0	0	9	0	0
108	C-T 245	1 MSS	0.9	9	8.19	182.5	0.11
109	C-T 223	2 MRMS	0.6	12	7.92	347.5	0.21
110	Y2-246	ZERO	0	0	9	0	0
111	Y2-248	ZERO	0	0	9	0	0
112	Y2-490	ZERO	0	0	9	0	0
113	Y2-499	ZERO	0	0	9	0	0
114	C-T 225	3 SS	1	30	6.3	445	0.27
115	C-T 211	ZERO	0	0	9	0	0
116	C-T 233	ZERO	0	0	9	0	0
117	F1-456	5 MR	0.4	20	7.2	672.5	0.4
118	Y2-415	ZERO	0	0	9	0	0
119	Y2-477	ZERO	0	0	9	0	0
120	C-T 1181	ZERO	0	0	9	0	0
121	Y2-274	4 SS	1	40	5.4	645	0.39
122	K-456 × Y-306	5 MRMS	0.6	30	6.3	812.5	0.49
123	K-78 × Y2-306	3 MSS	0.9	27	6.57	462.5	0.28
124	K-78 × Y2-288	7 MSS	0.9	63	3.33	1142.5	0.68
125	K-78 × Y2-408	1 MRMS	0.6	6	8.46	182.5	0.11
126	K-456 × Y2-164	8 S	1	80	1.8	1290	0.77
127	K-78 × Y2-223	4 MSS	0.9	36	5.76	645	0.39
128	K-456 × Y2-187	4 MRMS	0.6	24	6.84	645	0.39

129	K-78 × Y2-63	25 MRMS	0.6	15	7.65	397.5	0.24
130	K-78 × Y2-217	4 MRMS	0.6	24	6.84	645	0.39
131	Y1-9 × C-T225	ZERO	0	0	9	0	0
132	K-78 × Y2-204	4 MRMS	0.6	24	6.84	645	0.39
133	K-78 × Y2-287	6 MSS	0.9	54	4.14	960	0.57
134	K-78 × Y2-382	ZERO	0	0	9	0	0
135	K-456 × Y2-117	6 MSS	0.9	54	4.14	960	0.57
136	K-78 × C-T 888	6 MSS	0.9	54	4.14	960	0.57
137	K-456 × Y2-118	ZERO	0	0	9	0	0
138	K-78 × Y2-259	7 MRMS	0.6	42	5.22	1107.5	0.66
139	K-456 × Y2-321	4 MSS	0.9	36	5.76	645	0.39
140	K-456 × Y2-408	1 MR	0.4	4	8.64	182.5	0.11
141	K-78 × Y2-111	3 MSS	0.9	27	6.57	462.5	0.28
142	K-78 × C-T 232	ZERO	0	0	9	0	0
143	K-456 × Y2-66	7 MRMS	0.6	42	5.22	1175	0.7
144	YY3-118 × DF-13	ZERO	0	0	9	0	0
145	C-T 280	ZERO	0	0	9	0	0
146	Y2-272	ZERO	0	0	9	0	0
147	Y2-43	ZERO	0	0	9	0	0
148	Y2-72	ZERO	0	0	9	0	0
149	Y2-196	ZERO	0	0	9	0	0
150	Y2-193	ZERO	0	0	9	0	0
151	Y2-164	ZERO	0	0	9	0	0
152	Y2-154	ZERO	0	0	9	0	0
153	Y2-139	ZERO	0	0	9	0	0
154	Y2-137	ZERO	0	0	9	0	0
155	Y2-288	ZERO	0	0	9	0	0
156	Y2-136	5 MR	0.4	2	8.82	100	0.06
157	Y2-357	3 MSS	0.9	27	6.57	462.5	0.28
158	Y2-306	ZERO	0	0	9	0	0
159	Y2-218	ZERO	0	0	9	0	0
160	Y2-217	ZERO	0	0	9	0	0
161	Y2-204	ZERO	0	0	9	0	0
162	Y2-197	ZERO	0	0	9	0	0
163	Y2-31	ZERO	0	0	9	0	0
164	Y2-4	ZERO	0	0	9	0	0
165	K456	ZERO	0	0	9	0	0
166	K456 × Y2-222	ZERO	0	0	9	0	0
167	Y2-32 × C-1102	6 MSS	0.9	54	4.14	870	0.52
168	Y2-32 × C-1181	ZERO	0	0	9	0	0
169	K456 × C-232	2 MRM	0.5	10	8.1	347.5	0.21
170	K-456 × C-290	4 MRMS	0.6	24	6.84	645	0.39
171	Y1-478 × C-244	5 MR	0.4	2	8.82	100	0.06
172	Y1-236 × C-245	5 MR	0.4	2	8.82	100	0.06
173	YY3-114 × DF-11	ZERO	0	0	9	0	0

