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ANNUAL PROGRESS REPORT

FY (2019-20)

Agricultural Biotechnology Research Institute, AARI, Faisalabad.

TABLE OF CONTENTS

List of tables

List of Figures

OVERVIEW

All living organisms have the ability to improve themselves through natural means in order to adapt to changing environmental conditions. However, it takes hundreds of years before any detectable improvement is obtained. Man then learned how to domesticate and breed plants in order to develop crops to his own liking and needs using various means including biotechnology. Biotechnology is defined as a set of tools that uses living organisms (or parts of organisms) to make or modify a product, improve plants, trees or animals, or develop microorganisms for specific uses. Agricultural biotechnology is the term used in crop and livestock improvement through biotechnology tools. This monograph will focus only on agricultural crop biotechnology. Biotechnology encompasses a number of tools and elements of conventional breeding techniques, bioinformatics, microbiology, molecular genetics, biochemistry, plant physiology, and molecular biology. The biotechnology tools that are important for agricultural biotechnology include: Conventional plant breeding, Tissue culture and micro-propagation, Molecular breeding or marker assisted selection, Genetic engineering and GM crops, Molecular Diagnostic Tools and Soil Bacteriology. Agricultural Biotechnology Research Institute is improving major cash crops including cereals, fiber crops, sugarcane, fodders, oilseeds, pulses and vegetables using the above mentioned techniques. ABRI is also helping other institutes of AARI in conducting their research programs like detection of rust resistant genes in wheat through molecular markers, testing of biotech crops, incorporation of genes of various stresses in crops through genetic engineering like Roundup Ready gene, production of better somaclones, assessment of genetic diversity on different cross pollinated crops, screening of different genotypes for quality related genes, disease free seed multiplication etc. ABRI also has Soil Bacteriology Section which deals with microbial biotechnologies for restoration of soil and plant health. During 2019-20, more than 120 internship students were trained in various fields of biotechnology whereas many M.Sc. and Ph.D. students carried out part of their thesis research at this institute. Soil Bacteriology Section deals with beneficial microbial restoration of soil and plant health. Soil microbes exert positive effects on plant growth through biological N2-fixation, P-solubilization, production of growth hormones, antibiotics and siderophore etc. Soil Bacteriology Section also functions as Bio-fertilizer Testing Lab and works on Soil-microbe-plant interactions are the main areas of research. During the year, 2019-20, 12,180 inoculants of different Rhizobium sp and PGPR were supplied to the farmers.

RESEARCH WORK

1. CRISPR/CAS9-mediated knock out of FON4 gene in rice for induction of multi-floret character

The objective of this experiment was the knocking out of the FON_4 gene in rice which inhibits the multi-floret determination in rice. The protocol for regeneration of rice was optimized by using different concentration of plant hormones. Vector pC1300-Cas9 was used for edited FON4 gene of rice (Fig 1) and target site was selected and its primers were synthesized (Fig 2) for the knocking of FON4 gene in rice.

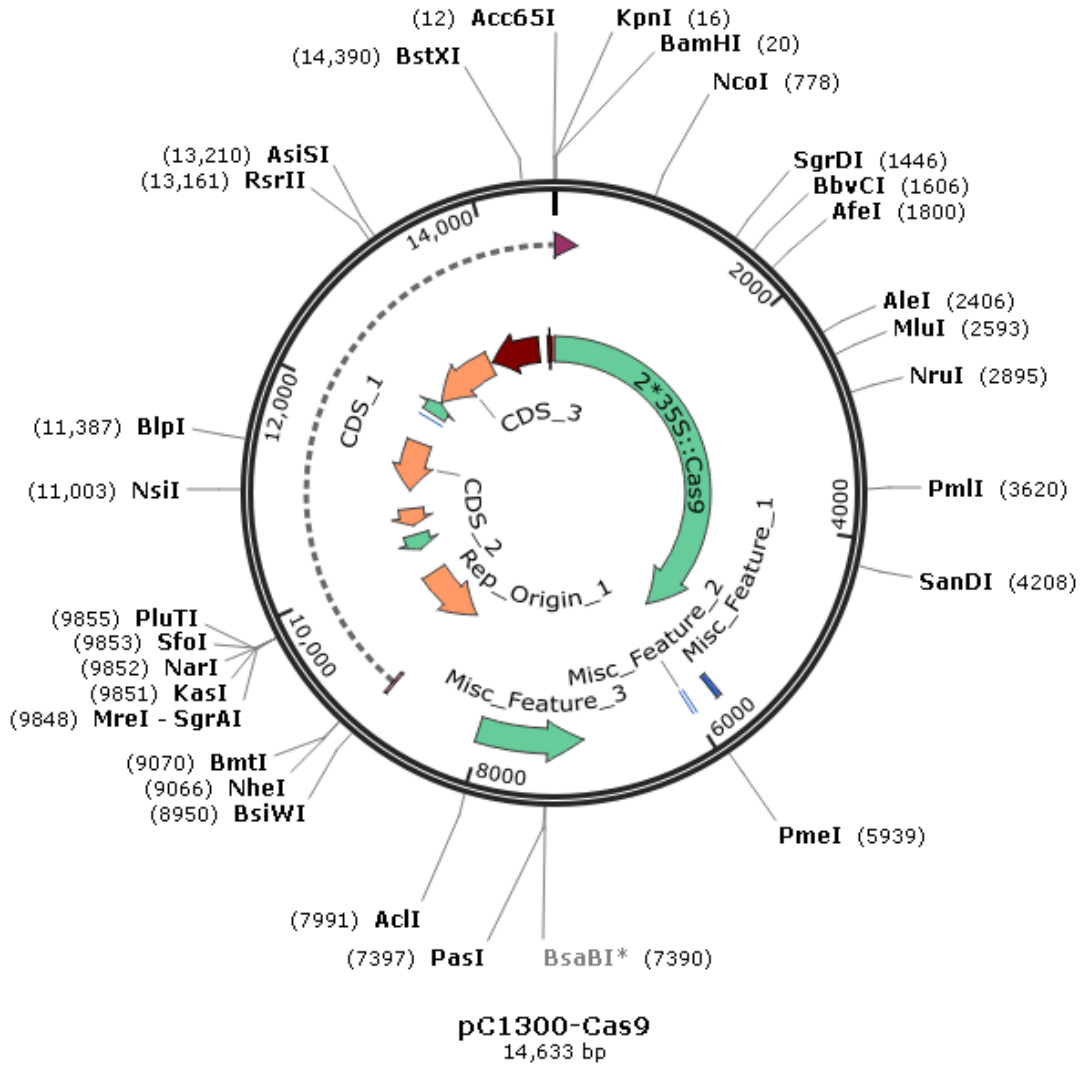


Fig 1. The CRISPR/Cas09 vector pC1300-Cas9 for editing of FON4 gene in rice. The vector indicates the different restriction site and guide RNA (gRNA) attachment site.

Target sites and its primers

Target 1 (in exon 2)
GGTGACCAGAGGCCAGTCCCGG

1st
F Primer: ggcaGGTGACCAGAGGCCAGTCC
R Primer: aaacGGACTGGCCTCTGGTCACC

Target 2 (in exon 3)
ATGAGAGAGATAGACTCAAAGG

2nd
F Primer: ggcaATGAGAGAGATAGACTCAA
R Primer: aaacTTGAGTCTATCTCTCTCAT

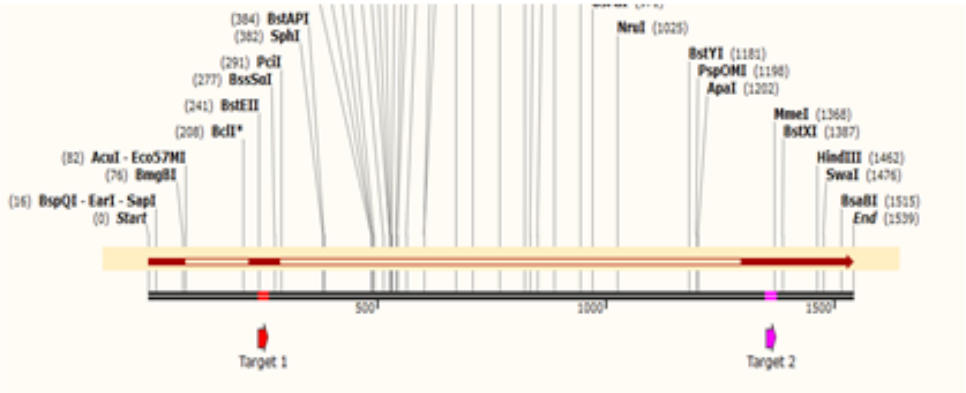


Fig 2. The Target site of FON4 gene of rice and its primers.

