

FINAL RESEARCH PROGRESS REPORT

For

PARB's CGS PROJECT NO.161

**SAFEGUARDING PAKISTANI WHEAT FROM POTENTIAL
DISEASE**

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Name of Host Institution: Wheat Research Institute Faisalabad



(2016)



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Basic Information of the Project:

Name of the project	Safeguarding pakistani wheat from potential disease
Project period	15 February 2010 to 14 February , 2015
Total project duration	60 Months
Total Project cost	Rs.31.547 Millions
Total Expenditures	Rs. 27.540 Millions
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Executive Summary

In Pakistan wheat is the most important crop. It covers 6.979 million hectare in Punjab with production of 1.8 million tonnes. Most of the dietary requirement of the peoples is met from the wheat because it is the staple food for the people of Pakistan. Biotic and abiotic stresses are most important factors and play vital role in the production of wheat. The low yield of wheat crop is due to many factors but biotic factors are most important. Among the biotic factors rust play a major role for the low yield. Stem rust caused by *Puccinia graminis* has been successfully controlled by the cultivation of genetically resistant varieties until 1999. With the emergence of a highly virulent race in Uganda (Ug99) a catastrophic situation in adjoining wheat growing countries has developed and it spread further in the region is predicted. The Ug99 race which is devastating and continuously changing is expected to migrate further into Pakistan and beyond. All Pakistani wheat germplasm were sent to Kenya and Ethiopia for testing against Ug99 over the last few crop cycles has generally shown susceptibility causing an alarming situation in research circles. In southern Punjab, a local race prevails that was observed in 2006 with recurrence each year. Its effect showed that all mega-varieties of Sindh and Punjab were attacked. Consequently an added facet of this project will be the development of wheat varieties resistant to both Ug99 and our local race which is not Ug99 (Robert Park, Personal Communication, 2009).

Five hundred lines (300 from WRI, Faisalabad and 200 from RARI, Bahawalpur) developed were screened every year. On the basis of resistance, new crosses were raised. Only resistant parents were hybridized to develop the F6 lines. Under the project, 1500 lines were developed and screened for leaf and yellow rust out of which 150 lines were promoted on yield and plant type basis.

150 lines were tested in PARB preliminary yield trial and 16 lines out yielded the commercial check varieties. Sixty lines were tested in the regular yield trials and 15 lines produced the good yield as compared the check varieties. The high yielding lines were promoted to MWYT and NUWYT trials. Under the Project No.161, two advance lines V-12304 and V-11138 were planted 21 location in Punjab. These lines produced 3854 and 3918 kg per ha which is higher than the local check Faisalabad. Total ten lines (V-09006, V-09031, V-12304, V-11138, V-09087, V-09082, V-10031, V-07076 V-08173 and V-10025) were tested in the project in NUWYT trials.

Nine hundred lines were genotyped for the rust resistant genes using molecular genotyping technique for different genes. The screening of the 100 lines for Ug99 were done in Kenya. The demonstration of four lines line were conducted on farmer trials during 2013 and 2014.

The project was initially awarded to Wheat Wide Crosses , NARC, Islamabad later on it was handed over to the Wheat Research Institute Faisalabad. The project was completed and its all milestones were achieved. Several lines were developed and tested in the project. Finally two durable rust resistant varieties are being tested in the NUWYT trial along with the check variety which was planted throughout the Pakistan

1. Introduction:

Wheat is a very important crop and full fill the dietary requirement of the people of Pakistan. It occupies primary position in food and in the food security of people of the Pakistan. Wheat contribute 10.10 % in the value added in agriculture and 2.1 % in the GDP. Area under wheat in 2014-15 was 6.979 million hectares , which is 1.13 % higher than the last year's area of 6.901 million hectares. The production of wheat was 2.4735 million tons with an average yield of 2775 kg ha.

The production of the wheat yield is very low as compared to the other countries of the world. The low yield causes a major lose to the economy of the country. The major damaged the crop lose in the wheat production is due to the leaf diseases which cause the lose up to 30%. Among the diseases the stem rust caused by *Puccinia graminis* is very devastating disease which can damaged the whole crop .

The project was designed to study the behavior of the rusts in wheat crop. The wheat varieties developed at wheat research institute were screened for the leaf rust. The varieties showed resistant were send to kenya for study the stem rust.