174	Y1-236 × C-225	ZERO	0	0	9	0	0
175	Y1-478 × C-223	5 MRM	0.5	2.5	8.775	100	0.06
176	Y1-360 × C-233	ZERO	0	0	9	0	0
177	K456 × Y2-204	7 MRMS	0.6	42	5.22	1142.5	0.68
178	K-78 × Y2-477	6 MSS	0.9	54	4.14	925	0.55
179	K-78 × Y2-66	6 SS	1	60	3.6	992.5	0.59
180	Y1-550 × C-T 247	8 MSS	0.9	72	2.52	1307.5	0.78
181	K-78 × Y2-272	1 MRMS	0.6	6	8.46	182.5	0.11
182	K-456 × Y2-288	4 MR	0.4	16	7.56	645	0.39
183	K-456 × Y2-361	4 MSS	0.9	36	5.76	645	0.39
184	K-456 × Y2-406	5 MRM	0.5	2.5	8.775	100	0.06
185	K-78 × Y2-197	7 MSS	0.9	63	3.33	1107.5	0.66
186	K456 × Y2-246	4 MRM	0.5	20	7.2	645	0.39
187	K-456 × Y2-274	7 MSS	0.9	63	3.33	1107.5	0.66
188	Y1-613 × C-T 1003	ZERO	0	0	9	0	0
189	K456 × Y2-136	ZERO	0	0	9	0	0
190	K-78 × Y2-4	ZERO	0	0	9	0	0
191	K-456 × Y-223	1 MRMS	0.6	6	8.46	182.5	0.11
192	K-78 × Y2-122	5 MSS	0.9	45	4.95	810	0.48
193	J-78 × 137	7 MSS	0.9	63	3.33	1140	0.68
194	K-78 × Y2-278	4 MSS	0.9	36	5.76	645	0.39
195	K-465 × C888	1 MR	0.4	4	8.64	182.5	0.11
196	Y-431 × CIMMYT 233	5 MR	0.4	2	8.82	100	0.06
197	K78 × Y2-154	5 MRMS	0.6	3	8.73	100	0.06
198	Y2-18 × C1003	4 MRMS	0.6	24	6.84	645	0.39
199	YY3116 × DF8	6 MS	0.8	48	4.68	940	0.56
200	BORL-16	ZERO	0	0	9	0	0
201	MARK-19	ZERO	0	0	9	0	0
202	CHINESE CROSS	8 SS	1	80	1.8	1307.5	0.78
203	PAK-13	ZERO	0	0	9	0	0
204	ZIN-16	ZERO	0	0	9	0	0

DR: Disease Reaction, RV: Response Value **CI**: Coefficient of Infection, **RRI**: Relative Resistance Index, **AUDPC**: Area under disease progress curve, **RAUDPC**: Relative area under disease progress curve.