However the experiment was later on shifted to brassica as GMOs are not allowed in cereals hence crop and gene was shifted.

2. Assessment of the genetic diversity and drought and heat tolerance status of soybean genotypes

The objective of this experiment was to assess genetic variation in 100 genotypes of soybean by using 35 DNA markers which can accelerate soybean breeding program to improve quality and yield traits. A total of 100 soybean genotypes seeds/leaf samples were collected from Director Oilseed Research Institute for genetic diversity Assessment as detailed below in Table 1. DNA extraction was performed using standard CTAB method and was quantified using Nanodrop spectrophotometer. The quality of extracted DNA was also assessed by loading DNA 20 ng/ µl on 0.8% (w/v) agarose gel stained with ethidium bromide. All the DNA extracts were stored at -40°C for further use.

Table 1. List of Soybean genotypes used in the study.

Sr. No.	Name	Sr. No.		Sr. No.	Name
1.	E-1467	34.	E-358	67.	HS-17
2.	SBL	35.	MS-5	68.	ESSEA
3.	SL-4	36.	Pershing	69.	Chasie
4.	SS-129	37.	E-302	70.	Fabalon
5.	Kura	38.	Aust-94-2	71.	FS-85
6.	PKN-38-2-1	39.	Faisal Soybean	72.	E-1216
7.	HS-16	40.	No. 8	73.	Ford
8.	Harper	41.	S-39-40	74.	Davis
9.	UDA	42.	E-1097	75.	Adams
10.	141-PKN-252-8	43.	SS-129	76.	Sueshine
11.	SK-5	44.	E-402	77.	AGS-66
12.	Hong Kong	45.	E81-77	78.	SEMMES
13.	Columbus	46.	R-313	79.	Braton
14.	E-1336	47.	HB-17	80.	ADA
15.	Bay	48.	E-1490	81.	AKKMESS
16.	PKN-140-3-1	49.	F-Soybean	82.	Clay
17.	NO-37	50.	HM-8468	83.	Lakota
18.	T18-50-32	51.	Ajmeri	84.	6GG
19.	Desto	52.	HM-2437	85.	Gail
20.	A-4268	53.	249-313-D	86.	A-3127
21.	TN81-142	54.	Elazarz-20	87.	Lorsoy
22.	E-976	55.	E-1092	88.	MS-4
23.	LEE	56.	Lafe-40	89.	E-418
24.	TN-77 (iii)	57.	BSR-301	90.	Carlin
25.	UFV-1	58.	CN-5	91.	Amsoy
26.	Aksar Bean	59.	FS-10	92.	KWARYGYO
27.	CH-96	60.	S-11-1274	93.	MS-6
28.	1	61.	TN-81-27-32	94.	Craw-Ford
29.	R X (14-16-1)	62.	SPK-16	95.	FS-60
30.	TD-321	63.	DGS=16	96.	Bossier
31.	Perry	64.	95-2	97.	Willikin
32.	E-1284	65.	95-1-14	98.	Early Shoot Foot
33.	E-1406	66.	MCH-5	99.	Hack
34.		67.		100.	Cutlos

PCR reaction was conducted in thermal cycler with total reaction volume of 25 µl including 20 ng/µl genomic DNA of each variety, 0.6 µM of each forward and reverse primers and 12 µl of green master mix for different SSR markers. The following temperature conditions were applied for amplification: initial denaturation 94ºC for 5 minutes, 50 cycles of denaturation 94 ºC for 1 minute, annealing at variable temperatures according to primers for

1 minute (Table 2), extension at 72 ºC for 1 minute. Final extension at 72 ºC for 7 minutes. The amplified products were stored at 4ºC.

A total of Thirty two SSR markers were used to assess the genetic diversity among 100 soybean genotypes. Among these SSR markers highest number of alleles were amplified by Satt-411 (19) all of which were polymorphic similarly other discriminating markers were BE806308, Satt-163, Satt-184, Satt-687, Satt-126, Satt-310 with 18, 17, 17, 17, 15 and 15 alleles respectively. All of these alleles were polymorphic. Only one SSR marker Sat-320 amplified 08 alleles among which 06 were monomorphic and 02 were polymorphic whereas rest al the markers amplified polymorphic alleles (Table 2).

Table 2. Polymorphism of all SSR markers used in the study.

Primer Name	Polymorphic Status	NoA	PA	PIC	TA
BE806308	Polymorphic	18	18	0.94	55
Satt-126	Polymorphic	15	15	0.91	55
Satt-127	Polymorphic	12	12	0.87	55
Satt-129	Polymorphic	4	4	0.74	55
Satt-155	Polymorphic	8	8	0.85	55
Satt-163	Polymorphic	17	17	0.93	55
Satt-164	Polymorphic	7	7	0.80	55
Satt-184	Polymorphic	17	17	0.89	55
Satt-200	Polymorphic	10	10	0.87	55
Satt-218	Polymrophic	6	6	0.75	55
Satt-230	Polymorphic	8	2	0.84	55
Satt-252	Polymorphic	14	14	0.91	55
Satt-310	Polymorphic	15	15	0.90	55
Satt-362	Polymorphic	8	8	0.78	55
Satt-396	Polymorphic	11	11	0.87	55
Satt-406	Polymorphic	11	11	0.88	55
Satt-409	Polymorphic	8	8	0.87	55
Satt-411	Polymorphic	19	19	0.90	55
Satt-687	Polymorphic	17	17	0.90	55
Satt-557	Polymorphic	14	14	0.86	55
Satt-666	Polymorphic	11	11	0.88	55
Satt-717	Polymorphic	11	11	0.84	55
Satt-517	Polymorphic	11	11	0.90	55
Satt-484	Polymorphic	6	6	0.80	55
Satt-309	Polymorphic	14	14	0.90	55
Satt-160	Polymorphic	13	13	0.90	55
Satt-005	Polymorphic	5	5	0.70	55
Satt-171	Polymorphic	8	8	0.85	55
Satt-173	Polymorphic	14	14	0.91	55

Satt-183	Polymorphic	8	8	0.86	55
Satt-285	Polymorphic	7	7	0.80	55
Satt-307	Polymorphic	7	7	0.98	55

To examine the organization of genetic diversity within 100 soybean accessions, UPGMA (Unweighted Pair Group of Arithmetic Averages) based cluster analysis was conducted based on genomic-SSR data. For this purpose, Similarity matrix was first generated according to SHAN similarity index, followed by the dendrogram construction (Fig 4). The population was broken in to seven major groups representing strong clustering patterns with similarity co-efficient ranging from 0.61 to 0.90. Maximum 28 genotypes were found in Cluster IV followed by Cluster V with 25 genotypes, Clsuter I with 24 genotypes, Cluster II with 13 genotypes and Cluster VI with 6 genotypes. Minimum genotypes were found in cluster III with 4 genotypes i.e. ESSEA, E-1216, Adams and Chasie (Table 3). Further genetic similarity coefficients of genotypes are given in excel file for more detailed information about the genetic relatedness of genotypes.