Under the Project No.161 two advance lines V-12304 and V-11138 were planted 21 location in Punjab. These lines produced 3854 and 3918 kg per ha which is higher than the local check Faisalabad. Total eleven lines, six from WRI, Faisalabad (V-12304, V-11138, V-09087, V-09082, V-10031 and V-10025) and 5 from RARI, Bahawalpur (V-076422, V-076346, V-099114, V-099172 and V-099384) were tested in the project in NUWYT trials.

Ninety lines were tested in the PARB in preliminary yield trial and 45 were tested in the regular yield trials. On over all basis 16 and 15 lines produced the good yield as compared the check varieties.

Project Objective:

Development of wheat varieties resistant to Rusts, and Helminthosporium blight

2. Outputs planned for the project:(As per project document)

a) Wheat Research Institute Faisalabad

Output-1 Development of durable rust resistant variety with desirable yield and quality traits for central Punjab

Activity-1 and Activity-2

200 wheat lines were screened every year. One set was planted at Wheat Research Institute Faisalabad and 2nd set was planted at Regional Agriculture research Institute Bahawalpur. The lines were also planted at Kaghan to note the data of powdery mildew none of line were found resistant The data for leaf and yellow rust was recorded and sharer among the collaborator. The data recorded by the wheat Research Institute Faisalabad is as under. The detail is attached as annexure I,II,III and IV

YEAR	No. OF LINES TESTED	Resistant		Moderately resistant		Susceptible	
		Lr	Yr	Lr	Yr	Lr	Yr
1 ST Year	100	94	91	2	9	4	0
2 nd Year	300(10-11)	255	146	25	98	20	56
3 rd Year	220(11-12)	205	220	0	0	15	0
4 th Year	220(12-13)	201	57	2	55	17	108
5 th Year	200	150	30	25	50	25	120

Activity-4

300 wheat advanced lines each year were provided to the university of Agriculture for molecular genotyping of the lines against the Lr.34, Lr46, Yr10, Yr18, Yr29 Sr2, Sr25, Sr26, Sr31, Sr36 and Sr 39 at the university of Agriculture Faisalabad the summary of the results are presented in the following table . The detail is provided in the annexure

Name of Marker	2012-13 1st year		year-2-2013-14	
	Present	Absent	Present	Absent
Sr2	304	56	249	53
Sr25	349	11	287	15
Sr26	348	12	287	15
Sr31	355	5	259	43
Sr36	347	13	294	8
Sr39	356	4	191	111
Lr34	340	20	247	55
Lr46	343	17	261	41
Yr10	354	6	251	51
Yr18	340	20	247	55
Yr29	343	17	261	41
Total	360		302	

Activity-5

Every year new cross combination were constituted among the lines found resistant. More than 200 F6 advanced lines developed were provided for screening against rusts under the activity 1 &2

Activity-6

A total of 150 lines were evaluated in the preliminary yield trials under the PARB project.

1st (2009-10) year only 12 lines performed better than the standard check variety Seher.06. the most prominent lines V-09129, V-09130, V-09137 V-09138, V-09082 V-09083, V-9085, V-09086, V-09087, V-09088 ,V09091 and V-09092 lines were selected on the base of their yield performance and promoted to the next yield trials. The detail result are presented in annexure.5

During 2nd year (2010-11) 32 advanced lines were tested in the preliminary yield trials. More than 15 lines passed the check variety Seher.06. More than 15 lines passed the check variety Seher.06. the most prominent lines are V-10022, V10025, V-10028, V-10031, V-10035, V-10037, V-10039, V-10041, V-10042, V-10043, V-10044 and V-10048. These lines showed the better performance in the field. The detail result are presented in annexure.6

During 3rd year (2011-12) 42 newly developed resistant lines were tested in the preliminary yield trial. Among the tested lines more than 12 lines out yielded the check variety Seher.06. The most promising lines which out yielded the check variety Seher.06 are V-1101, V-11002, V-1104, V-11009, V-11022, V-11023, V-11024, V-11025, V-11027 V-11032, V-11034, V-11035 and V-11042. The detail result are presented in annexure.7

During 4th year (2012-13) 30 lines were tested preliminary yield trials against the standard check varieties, Faisalabad.08, Millat.11 and Punjab11. Many lines out yielded the check varieties. A total Fifteen lines were selected on the basis of yield and disease reaction basis. Four wheat advanced lines V-12304, V-11320, V.11338, V-11356 and V-11365 were high yielder and rust resistant to diseases. The detailed result are presented in annexure.8

During 5th year (2013-14) 30 lines were tested preliminary yield trials against the standard check varieties, Faisalabad.08, Millat.11 and Punjab11. More than 15 lines out yielded the check varieties. A total Fifteen lines were selected on the basis of yield and disease reaction basis. Four wheat advanced lines V-13017, V-13019, V-13032, V-13032, V-13028, V-13029 V-13030 V-13031 and V-13045 were high yielder and rust resistant to diseases. The detailed result are presented in annexure.9

Activity-7

A total of 60 lines were tested in the regular yield trials for 4 years in the project.