Appendix 5.1: Data scoring sheet of 1st thirteen primers

Genotype/ Primer	P 1	P 2	P 3	P 4	P 4.1	P 5	P 5.1	P 6	P 7	P 8	P 8.1	P 8.2	P 9	P 10	P 10.1	P 11	P 12	P 12.1	P 13
1	0	1	1	1	0	1	0	0	1	1	0	0	0	0	1	0	1	1	1
2	0	1	1	1	0	1	0	0	0	1	0	0	0	0	1	0	1	1	1
3	0	1	1	0	0	1	0	0	1	1	0	0	1	0	1	0	1	1	1
4	0	1	0	1	0	1	0	1	1	1	0	0	0	0	1	1	1	1	0
5	0	1	1	1	0	1	0	0	1	1	0	0	1	0	0	0	1	1	1
6	0	1	1	1	0	1	0	1	1	0	1	0	1	0	0	0	1	1	1
7	0	1	0	1	0	1	0	0	0	0	1	0	0	0	0	0	1	1	1
8	0	1	1	1	0	1	0	0	1	0	1	0	0	0	0	0	1	1	1
9	0	1	1	0	0	1	0	1	1	1	0	0	0	0	0	0	1	1	1
10	0	1	1	0	0	1	0	1	1	0	1	0	0	0	0	0	1	1	1
11	1	1	0	1	0	1	0	0	1	1	0	0	0	0	1	1	1	1	1
12	0	0	1	1	0	1	0	0	1	0	1	0	0	0	0	0	1	1	1
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14	0	1	1	0	1	1	1	1	1	0	0	0	0	0	1	0	1	1	1
15	0	1	1	1	0	1	0	1	1	0	0	0	0	0	1	0	1	1	1
16	0	1	1	1	0	1	0	1	1	0	0	0	0	0	0	1	1	1	1
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22	1	1	1	1	0	1	0	1	1	0	1	0	0	0	0	0	1	1	1
23	0	1	1	1	0	1	0	1	0	1	0	0	0	0	0	1	1	1	1
24	0	1	1	0	1	1	0	1	1	1	0	0	1	0	0	0	1	1	1
25	1	0	1	1	0	1	0	1	1	0	0	0	0	0	0	1	1	1	1
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29	0	1	0	1	0	1	0	1	1	0	0	0	0	0	0	0	0	0	1
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188	0	1	1	0	1	1	0	1	0	1	0	0	0	0	1	1	1	1	1
189	1	1	0	0	1	1	0	1	1	1	0	0	0	0	0	1	1	1	1
190	1	1	0	1	0	1	0	1	1	1	0	0	0	0	1	0	1	1	1
191	1	1	0	0	1	1	0	1	1	1	0	0	0	0	0	1	1	1	1
192	1	1	0	0	0	1	0	1	1	1	0	0	0	0	0	0	1	1	1
193	1	0	0	0	0	1	0	1	1	1	1	0	0	0	0	0	1	1	1
194	1	1	0	0	0	1	0	1	1	1	0	0	0	0	0	1	1	1	1
195	1	0	0	0	0	1	0	1	1	1	1	0	0	0	0	1	1	1	1
196	1	0	0	0	0	1	0	1	1	0	1	0	0	0	0	1	1	1	1
197	1	0	0	0	0	1	0	1	1	0	0	0	0	0	0	1	1	1	1
198	1	1	0	0	0	0	0	1	1	1	0	0	0	0	0	1	1	1	1
199	1	1	0	0	0	1	0	1	1	1	0	0	0	0	0	1	1	1	1
200	1	0	0	1	0	1	0	1	1	0	1	0	0	0	1	1	1	1	1
201	0	0	0	0	1	1	0	1	1	1	0	0	1	0	1	1	1	1	1
202	1	0	0	1	0	1	0	1	1	0	0	0	0	0	1	1	0	0	1
203	1	0	0	1	0	1	0	1	0	0	0	0	0	0	1	1	1	1	1
204	1	0	0	1	0	1	0	1	0	1	0	0	0	0	1	1	1	1	1