Table 3. Cluster wise distribution of 100 soybean accessions

Cluster Name	Number of genotypes	Genotypes Name
Cluster 1	24	E1467, PKN-38-21, UDA, 141-PKN-2528, SK-5, Hong Kong, LEE, Columbus, E-1336, HS-16, Bay, PKN-14031, NO-37, Desto, A-4268, T-1850032, TN-77(iii), TN-81142, E-976, SBL, SL-4, SS-129, Harper, Kura
Cluster II	13	UFV-1, Aksar-Bean, CH-96, I, TD-321, RX-14161, Perry, Faisal-Soybean, No.8, E-358, MS-5, Pershing, E-302
Cluster III	4	ESSEA, E-1216, Adams, Chasie
Cluster IV	28	S-3940, E-402, SS-129, E-1490, E-1097, E-8177, HM8468, R-313, Ajmeri, E-1092, 249-313-D, Davis, HM-2437, Lafe-40, Elazar-20, BSR-301, SPK-16, FS-10, 95-114, FS-85, TN-812732, Fabalon, DGS-16, 952, MCH-5, CN-5, Ford, HS-17
Cluster V	25	SueShine, 6GG, Gail, Braton, AKKMESS, E-1284, Ausr-982, FSoybean, HB-17, E-1406, Bossier, Willikin, Early Shoot Foot, Hack, Cutlos, AGS-66, A-3127, MS-4, E-418, Carlin, Amsoy, KWARYGYO, MS-6, FS-60,
Cluster VI	6	SEMMES, Craw Ford, Lorsoy, ADA, Clay, Lakora, S-111-274

The genetic diversity was assessed among 100 soybean genotypes using 33 polymorphic SSR markers. The genotypes were divided to seven distinct groups as is evident from the structure analysis give in the Fig 3.

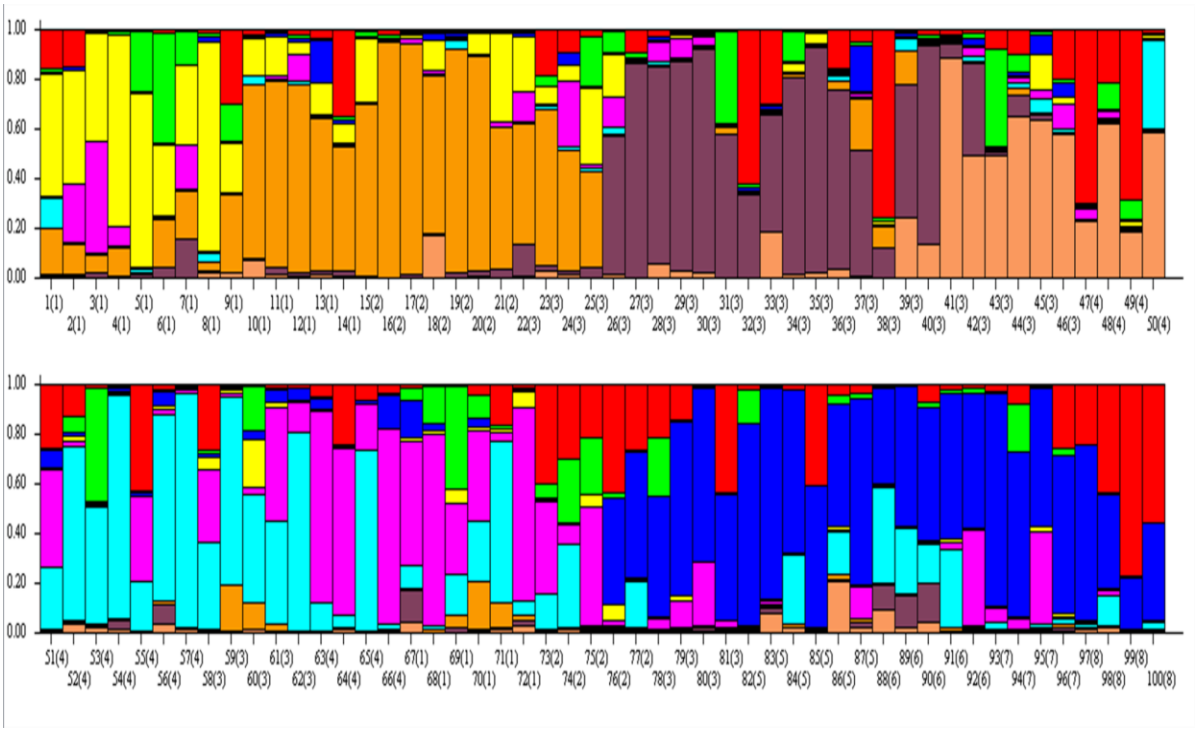


Fig 3. The structure analysis of 100 soybean accessions showing the genetic relatedness and genetic differences sketched using the binary data of 33 polymorphic SSR markers

For assessment of drought and heat tolerance status of 100 soybean accessions/genotypes leaves were collected from Oilseed Research Institute, AARI, Faisalabad and DNA was extracted PCR was completed with 10 SSR markers. However new gene linked DNA markers were searched and sent to supplier for synthesis and experiment is still under progress.

3. Identification of Cytoplasmic male sterile lines and maintainer lines in onion genotypes

The experiment was aimed to screen 210 plants of 21 genotypes (10 plats from each genotypes) to identify cytoplasmic male sterile and maintainer lines using linked DNA markers.

For this purpose leaves of 210 plants belonging to 21 genotypes of onion were collected from Vegetable Research Institute, AARI, and Faisalabad. The DNA was extracted and all 210 plants were surveyed using 01 DNA marker ORF-725 for identification of cytoplasmic type. The results indicated that 146 plants possessed Normal cytoplasm (N), whereas

remaining 64 plants possessed sterile cytoplasm 38 of which belonged to T cytoplasm and 26 to S cytoplasm type. However assessment of nuclear genes for male fertility/sterility remained in progress. The detailed results are given below in Table 4.

Table 4. The characterization of cytoplasm type in 21 genotypes (210 plants) through PCR based identification methods.

Sr. No.	G. Name	Plant No.	Cyto Type	Sr. No.	G. Name	Plant No.	Cyto Type
1	Golden ORB	P1	N	106	Kessar	P6	T
2	Golden ORB	P2	N	107	Kessar	P7	S
3	Golden ORB	P3	N	108	Kessar	P8	T
4	Golden ORB	P4	N	109	Kessar	P9	T
5	Golden ORB	P5	N	110	Kessar	P10	T
6	Golden ORB	P6	N	111	Mirpurkhas S_3	P1	N
7	Golden ORB	P7	N	112	Mirpurkhas S_3	P2	N
8	Golden ORB	P8	N	113	Mirpurkhas S_3	P3	N
9	Golden ORB	P9	N	114	Mirpurkhas S_3	P4	N
10	Golden ORB	P10	N	115	Mirpurkhas S_3	P5	N
11	White Pearl	P1	N	116	Mirpurkhas S_3	P6	N
12	White Pearl	P2	N	117	Mirpurkhas S_3	P7	N
13	White Pearl	P3	N	118	Mirpurkhas S_3	P8	N
14	White Pearl	P4	T	119	Mirpurkhas S_3	P9	N
15	White Pearl	P5	N	120	Mirpurkhas S_3	P10	N
16	White Pearl	P6	N	121	Desi Red S_4	P1	N
17	White Pearl	P7	N	122	Desi Red S_4	P2	N
18	White Pearl	P8	N	123	Desi Red S_4	P3	N
19	White Pearl	P9	N	124	Desi Red S_4	P4	N
20	White Pearl	P10	N	125	Desi Red S_4	P5	N
21	Red Imposta S_3	P1	N	126	Desi Red S_4	P6	N
22	Red Imposta S_3	P2	N	127	Desi Red S_4	P7	N
23	Red Imposta S_3	P3	N	128	Desi Red S_4	P8	N
24	Red Imposta S_3	P4	S	129	Desi Red S_4	P9	N
25	Red Imposta S_3	P5	N	130	Desi Red S_4	P10	N
26	Red Imposta S_3	P6	N	131	Nasarpuri S_4	P1	N
27	Red Imposta S_3	P7	N	132	Nasarpuri S_4	P2	N
28	Red Imposta S_3	P8	N	133	Nasarpuri S_4	P3	N
29	Red Imposta S_3	P9	N	134	Nasarpuri S_4	P4	N
30	Red Imposta S_3	P10	N	135	Nasarpuri S_4	P5	N