During 2010-11, 16 advanced lines were tested in the regular yield trial and 8 lines out yielded the check variety Seher.06. The most promising lines were V-09006, V-09007, V-09029, V-09040, V-09082, V-09087, V-9088 V-09082, V-09087 and V-09091. These lines were better yielder and resistant to rust. The detailed data is attached as annexure.10

During 2011-12, 21 advanced lines were tested in the regular yield trial and only three lines passed the check varieties Seher.06. The most promising lines were V-10035, V-10041 and V-10062. These lines were better yielder and resistant to rust. The detailed data is attached as annexure.11

During 2012-13, 15 advanced lines were tested in the regular yield trial and only six lines passed the check variety Millat.11. The most promising lines were V-11001, V-11006, V-11022 V-11032, V-11034 and V-11041. These lines were better yielder and resistant to rust. The detailed data is attached as annexure.12

During 2013-14, 15 advanced lines were tested in the regular yield trial and only six lines passed the check variety Millat.11. The most promising lines were V-13002, V-13005, V-13007, V-13012, V-13013 and V-13016. These lines were better yielder and resistant to rust. The detailed data is attached as annexure.13

Activity-8

During 2009-10 Two lines viz V-07076 and V-07096 were tested in Punjab on 24 locations. The advance line V-07076 and V-07096 produced 3863 and 3861 kg/ha. Which is higher than the check variety Lasani and Faisalabad.08. The detailed data is presented in the annexure 14

During 2011-12 three lines were tested in MTWV trials Viz, Three lines viz V-09031, V-09087 and V-09006 were tested in Punjab on 21 locations during 2011-12. The Line V-09087 produced 4952 kg/ha exceeding the check variety Lasani.08. The detail data is presented in the following annexure.15

During 2012-13 Two lines V-10025 and V-10031 were tested in MTWV trials conducted through the Punjab Province. None of the lines passed the check varieties Lasani.08 and Punjab.11 which produced 3789 and 3594 kg/ha respectively. The detail data is presented in the following annexure.16

During 2013-14 two wheat advanced lines V-11138 and V-12304 were tested in the Punjab province on 21 locations. The data revealed that both the lines V-11138 and V-12304 performed well and produced 4088 and 4183 kg/ha respectively which is higher than both the check varieties Punjab.11 and Faisalabad.08. The detailed data is presented in the annexure No.17

Activity-9

During 2010-11 two lines viz V-07096 and V-07076 were tested in NUWYT trials. These lines performed well. The wheat line V-07096 produced 4414 kg/ha as compared to the check variety Seher.06. The detailed data is presented in the Annexure no.18

During 2011-12 the two wheat advanced lines V-07096 and V-08173 were tested in the NUWYT trials. These two lines produced excellent yield and out yielded the check varieties. The detail data is presented in annexure No.19

During 2012-13 the wheat line V-09087 was tested in the NUWYT trial. The line produced the 3876 kg/ha yield as compared to the check variety Seher.06 which produced 3777 kg/ha. The detailed data is presented in the annexure no.20

During 2013-14 two wheat advanced lines V-09082 and V-09087 were tested in the NUWYT trials against the standard check variety Faisalabad.08. These two lines produced 4175 and 3876 kg/ha yield as compared to the check variety Faisalabad.08 which produced only 3884 kg/ha. The detailed data is presented in the annexure no.21

Activity-10

The demonstration plots were planted for two years at farmers' fields and packages of technology were delivered to the farmers. The names of varieties planted along with the location are as under.