Appendix 5.2: Data scoring sheet from Primer 14 to 25

Genotype/ Primer	P 14	P 15	P 16	P 17	P 18	P 18.1	P 19	P 19.1	P 19.2	P 20	P 21	P 22	P 23	P 24	P 24.1	P 25	P 25.1
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2	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	0	0
3	1	0	0	0	1	1	1	1	1	0	1	1	0	1	1	0	0
4	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	0
5	1	1	0	1	1	1	1	1	1	0	1	0	0	1	1	1	0
6	1	1	0	1	1	1	1	1	1	1	1	0	0	1	1	0	0
7	1	1	0	0	1	1	1	1	1	0	1	1	0	1	1	0	0
8	1	0	0	0	1	1	1	1	1	1	1	1	0	1	1	0	1
9	1	0	0	1	1	1	1	1	0	1	1	1	1	1	1	0	1
10	1	0	0	0	1	1	1	1	0	1	1	1	0	1	1	0	0
11	1	1	0	1	1	1	1	1	0	1	1	1	0	1	1	0	0
12	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0
13	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1	0	0
14	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	0	0
15	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	0	0
16	1	0	0	1	1	1	1	1	1	0	1	1	1	1	1	0	0
17	1	1	0	1	1	1	1	1	1	0	1	1	1	1	1	0	0
18	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0
19	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1	0	0
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21	1	1	0	1	1	1	1	1	1	0	1	1	1	1	1	0	0
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24	1	0	0	0	1	1	1	1	1	0	1	1	0	1	1	0	0
25	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	0	0
26	1	0	1	0	1	1	1	1	1	0	1	1	1	1	1	0	0
27	1	0	0	0	1	1	1	1	1	0	1	1	1	1	1	0	0
28	1	1	0	0	1	1	1	1	1	0	1	1	1	1	1	1	0
29	1	0	1	1	1	1	1	1	1	0	1	1	1	1	1	1	0
30	1	0	0	0	1	1	1	1	1	1	1	1	1	1	1	0	0
31	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	0	0
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199	1	0	1	1	1	1	1	1	1	0	0	1	0	1	0	1	0
200	1	0	1	1	1	1	1	1	1	0	0	1	1	1	0	1	0
201	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0
202	1	0	0	1	1	1	1	1	1	1	1	1	1	1	0	1	0
203	1	1	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1
204	1	0	1	1	1	1	1	1	1	0	1	1	1	1	0	0	1

Appendix 5.3: Data scoring form Primer 35 to 54:

Genotype/ Primer	P 35	P 35.1	P 36	P 36.1	P 37	P 39	P 40	P 44	P 44.1	P 44.2	P 45	P 45.1	P 46	P 46.1	P 49	P 50	P 51	P 54
1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	1	1	1	1
2	1	1	1	1	1	1	1	1	1	1	0	0	0	0	1	1	1	1
3	1	1	1	1	1	1	1	1	1	1	0	0	1	0	1	1	1	1
4	1	1	1	1	0	1	1	1	1	1	1	0	0	1	1	1	1	1
5	1	1	1	1	1	1	1	1	1	1	0	0	0	1	1	1	1	1
6	1	1	0	0	1	1	1	1	1	1	1	0	1	0	1	1	1	1
7	1	1	1	1	0	1	1	1	1	1	0	0	0	1	1	1	1	0
8	1	1	1	1	1	1	1	1	1	1	0	0	0	1	1	1	1	1
9	1	1	1	1	1	1	1	1	1	1	0	0	0	1	1	1	1	1
10	1	1	1	1	1	1	1	1	1	1	0	0	0	1	1	1	1	1
11	1	1	1	1	1	1	1	1	1	1	0	0	0	1	1	1	1	1
12	1	1	1	1	1	1	1	1	1	1	0	0	1	0	1	1	1	1
13	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	0
14	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1	1
15	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1
16	1	1	1	1	0	1	1	1	1	1	1	0	1	1	1	1	1	1
17	1	1	1	1	0	1	1	1	1	1	1	0	0	1	1	1	1	1
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19	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1
20	1	1	0	0	1	1	1	1	1	1	1	0	0	1	1	1	1	1
21	1	1	1	1	0	1	1	1	1	1	1	0	1	0	1	1	1	1
22	1	1	1	1	0	1	1	1	1	1	0	0	0	1	1	1	1	1
23	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1
24	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1
25	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1
26	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1
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34	1	1	0	0	1	1	1	1	1	1	1	0	1	1	1	1	1	1
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153	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1
154	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1
155	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1
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180	1	1	0	0	1	1	1	1	1	1	1	0	0	1	1	1	1	1
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201	1	1	1	1	1	1	0	1	1	1	1	0	1	1	1	1	1	1
202	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	0
203	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1	0
204	1	0	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1

Appendix 6:

Comparison on the base of height:

Comparison on the base of rust:

Immune

Moderate Attack

Succeptible

Comparison on the base of Awn:

Awn less line

Line having Awns

Comparison

Thesis on wheat exotic lines

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