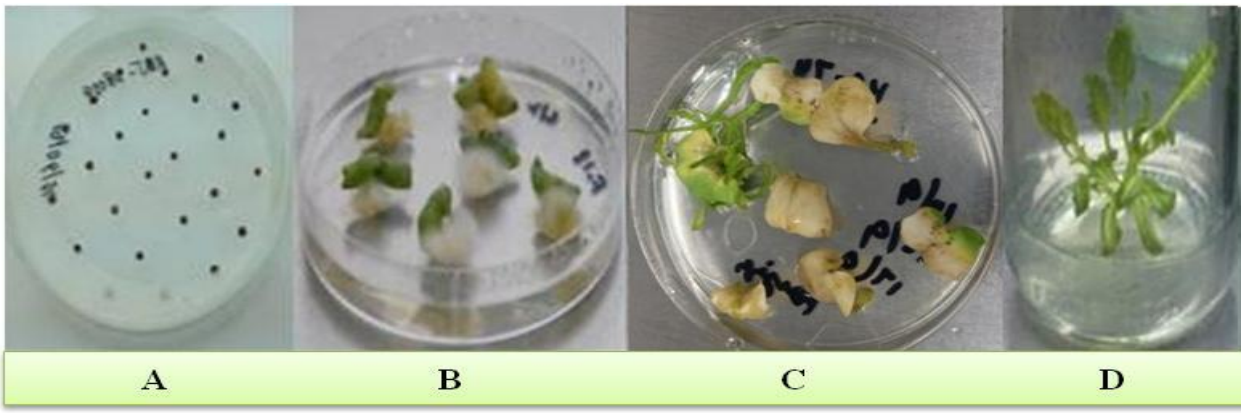
31	Phulkara	P1	N	136	Nasarpuri S_4	P6	N
32	Phulkara	P2	N	137	Nasarpuri S_4	P7	N
33	Phulkara	P3	N	138	Nasarpuri S_4	P8	N
34	Phulkara	P4	N	139	Nasarpuri S_4	P9	N
35	Phulkara	P5	N	140	Nasarpuri S_4	P10	N
36	Phulkara	P6	N	141	Sultan	P1	N
37	Phulkara	P7	N	142	Sultan	P2	N
38	Phulkara	P8	N	143	Sultan	P3	N
39	Phulkara	P9	N	144	Sultan	P4	N
40	Phulkara	P10	N	145	Sultan	P5	N
41	VRIO-2	P1	N	146	Sultan	P6	N
42	VRIO-2	P2	N	147	Sultan	P7	N
43	VRIO-2	P3	N	148	Sultan	P8	N
44	VRIO-2	P4	N	149	Sultan	P9	N
45	VRIO-2	P5	N	150	Sultan	P10	N
46	VRIO-2	P6	S	151	VRIO-10	P1	N
47	VRIO-2	P7	N	152	VRIO-10	P2	N
48	VRIO-2	P8	S	153	VRIO-10	P3	N
49	VRIO-2	P9	N	154	VRIO-10	P4	N
50	VRIO-2	P10	S	155	VRIO-10	P5	N
51	Glory	P1	S	156	VRIO-10	P6	N
52	Glory	P2	S	157	VRIO-10	P7	N
53	Glory	P3	S	158	VRIO-10	P8	N
54	Glory	P4	S	159	VRIO-10	P9	N
55	Glory	P5	T	160	VRIO-10	P10	N
56	Glory	P6	S	161	Desi Red	P1	N
57	Glory	P7	S	162	Desi Red	P2	N
58	Glory	P8	N	163	Desi Red	P3	N
59	Glory	P9	S	164	Desi Red	P4	N
60	Glory	P10	S	165	Desi Red	P5	N
61	1122 VRI	P1	S	166	Desi Red	P6	N
62	1122 VRI	P2	S	167	Desi Red	P7	N
63	1122 VRI	P3	S	168	Desi Red	P8	N
64	1122 VRI	P4	N	169	Desi Red	P9	N
65	1122 VRI	P5	S	170	Desi Red	P10	N
66	1122 VRI	P6	S	171	HIKE	P1	T
67	1122 VRI	P7	S	172	HIKE	P2	T
68	1122 VRI	P8	S	173	HIKE	P3	T
69	1122 VRI	P9	S	174	HIKE	P4	T
70	1122 VRI	P10	S	175	HIKE	P5	T
71	Yellow S_3	P1	N	176	HIKE	P6	T
72	Yellow S_3	P2	N	177	HIKE	P7	T
73	Yellow S_3	P3	N	178	HIKE	P8	T

74	Yellow S_3	P4	N	179	HIKE	P9	T
75	Yellow S_3	P5	N	180	HIKE	P10	N
76	Yellow S_3	P6	N	181	White Pearl VRI	P1	N
77	Yellow S_3	P7	N	182	White Pearl VRI	P2	N
78	Yellow S_3	P8	N	183	White Pearl VRI	P3	N
79	Yellow S_3	P9	N	184	White Pearl VRI	P4	N
80	Yellow S_3	P10	N	185	White Pearl VRI	P5	N
81	VRIO-6	P1	N	186	White Pearl VRI	P6	N
82	VRIO-6	P2	N	187	White Pearl VRI	P7	N
83	VRIO-6	P3	N	188	White Pearl VRI	P8	N
84	VRIO-6	P4	N	189	White Pearl VRI	P9	N
85	VRIO-6	P5	N	190	White Pearl VRI	P10	T
86	VRIO-6	P6	T	191	Sultan Ad	P1	T
87	VRIO-6	P7	N	192	Sultan Ad	P2	T
88	VRIO-6	P8	N	193	Sultan Ad	P3	T
89	VRIO-6	P9	N	194	Sultan Ad	P4	T
90	VRIO-6	P10	N	195	Sultan Ad	P5	T
91	T.E.G VRI	P1	N	196	Sultan Ad	P6	T
92	T.E.G VRI	P2	N	197	Sultan Ad	P7	T
93	T.E.G VRI	P3	N	198	Sultan Ad	P8	T
94	T.E.G VRI	P4	N	199	Sultan Ad	P9	T
95	T.E.G VRI	P5	N	200	Sultan Ad	P10	T
96	T.E.G VRI	P6	N	201	HON300A	P1	T
97	T.E.G VRI	P7	N	202	HON300A	P2	T
98	T.E.G VRI	P8	N	203	HON300A	P3	T
99	T.E.G VRI	P9	N	204	HON300A	P4	T
100	T.E.G VRI	P10	N	205	HON300A	P5	T
101	Kessar	P1	S	206	HON300A	P6	T
102	Kessar	P2	T	207	HON300A	P7	S
103	Kessar	P3	T	208	HON300A	P8	T
104	Kessar	P4	S	209	HON300A	P9	T
105	Kessar	P5	T	210	HON300A	P10	S

4. Integration of modified GT gene in *Brassica juncea* through agrobacterium mediated transformation method

Integration of modified GT gene in brassica was planned for the development of glyphosate herbicide resistance for the effective weeds control. The treatment plan was comprised of two brassica genotypes RBJ-96026 and KS-74, the targeted gene was EPSPS whereas pB7WG2D,1 and pK2GW7,D were the expression vectors used and LBA4404 agrobacterium strain was used for genetic transformation of the brassica.

13573, four days old cotyledonary leaf petioles were inoculated with Agrobacterium strain LBA4404 having modified GT gene with selection marker. 277 antibiotic resistant shoots were screened on selection media containing respective antibiotic (PPT @ 3mg/L & Kanamycine @ 50 mg/L.81 putative antibiotic resistant plantlets were survived on regeneration and rooting media containing antibiotic for selection 26 putative transgenic plants were developed. All putative transgenic brassica plants were subjected to glyphosate assay and five plants found positive. Previously found positive seven plants seeds T0 were grown in pots however, in next generation none of the plant was found positive in PCR. The procedure for tissue culture of brassica for genetic transformation is elaborated in Fig 4.