During 2012-13 the newly approved variety were planted at the farmers field in four districts of the Punjab province. The detail is as under

S.No	Name of grower	Variety planted
1	Haji Muhammad Sharif 35-Burj Malam, Pattoki	AARI-11
2	Haji Hashim Ali Mashallah Property Center Raiwind Road, Taroki Gill, Lahore	AARI-11
3	Master Asmatullah Cheema Pano Pani , Daska	Millat-11
4	Syed Saadat Ali Ali Pur Syedan, Narowal	Millat-11
5	Prof. Saifullah Khan Moza Jugyal, P.O. Kot Nainan Shakar Garh, Narowal	Millat-11

During 2013-14 the newly developed wheat advanced line V-09082 and V-09087 were planted at the farmers field in two districts of the Punjab province. The package of technology were delivered to the farmers and importance of new variety were explained. The detail is as under

S.No	Name of grower	Yield Md/AC	Variety planted
1	Asad Qayyum,104/JB, Faisalabad	40	V-09082
2	Mehr Muhammad Tariq, 57/JB, Faisalabad	45	V-09087
3	Muhammad Sarwar,205/RB, Faisalabad	47	V-09082
4	Haji Muhammad Aslam 18/WB, Vehari,	49	V-09087
5	Muhammad Imran, 63/JB,Faisalabad,	50	V-09082

Activity-11 The selected material was screened at Kenya.

First year The seed of 43 advance lines were send to Kenya for screening against Ug99. The 18 lines were contributed by Regional Agriculture Research Institute, Bahawalpur and remaining were from Wheat Research Institute, Faisalabad. The detail of these lines is as under:-

Sr.#	Name of Line	Reaction noted on 17.10.2011	Reaction noted on 25.10.2011
1.	V-029221	10MSS	30M
2.	V-10293	20M	50MS
3.	V-10306	15MS	30MR
4.	V-10309	5MS	20M
5.	V-09196	20M	40M

During first year Out of 25 lines sent to Kenya these lines i.e. V-10293, V-10306, V-10309 and V-09196 showed good resistance against ug-99 The detailed data is presented in the annexure No.22

Second year 24 were send to Kenya for screening against stem rust screening. Out of 24 lines 9 lines showed the moderate reaction among the 24 lines send to Kenya. The detailed data is presented in the annexure No.23

S#	Name of entry	Parentage	Stem Rust
1	70010	AUQAB 2000/CHAM 6	10 M
2	70019	KOHISTAN 97/4/PASTOR/3/VEE # 5//DOVE/BUC	10 M
3	70024	KOHISTAN 97/4/PASTOR/3/VEE # 5//DOVE/BUC	10 M
4	70064	PRL'S'/PVN//HUW 234 + LR-34	30 M
5	70093	SHALIMAR. 88/ 87094/ MH. 97/3/ LU. 26/HD.2179//2*INQ. 91	15 M
6	70134	PASTOR/HXL 7573// 2* BAU/3/YECORA-70	10 M
7	70178	PB 96/V 87094// MH.97/3/UQAB.2000	15 M
8	70186	MUNIA/3/RUFF/FGO//YAV. 79/4/PASTOR/5/BL 1724	1 MR
9	70203	BOW/URES// 2* WEAVER/3/ CROC-1/AE.SQ (213)// PGO/4/ SH. 88	5M

b) Regional Agriculture Research Institute Bahawalpur

Output-1: Development of durable rust resistant variety with desirable yield and quality traits for southern Punjab.

Screening of wheat germplasm

Activity 1 & 2

According to the project seed of 100 lines from RARI, Bahawalpur was to be sent for screening. A set of wheat strains received from concerned components were planted at RARI, Bahawalpur. The data was recorded and shared with concerned components. The data of entries from RARI, Bahawalpur is given as under;

Year	No. of lines tested	Resistant		M-Resistant		susceptible	
		Lr	Yr	Lr	Yr	Lr	Yr
1 st	100	95	E*	3	E	2	E
2 nd	100	78	80	4	5	18	15
3 rd	100	84	92	9	5	7	3
4 th	100	85	87	4	7	11	6
5 th	100	83	81	8	12	9	7

(E* = Escape)

The detail is attached as Annexure I, II & III.

Molecular Genotyping (Activity-4)

In this activity, total 602 entries from RARI, Bahawalpur were sent for genotyping. Summary of genotyping of entries from RARI, Bahawalpur is as under;

Name of marker	2012-13		2013-14		2014-15	
	Present	Absent	Present	Absent	Present	Absent
Sr-2	160	42	163	37	177	23
Sr-25	186	12	180	20	192	8
Sr-26	195	7	191	9	177	23
Sr-31	190	12	193	7	192	8
Sr-36	192	10	186	14	197	3

Sr-39	197	5	135	65	199	1
Lr-34	198	4	147	53	190	10
Yr-10	194	8	138	62	192	8
Yr-18	198	4	158	42	191	9
Yr-29	191	11	147	53	191	9
Total	1905	115	1638	362	1898	102

Detailed data of entries from RARI, Bahawalpur is given in Annexure XXV, XXVII, XXIX.

Preliminary Wheat Yield Trials (Activity-5)

Total 104 entries were tested in preliminary wheat yield trial and selected entries were promoted to regular wheat yield trials. During 2009-10, 20 entries were tested for grain yield in one “A” trials, 6 genotypes viz; 99103, 99104, 99106, 99111, 99125 and 99129 gave better performance than the check varieties “Sehar-06” and Fareed-06. These entries were promoted for further testing. Detail is given in Annexure V-B.

During 2010-11, 22 entries were tested for grain yield in 2 “A” trials, 6 genotypes viz; 10B9369, 10B9370, 10B9390, 10B9381, 10B9383 and 10B9384 gave better performance than the check variety “Sehar-06”. These entries were promoted for further testing. Detail is given in Annexure VI-B.

During the year 2011-12, 22 entries were tested for grain yield in “A” trials, only two strains viz; 11B-2098 and 11B-2017, out yielded the best check (Aas-11). While 8 entries were promoted for further testing. Detail is given in Annexure VII-B.

During the year 2012-13, 20 genotypes with 2 checks i.e. “Mairaj-08” and “Aas-11” were put up in yield trials, only 3 genotypes viz; 12B2707, 12B2708 and 12B2717 out yielded the best check i.e. Aas-11 and were promoted to next year testing. Detail is given in Annexure VIII-B.

During the year 2013-14, 20 entries were tested in 2 preliminary wheat yield trials, 3 strains viz; 13B-3010, 13B-3009 and 13B-3020 gave better yield than both checks i.e. Aas-11 and Sehar-06. While five strains were further promoted to regular wheat trials (Annexure IX-B).

Regular Wheat Yield Trials (Activity-6)

This activity relates to evaluation of selected lines in regular wheat yield trials. A total of forty (40) strains were tested in these trials. A brief description is as under;

During 2010-11, thirteen entries were put in regular wheat yield trial, out of which only three (3) entries 10B-9384, 10B-9383, and 10B-9379 gave better yield (3850, 3550 and 3550 Kg/ha, respectively than all the three checks i.e. Mairaj-08, Fareed-06 and Sehar-06 (Annexure-X-B).

During the year 2011-12, fourteen strains were tested, out of which 2 strains (10B-9393 and 10B-2003) showed higher yield (5976 and 5956 kg/ha, respectively) than the best check Aas-11 with grain yield of 5918 kg/ha (Annexure-XI-B).

During 2012-13, ten (10) genotypes were tested in regular wheat yield trials, only two strains i.e. 12B-2604 and 12B-2043 gave respectively 5166 and 5138 kg/ha as compared with the check variety Aas-11 with grain yield of 5027 kg/ha (Annexure-XII-B).

During 2013-14, total 10 entries were tested in regular wheat yield trials, only one strain (13B-2717) gave better yield (8862 kg/ha) than both checks i.e. Aas-11 and Sehar-06 (Annexure XIII-B).

Micro Wheat Yield Trials (Activity 7)

During 2010-11, two strains i.e. 076422 and 088132 were put up in MWYT, 088132 showed better yield (4923 kg/ha) than Lasani-08 (check 1) but less than Sehar-06 (Check 2). Detailed results are given in Annexure-XIV-B.

During 2011-12, the strain 076346 gave better yield (6157kg/ha) than best check (Fsd-08 with 6143kg/ha grain yield) and was further tested in second year in National uniform wheat yield trial. Detailed results are given in Annexure-XV-B.

During 2012-13, two wheat strains (099172 and 099110) were evaluated in MWYT, both strains gave better yield (4038 and 3929 kg/ha respectively) than the check 'Punjab-11' which gave 3786 kg/ha (Annexure-XVI-B).

During 2013-14, two strains i.e. 11B-2049 and 11B-2074 were tested on Punjab level, both could not perform better than the check varieties (Annexure-XVII-B).