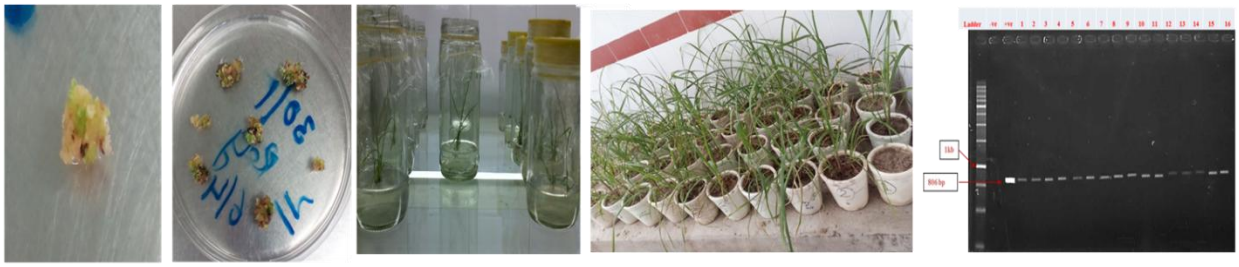
(A) Brassica seeds for Explant
(B) Regenerated cotyledonary petioles
(C) Antibiotic resistant shoots
(D) Plantlets

Fig 4. The overview of the tissue culture procedure of Brassica for genetic transformation.

5. Genetic transformation of herbicide (glyphosate) resistant gene (EPSPS) in sugarcane

The objective of this experiment is the development of herbicide tolerant sugarcane plants through genetic engineering. The treatment plan was comprised of two sugarcane genotypes CPF-246 and CPF-249, the targeted gene was EPSPS whereas pB7WG2D,1 and pK2GW7,D were the expression vectors used and LBA4404 agrobacterium strain was used for genetic transformation of the sugarcane.

2625 three Weeks old calli were inoculated with Agrobacterium strain LBA4404 having modified GT gene with selection marker. 239 putative antibiotic resistant plants were screened on selection media containing Kanamycin @ 50 mg/L. 97 putative antibiotic resistant plantlets were survived on regeneration and rooting media containing antibiotic for selection. 86 putative transgenic plants were developed and their confirmation was under process. Previous year two month old putative sugarcane transgenic plants were subjected to herbicide (Glyphosate) spray trial @ 1900 ml/acre. Observations were recorded for 40 days on regular basis and 4 herbicide tolerant plants were observed and their confirmation via PCR is under process. The procedure for tissue culture of sugarcane for genetic transformation is elaborated in Fig 4.



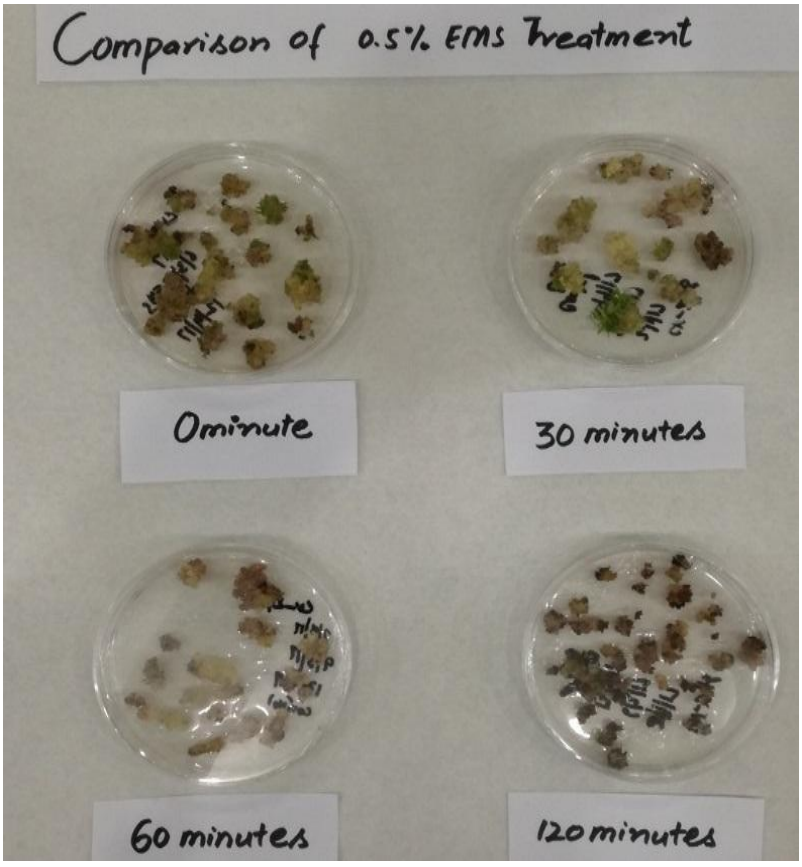
(A) Inoculated calli
(B) Screening of regenerated shoots
(C) Antibiotic resistant plants in pots
(D) Transgene confirmation through PCR

Fig 5. The overview of the tissue culture procedure of sugarcane for genetic transformation.

6. Mutagenic effect on sugarcane treated by EMS for drought tolerance

The objective of the experiment was to develop drought tolerant mutant plants of sugarcane through somaclonal variation in combination with mutagenesis. 3-4 weeks old embryogenic calli were treated with EMS (0.5%) for 120 min (selected on previous year results basis). Screened EMS treated calli on selection media supplemented with 10 % PEG (selected on previous year results basis) as shown in Fig 6.

5650 sugarcane spindles (inner most leaf) were cultured for callus induction. Four weeks old (2840 calli) were treated with 0.5% EMS for 120 minutes. 236 survived regenerated calli were transferred to regeneration and multiplication media supplemented with PEG (6000). 147 plants were shifted to pots for hardening. 168 putative drought tolerant plantlets were transferred to pots for hardening and Physiological and molecular analysis.



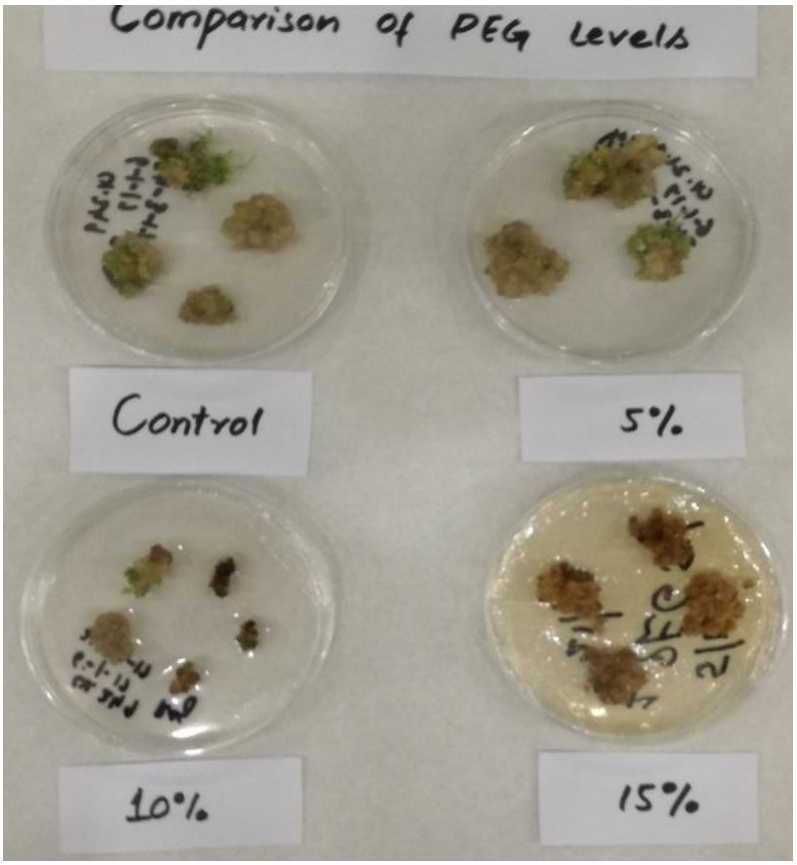


Fig 6. Mutagenic effect of EMS on sugarcane calli treated with 0.5% concentration for different period of time i.e. o minutes, 30 minutes, 60 minutes and 120 minutes and selection on different levels of PEG.