National Uniform Wheat Yield Trial (Activity 8)

During 2011-12, two wheat strains i.e. 076422 and 076346 were tested in national uniform wheat yield trial on 9 locations of southern Punjab. The strain 076346 gave yield of 6637kg/ha with 6% increase over the local check (Mairaj-08). (Annexure XIX-B)

During 2012-13, again these two strains i.e. 076346 and 076422 were included in national uniform wheat yield trials, the strain 076346 gave better yield averaged on 9 locations of southern Punjab and produced 5218kg/ha as compared to the local check with average grain yield of 4851kg/ha. (Annexure XX-B)

During 2013-14, three wheat genotypes i.e. 099114, 099346 and 10B-9384 were tested in NUWYT, all three strains gave better yield than the local check, while 099346 gave 9% better

yield than the local check with grain yield of 4998kg/ha and 4593kg/ha, respectively. This performance was on 9 locations of southern Punjab. (Annexure XXI-B)

Demonstration (Activity-9)

Demonstration plots were to be planted for two years on 5 locations in southern Punjab, detail of work done is given year wise as under;

First year (2013)

Sr.No.	Variety	Yield (kg/ha)					
		Multan	Khanpur	RARI, Bahawalpur	Jalla Arain, Lodhran	D.G.Khan	Chak 11BC, Bahawalpur
1	10B-9384	5300	5200	4850	3700	4270	4490
2	076422	5490	5450	4700	3900	4450	4490
3	Fareed-06	5170	5300	4950	3750	4190	4350
4	Mairaj-08	5400	5450	4700	3550	4300	4300

Second year (2014)

Sr.No.	Variety	Yield (kg/ha)				
		Multan	Khanpur	RARI, Bahawalpur	Jalla Arain, Lodhran	D.G.Khan
1	076346	5444	4650	3660	4400	5800
2	076422	4760	4255	3420	4550	5155
3	Aas-11	5222	4830	3385	5160	5260
4	099346	4860	4725	3680	4740	5655
5	Fareed-06	5300	4720	3700	4955	5720

Testing of wheat germplasm at Kenya (Activity 3 & 10)

First year (2010) the seed of 20 entries was sent to Kenya for screening against Ug-99 (a fungal disease). Out of which, 12 were found as resistant. Reaction of a few resistant genotypes is given as under;

Sr. No.	V-code	Stem rust
1	V-2862 (Aas-11)	15MS
2	076346 (Gold-16)	10MRMS
3	076422	10MSS
4	06377	10MSS
5	088148	10MSS
6	088186	10MSS

7	088200	10MSS
8	099199	20MSS

Detail is given in Annexure XXI-B

During 2011, 18 entries were sent, out of which 5 were resistant to Ug-99.

Sr. No.	V-code	Stem rust
1	099108	20M
2	099164	30M
3	099157	20MS
4	109384	30M
5	099104	40M

Detail is given in Annexure XXII

During 2012, 21 entries were sent for screening, 14 were found resistant, lines with better reaction are given below;

Sr. No.	V-code	Stem rust
1	11B-2024	10MR
2	11B-2049	5M
3	11B-2057	20MR
4	11B-2058	10MR
5	11B-2016	20M
6	11B-2061	10M

Detail is given in Annexure XXIII

(Note: One line was given as double star and two were allotted single star.)

During 2013, seed of 20 entries was sent to PM. Data of 14 lines was available. Out of 20 lines, only 4 were resistant. Reaction of a few better performing lines is given as under;

Sr. No.	V-code	Stem rust
1	11B-2024	20RMR
2	11B-2049	20M
3	11B-2057	40MR
4	11B-2061	40MS

Detail is given in Annexure XXIII-C

C) University of Agriculture Faisalabad

Output-1: Identification and validation of wheat germplasm/ segregating generations for rust genes at molecular level

1. Detailed component wise methodology adopted, data analyzed and results obtained (Attach raw data as annexure)

Primer Designing:

The primer sequences of published markers linked to wheat rust resistance genes Lr 34, Lr 46, Yr 10, Yr 18, Yr 29, Sr 2, Sr 25, Sr 26, Sr 31, Sr36, Sr 39 were identified and synthesized for further PCR analysis. List of primers is given below in Table. 2.