7. Testing of biotech crops

This experiment is aimed at testing of different genetically modified crops for their approval by National Biosafety committee or some or intended for import and export purpose. 901 samples of different crop plants i.e. Rice, Maize, Brassica, Chickpea, Sorghum sudan grass, Peas, onion, beet, chilies, bitter gourd, bottle gourd, ridge gourd, sponge gourd, pumpkin, spinach, watermelon, tar, brinjal, sunflower, cauliflower, cabbage, okra, raddish, turnip, bajra, tomato, cucumber, squash, beans, finecut grass, cloves, sweet pepper, hot pepper etcwere analyzed for two GM elements (35S Promoter & NOS Terminator). A total of 174 entries were tested through strip and PCR for four genes i.e. Cry1Ac, Cry2Ab, Vip3Aa and EPSPS (Fig 7). 30 samples were quantified for Cry1Ac through Enzyme Linked Immunosorbent Assay (ELISA).

In previous year 111 entries of NCVT were received and were tested for four genes Cry1Ac, Cry2A, Vip3Aa and RR genes and quantified for Cry1Ac through ELISA (Table 5). The results showed that 105 entries were positive for Cry1Ac and only 06 were found Non-Bt. Cry2Aab gene was identified in only 1 entry PC-1928. RR gene was found in 16 entries and no entry was found positive for Vip3Aa gene.

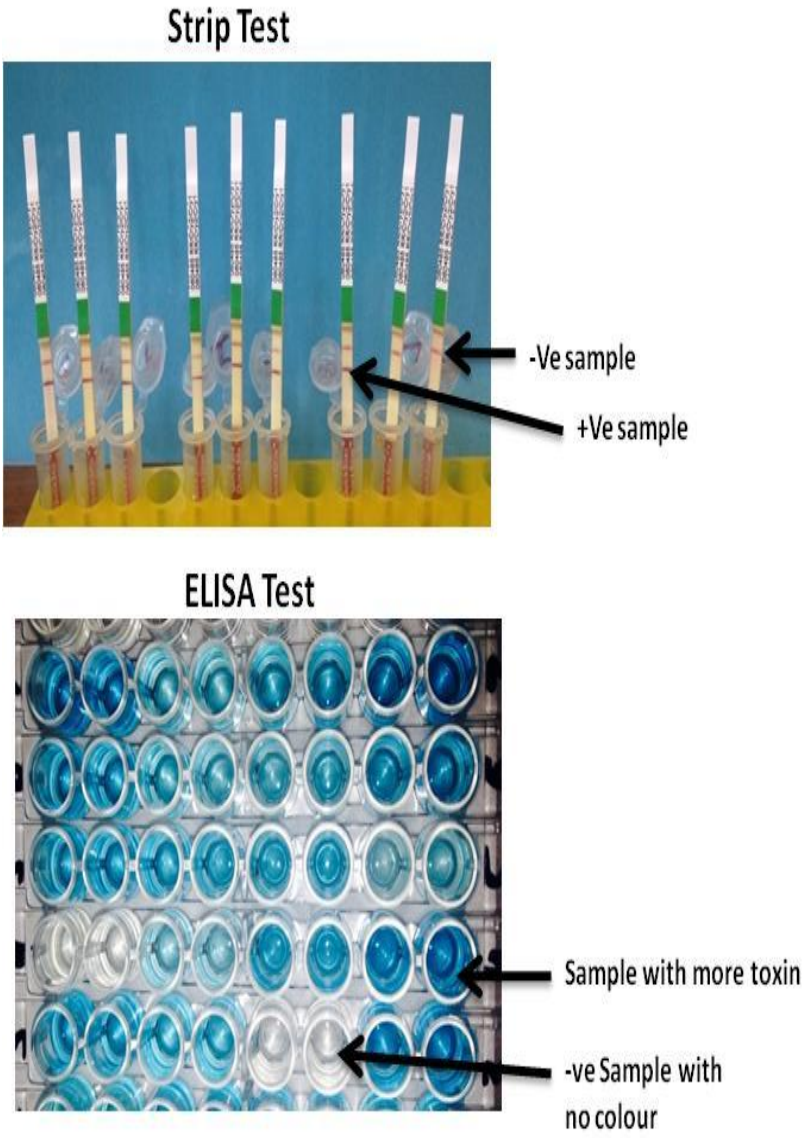


Fig 7. The pictorial view of results of the strip test and ELISA.

Table 5. The strip test results of NCVT trial for four genes i.e. Cry1Ac, Cry2Ab, Vip3A and RR genes along with trait purity and quantification of Cry1Ac in positive entries

Sr. No.	Sample code	Trait Confirmation (+/-)				Trait Purity			Toxin Expression (µg/g of fresh leaves 80 DAP)
		Cry 1Ac	Cry 2Ab	RR	Vip3A	Cry 1Ac	Cry2Ab	RR	
1	**PC-1901**	**+**	**-**	**-**	**-**	**100**	**0**	**0**	**1.68**
2	PC-1902	+	-	+	-	100	0	100	3.26
3	PC-1903	+	-	-	-	100	0	0	2.82
4	PC-1904	+	-	-	-	100	0	0	2.19
5	PC-1905	+	-	-	-	100	0	0	1.11
6	PC-1906	+	-	-	-	100	0	0	4.46
7	PC-1907	+	-	-	-	100	0	0	1.2
8	PC-1908	+	-	-	-	100	0	0	1.42
9	PC-1909	+	-	+	-	100	0	100	2.82
10	PC-1910	+	-	-	-	100	0	0	3.27
11	PC-1911	+	-	-	-	100	0	0	2.84
12	PC-1912	+	-	+	-	100	0	100	3.68
13	PC-1913	+	-	-	-	100	0	0	2.04
14	PC-1914	+	-	+	-	100	0	100	2.64
15	PC-1915	+	-	-	-	100	0	0	3.06
16	PC-1916	+	-	-	-	80	0	0	2.98
17	PC-1917	+	-	-	-	100	0	0	2.05
18	PC-1918	+	-	-	-	100	0	0	2.64
19	PC-1919	+	-	-	-	100	0	0	4.20
20	PC-1920	+	-	-	-	80	0	0	2.54
21	PC-1921	+	-	-	-	100	0	0	3.26
22	PC-1922	+	-	-	-	100	0	0	2.28
23	PC-1923	+	-	-	-	100	0	0	2.12
24	PC-1924	+	-	-	-	100	0	0	4.26
25	PC-1925	+	-	-	-	100	0	0	2.24
26	PC-1926	+	-	-	-	100	0	0	3.24
27	PC-1927	+	-	-	-	100	0	0	2.45
28	PC-1928	+	+	+	-	100	20	100	4.24
29	PC-1929	+	-	-	-	100	0	0	2.14
30	PC-1930	+	-	-	-	100	0	0	1.54
31	PC-1931	+	-	-	-	100	0	0	2.63
32	PC-1932	+	-	+	-	100	0	100	2.47
33	PC-1933	+	-	-	-	100	0	0	3.78
34	PC-1934	+	-	-	-	100	0	0	4.87
35	PC-1935	+	-	-	-	100	0	0	1.28
36	PC-1936	+	-	-	-	100	0	0	1.24
37	PC-1937	+	-	+	-	100	0	100	2.68
38	PC-1938	+	-	+	-	100	0	100	3.24
39	**PC-1939**	**+**	**-**	**-**	**-**	**100**	**0**	**0**	**2.46**
40	PC-1940	+	-	-	-	100	0	0	2.78
41	PC-1941	+	-	-	-	100	0	0	2.36
42	PC-1942	+	-	-	-	100	0	0	3.5
43	PC-1943	+	-	-	-	100	0	0	3.16
44	PC-1944	+	-	-	-	100	0	0	4.1
45	PC-1945	+	-	-	-	100	0	0	1.48
46	PC-1946	+	-	-	-	100	0	0	2.6
47	PC-1947	+	-	-	-	100	0	0	3.34
48	PC-1948	+	-	-	-	100	0	0	2.52