Table 2: List of primers used for evaluation of Wheat Rust Resistance

Serial No.	Primer Name	Primer Sequence
1	SR2-2F	AGCGCTCGAAAAGTCAG
2	SR2-2R	GGCAGGTCCAACCTCCAG
3	SR25-2F	CTTCACCTCCAAGGAGTTCCAC
4	SR25-2R	GCGTACCTGATCACCACCTTGAAGG
5	SR26-2F	AGCCGCGAAATCTACTTTGA
6	SR26-2R	TTAAACGGACAGAGCACACG
7	SR31-2F	TGGGATGCGAGAATATCCGG
8	SR31-2R	TGCGATGCCTAAAGCCTCTC
09	SR36-3F	ATGGTTTGTGTGTGTGTGTAGG
10	SR36-3R	AAACGCCCCAACCACCTCTCTC
11	SR39-1F	AGAGAAGATAAGCAGTAAACATG
12	SR39-1R	TGCTGTGATGAGAGGAACTCTG
13	LR34/YR18-1F	GTTGGTTAAGACTGGTGATGG
14	LR34/YR18-1R	TGCTTGCTATTGCTGAATAGT
15	LR46/YR29-1F	GGTCTTCTGGGCTTTGATCCTG
16	LR46/YR29-1R	GTTGCTAGGGACCCGTAGTGG
17	YR10/E2-3F	TGGTAGTAGAGTAATCGCAACA
18	YR10/E2-3R	TCTTCAGATTTGGAGGTAGG

Germplasm collection and DNA isolation

The seeds of 300 wheat genotypes were obtained from Wheat Research Institute (WRI), Faisalabad and 200 genotypes were obtained from Regional Agriculture Research Institute (RARI), Bahawalpur each year of project and 1564 genotypes in three years. The seeds of received genotypes/lines were planted in silica plates and kept under controlled temperature (20-25 °C) suitable for germination. At two leaf stage,

the leaf samples were used for DNA isolation. The DNA of all wheat genotypes were extracted using standard CTAB method. The DNA was dissolved in 1X TE buffer containing 16µg/ml RNAs-A to remove RNA and stored at -20 °C for downstream use.

For quantification of DNA, DNA samples were run on 1% agarose gel. Reference markers (1 kb DNA ladder and Lambda/HindIII DNA marker) were also run in the gel to estimate the quantity of DNA in the sample. The electrophoresed agarose gels were examined under ultra violet Transilluminator and images were captured on UVP® Gel Documentation System. The gel images of all DNA samples were analyzed in computer software “Image J” to quantify the amount of DNA in all samples as already mentioned. The Quantity of DNA was normalized to 15ng/ul for each sample make PCR analysis easier. The DNA dilutions were made in 96 well PCR plates.

PCR analysis of rust resistance markers

The DNA of all wheat genotypes was analyzed against rust markers. The PCR mixture was prepared in 96 well PCR plates and PCR was performed in Bio-Rad thermocycler. The recipe for 25 µl PCR reaction mixture is described below in the Table 3.

Table 3: Recipe for PCR mixture used for rust marker analysis

Sr. No	Ingredients	Concentration	Volume
1	DNA template	15 ng/µl	3.0 µl
2	<i>Taq</i> Buffer	10 X	2.5 µl
3	MgCl ₂	25 mM	2 µl
4	Forward Primer	50 ng/µl	0.5-1 µl
5	Reverse Primer	50 ng/µl	0.5-1 µl
6	DNTPs	10 mM	0.5 µl
7	<i>Taq</i> Polymerase	2.5 U/µl	0.5 µl
8	Deionized water (D3 H ₂ O)	D3 H ₂ O	14.5-15.5 µl
	Total		25 µl

The PCR amplification was carried out in thermal cycler (Bio-Rad C-100 and C-1000 thermal cycler) using the following profile

Thermal Cycler Profile for PCR analysis of Rust Marker genes

Initial Denaturation: 95 °C for 5 min

Loop 1: 10 cycles

Denaturation: 94 °C for 1 min

Annealing: 65°C for 1 min (Increment -1°C/cycle, Ramp to 72°C at 0.5°C/second)

Elongation: 72°C for 1 min

Loop 2: 30 cycles

Denaturation: 94 °C for 1 min

Annealing: 50-58 °C for 1 min (may be adjusted during condition optimization for each primer pair)

Elongation: 72 °C for 1 min

Final Extension: 72 °C for 5 min (1 time)

Hold 8 °C

Data analysis

The amplified PCR products were electrophoresed on agarose gel (2%) stained with Ethidium Bromide. Scoring was done on the basis of presence (+ sign) and absence (- sign) of band in the gel for each sample. Presence (+ sign) of band showed the presence of resistance marker/gene in the genotype while absence (- sign) band represents the absence of resistance marker/ gene. The results of all wheat genotypes representing presence of amplified bands are resistant to the specific marker genes.

Results of three year activities are given in annexure 24 to annexure 29.

Marker	2012-13 1st year		1st year 2012-13		year2- 2013-14		year-2-2013-14	
	Present	Absent	Present	Absent	Present	Absent	Present	Absent
Sr2	304	56	160	42	163	37	249	53
Sr25	349	11	190	12	180	20	287	15
Sr26	348	12	195	7	191	9	287	15
Sr31	355	5	189	13	193	7	259	43
Sr36	347	13	192	10	186	14	294	8
Sr39	356	4	197	5	135	65	191	111
Lr34	340	20	198	4	158	42	247	55
Lr46	343	17	191	11	147	53	261	41
Yr10	354	6	194	8	138	62	251	51
Yr18	340	20	198	4	158	42	247	55
Yr29	343	17	191	11	147	53	261	41
Total	360		202		200		302	

2. Component wise salient achievements

Wheat genotypes (1564 genotypes= 962 from WRI and 602 from RARI) were characterized for rust resistant markers. Presence of Sr, Lr and Yr genes in each genotype was recorded and submitted to PM for use in the breeding program.

3. Overall progress of the problem searched

Characterization of 1564 wheat genotypes obtained from collaborating institutes (WRI and RARI) was done successfully for Sr, Lr and Yr. These results were provided to them so that utilized in the breeding program for development of rust resistant line and varieties through gene pyramiding.

4. Varieties, breeds, vaccines or products developed and patented

The wheat advanced line V-12304 has completed all the stages of testing and is the final stage of approval

A wheat variety, Gold-16 has been approved and released for general cultivation in southern Punjab. Summarized results of the variety are given as under;

Resistance against rusts

The variety showed good resistance/tolerance against all three types of rusts (Lr, Yr and Sr) except Ug-99 (a new race of stem rust). Detailed results are given in Annexure XXII-B and XXIII-B.

Yield Potential

The variety performed better in preliminary, advance, MWYT and NUWYT trials. Detail evaluation is presented in Annexure V-B, VI-B, VII-B, VIII-B, XIX-B (preliminary yield trial results), X-B, XI-B, XII-B, XIII-B (advance yield trial results), XIV-B, XV-B, XVI-B, XVII-B (MWYT results), XVIII-B, XIX-B, XX-B and XXI-B (NUWYT results).

Quality evaluation

Quality evaluation tests were performed at NARC, Islamabad. Results of 2 years evaluation are given as under:

Quality evaluation during 2011-12						
Sr.No.	Genotype	1000 grain wt. (g)	Protein (%age)	Gluten (%age)		Test weight
				Dry	Wet	
1	V-076346 (Gold-16)	39	13.42	6.80	17	75
2	Galaxy-13	38	12.59	7.3	18	75
Quality evaluation during 2012-13						
1	V-076346 (Gold-16)	38	14.40	9.88	25	69
2	FSD-08	34	13.30	7.8	21	73

5. No. of national and international papers published

1. Khan, A.A., H.M.N. Cheema, Z. Ali, U. Aslam, M. Hussain and I. Karim. 2016. Genetic variance, population structure and gene flow among *Triticum aestivum* for rust resistance. Pak. J. Agric. Sci. 0: 00-00. (In review)

6. No. of Ph.D/M.Phil. produced

1. Mr. Ihsan Karim (2007-ag-2657) has completed his MSc(Hons) PBG thesis research "Genetic diversity analysis of wheat accessions for rust resistance" from this project

7. Any other achievement

The material has been developed which will be utilized in breeding programme future

8. Current status of commercialization of the project. How many stakeholders adopted this technology along with monetary benefits

The wheat variety developed. The seed of this variety is being distributed among the farmers through Punjab Seed Corporation..

9. impact of the project on strengthening of the institutional infrastructure, machinery, equipment and human resources

Results of this project have provided facilities for further research and breeding program on wheat rust. Refrigerated centrifuge, Micropipettes, Ice flake machine, water double distillation unit was purchase in this project which have strengthen the infrastructure for future research, projects, training and postgraduate students research of the university.

Two research associates, one MSc (Hons) and many graduate students were trained from this project which is most productive part for human resource development.

10. Constraints in the:

- (a) Implementation of the project**
- (b) Commercialization of the project**

11. Suggestions for future research and development

Wheat genotype and germplasm should evaluated for rust genes and pyramiding of these gene through breeding will be carried out continuously to beat the rusts as new rust strains are being reported.

Differential for recent strains should be developed to identify the rust strains prevailing at wheat farms. For this purpose, molecular techniques for strain identifications can also be the best solution because of low cost, efficient and time saving.