49	PC-1949	+	-	-	-	100	0	0	2.87
50	PC-1950	+	-	-	-	100	0	0	1.72
51	PC-1951	+	-	-	-	100	0	0	3.29
52	PC-1952	+	-	-	-	100	0	0	2.62
53	PC-1953	+	-	-	-	100	0	0	2.94
54	PC-1954	+	-	-	-	100	0	0	2.30
55	PC-1955	+	-	-	-	100	0	0	3.76
56	PC-1956	+	-	-	-	100	0	0	3.55
57	PC-1957	+	-	-	-	100	0	0	2.56
58	PC-1958	+	-	-	-	100	0	0	3.23
59	PC-1959	+	-	-	-	100	0	0	4.78
60	PC-1960	+	-	-	-	100	0	0	3.68
61	PC-1961	+	-	-	-	100	0	0	1.08
62	PC-1962	+	-	-	-	100	0	0	0.88
63	PC-1963	+	-	-	-	100	0	0	5.38
64	PC-1964	+	-	-	-	100	0	0	4.32
65	PC-1965	+	-	-	-	100	0	0	3.98
66	PC-1966	+	-	-	-	100	0	0	3.50
67	PC-1967	+	-	-	-	100	0	0	4.72
68	PC-1968	+	-	-	-	100	0	0	2.03
69	PC-1969	+	-	-	-	100	0	0	3.71
70	PC-1970	+	-	-	-	100	0	0	3.59
71	PC-1971	+	-	-	-	100	0	0	4.19
72	PC-1972	+	-	-	-	100	0	0	1.34
73	PC-1973	+	-	-	-	100	0	0	5.20
74	PC-1974	+	-	-	-	100	0	0	4.53
75	PC-1975	+	-	-	-	100	0	0	2.42
76	PC-1976	+	-	-	-	100	0	0	1.01
77	**PC-1977**	**+**	**-**	**-**	**-**	**100**	**0**	**0**	**0.94**
78	PC-1978	+	-	+	-	100	0	100	5.42
79	PC-1979	+	-	+	-	100	0	100	5.63
80	PC-1980	+	-	+	-	100	0	100	1.33
81	PC-1981	+	-	+	-	100	0	80	0.84
82	PC-1982	+	-	-	-	100	0	0	2.03
83	PC-1983	+	-	-	-	100	0	0	3.56
84	PC-1984	+	-	-	-	100	0	0	2.51
85	PC-1985	+	-	-	-	100	0	0	3.59
86	PC-1986	+	-	-	-	100	0	0	2.70
87	PC-1987	+	-	-	-	100	0	0	4.00
88	PC-1988	+	-	-	-	100	0	0	2.80
89	PC-1989	+	-	-	-	100	0	0	1.12
90	PC-1990	+	-	-	-	100	0	0	0.81
91	PC-1991	+	-	-	-	100	0	0	6.20
92	PC-1992	+	-	-	-	100	0	0	4.37
93	PC-1993	+	-	-	-	100	0	0	3.01
94	PC-1994	+	-	-	-	40	0	0	4.21
95	PC-1995	+	-	-	-	60	0	0	3.99
96	PC-1996	+	-	-	-	20	0	0	1.62
97	PC-1997	+	-	-	-	60	0	0	2.80
98	PC-1998	-	-	-	-	0	0	0	0
99	PC-1999	-	-	-	-	0	0	0	0
100	PC-2000	+	-	+	-	100	0	100	3.29
101	PC-2001	+	-	-	-	100	0	0	4.11
102	PC-2002	+	-	+	-	100	0	20	1.54
103	PC-2003	-	-	-	-	0	0	0	0
104	PC-2004	+	-	-	-	60	0	0	3.80
105	PC-2005	-	-	-	-	0	0	0	0
106	PC-2006	+	-	+	-	80	0	40	3.95
107	PC-2007	-	-	-	-	0	0	0	0

108	PC-2008	-	-	-	-	0	0	0	0
109	PC-2009	+	-	-	-	100	0	0	2.29
110	PC-2010	+	-	-	-	100	0	0	0.76
111	PC-2011	+	-	+	-	100	0	100	0.78

8. SSR markers as a tool for preliminary screening of maize lines for drought tolerance.

The objective of this experiment was to test 62 maize inbred lines for drought tolerance using linked DNA markers. The leaves of 62 maize genotypes were collected from Maize and Millets Research Institute, Yusafwala Sahiwal and DNA was extracted. 23 linked DNA markers were used to assess the drought tolerance status of 62 maize inbred lines the results of which are summarized in the Table 6.

Table 6. The highest drought tolerant and drought sensitive genotypes among 62 maize genotypes. The number of alleles amplified by each genotypes against 23 DNA markers are also given.

Genotype Name	No. of Alleles	Status
EL-106	40	Drought tolerant
EL-307	44	Drought tolerant
EL-322	41	Drought tolerant
YH-1898	44	Drought tolerant
YH-5482	40	Drought tolerant
DP-1	45	Drought tolerant
DP-3	43	Drought tolerant
DP-4	42	Drought tolerant
DP-5	40	Drought tolerant
DP-8	48	Drought tolerant
DP-9	40	Drought tolerant
DP-12	41	Drought tolerant
EL-23	24	Drought sensitive
EL-28	30	Drought sensitive
EL-48	26	Drought sensitive
EL-172	29	Drought sensitive

9. Biosafety trial 2018 for confirmation of insect resistance & herbicide tolerance genes and quantification of Bt protein in candidate cotton varieties.

The objective of this experiment was to check the status of *Cry1Ac*, *Cry2Ab*, *Vip3Aa* and *RR* genes in cotton genotypes received from Cotton Research Institute and Cotton Research

Stations and sub-stations. Sowing of 21 cotton varieties in GMO Containment for Biochemical tests 03 (CRI, Multan), 05 (CRI, Khanpur), 03 (CRS, Sahiwal), 03 (CRS, Veharil), 05 (CRS, Faisalabad) and 02 (NIBGE, Faisalabad). All plants were positive for Cry1Ac gene only. Event confirmation (PCR): Mon-531 Event was confirmed through qualitative DNA analysis method utilizing event specific primers for Cry1Ac gene.

10. DNA fingerprint study in tomato (*Solanum Lycopersicum* l) cultivars using simple sequence repeats (SSR) markers

Genetic structure of tomato varieties cultivated in Punjab Pakistan was studied using UPGMA (Unweighted Pair Group of Arithmetic Averages) based cluster analysis on the basis of genomic data from 206 SSR markers. Similarity matrix was first calculated using the approach of SHAN similarity index followed by construction of dendrogram. The population was broken down to two groups i.e. A and B with similarity index ranging from 0.76 to 0.88 with one genotype 17253 as outlier. Maximum genetic similarity up to 88% was observed between two hybrids Ahmar and Naqeeb whereas minimum genetic similarity 76% was observed between 17253 and all other 12 genotypes (Fig 8).

Group A was comprised of three genotypes Salar-F1, Sundar-Hybrid and Sandal-F1. Similarly Group B was comprised of nine genotypes i.e. Ahmar, Naqeeb, Nadir hybrids and 8502, 8505, 9108, 13195, 8504 and 68543 genotypes. Group B was subdivided to B1 with five genotypes i.e. Ahmar, Naqeeb, Nadir hybrids, 8502 and 8505. Whereas B2 was comprised of 04 genotypes i.e. 9108, 13195, 8504 and 68543. One genotype 17253 was not classified to any subgroup and was lying separately in the dendrogram. More closely related genotypes in Group B were Ahmar and Naqeeb whereas most distantly related genotypes in the B group were 68543 and Ahmar hybrid (Fig 8).

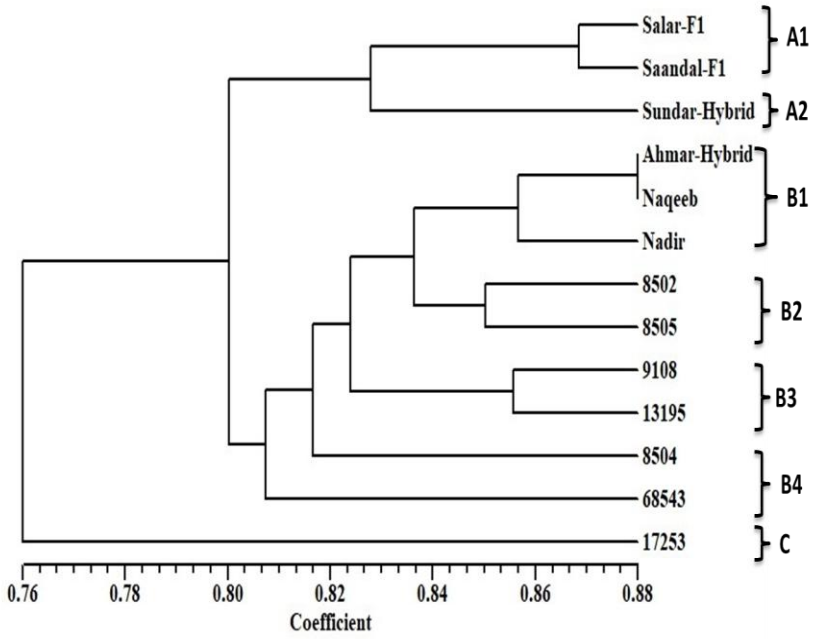


Figure 8. The UPGMA dendrogram showing the genetic relatedness of 13 tomato genotypes based on SHAN similarity matrix. The resulted showed that genotypes are divided to 3 groups which are further subdivided.

11. DNA Barcoding of wheat genotypes using SSR markers for rapid varietal identification.

To determine the genetic structure of wheat varieties cultivated in Punjab Pakistan UPGMA (Unweighted Pair Group of Arithmetic Averages) based cluster analysis was performed using genomic data from 228 SSR markers. Similarity matrix was first calculated using the approach of SHAN similarity index followed by construction of dendrogram. The population was broken down to three groups i.e. A, B and C with similarity index ranging from 0.60 to 0.80. Maximum genetic similarity up to 80% was observed between Millat-11 and Punjab-11 whereas minimum genetic similarity 63% was observed between Gold-16 and Ghazi-19 (Fig 9).

Group A was comprised of six genotypes i.e. AARI-11, FSD-08, Millat-11, Punjab-11, Ujala-16 and Galaxy-13. Group A was further subdivided to A_1 and A_2 with two varieties i.e. AARI-11 and FSD-08 in A_1 and four varieties viz. Millat-11, Punjab-11, Ujala-16 and Galaxy-13 in Group A_2. Millat-11 & Punjab-11 were closely related genotypes in Group A followed by Ujala-16 & Galaxy-13. Similarly Group B was comprised of seven genotypes i.e. Anaj-17, Chakwal-50, Johar-16, F-Bhakar, Barani-17, Dharabi-17 and Ihsan-16. Group

B was subdivided to B_1 with four genotypes i.e. Anaj-17, Chakwal-50, Johar-16 & F-Bhakar. B_2 was comprised of 03 varieties i.e. Barani-17, Dharabi-17 and Ihsan-16. Maximum genetic similarity in group B was observed between Barani-17 and Dharabi-17. Group C was comprised of 02 genotypes i.e. Gold-16 and Ghazi-19 which were distantly related to rest of the population and there was lowest genetic similarity (63%) among these two genotypes (Fig 10). Further the Fig 11 represents the gel images of two SSR markers of wheat i.e. GWM339 and GWM 350

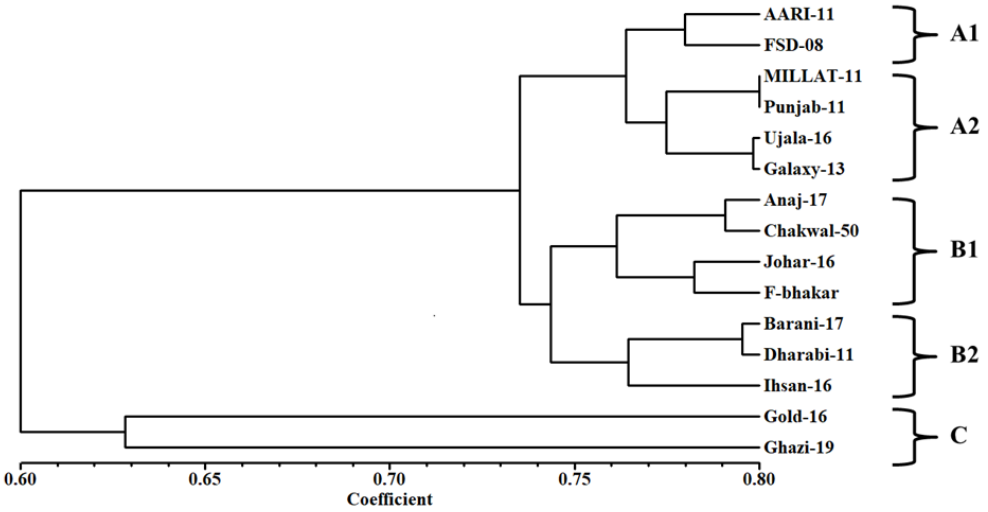
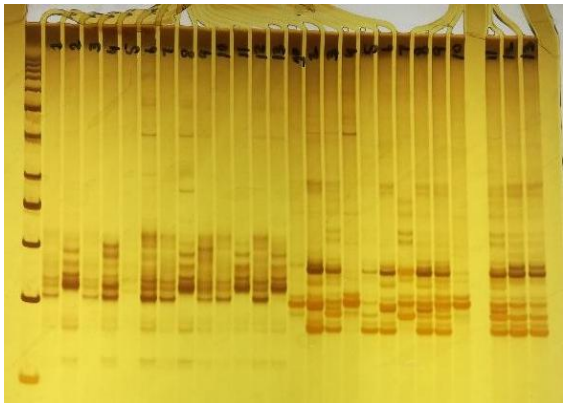


Fig 9. Cluster analysis of 13 wheat varieties using unweighted Pair Group Method with Arithmetic Averages.



GWM-339 GWM350

Fig 10. The representative gel images of the wheat barcoding showing the amplification pattern of two SSR markers i.e. GWM-339 and GWM-350 on the polyacrylamide gel electrophoresis.

12. Utilization of DNA markers based on SSR polymorphism for identification of potato varieties.

To determine the genetic structure of potato varieties cultivated in Punjab Pakistan UPGMA (Unweighted Pair Group of Arithmetic Averages) based cluster analysis was performed using genomic data from 217 SSR markers. Similarity matrix was first calculated using the approach of SHAN similarity index followed by construction of dendrogram. The population was broken down to three groups i.e. A, B and C with similarity index ranging from 0.69 to 0.84 with one genotype as outlier. Maximum genetic similarity up to 84% was observed between SH-5 and PRI-Red whereas minimum genetic similarity 69% was observed between FD78-51 and all other 11 genotypes (Fig 12).

Group A was comprised of five genotypes i.e. FSD-Red, FSD-White, SH-5, PRI-Red and Ruby. Group A was further subdivided to A_1 and A_2 with two varieties i.e. FSD-Red and FSD-White in A_1 and three varieties viz. SH-5, PRI-Red and Ruby in Group A_2. SH-5 & PRD-Red were closely related genotypes in Group A. Similarly Group B was comprised of three genotypes i.e. Sadaf, Sialkot-Red and FD73-110 among which Sialkot-Red and FD73-110 were more related to each other and compared to Sadaf. No subgroups were observed for B Group. Group C was also comprised of 03 genotypes i.e. FD76-67, FD73-49 and FD81-8. FD76-67 and FD74-49 were more related to each other in C group as compared to FD81-8 sharing 82% genetic similarity. Whereas FD78-51 was not related to any other genotype (Fig 12).

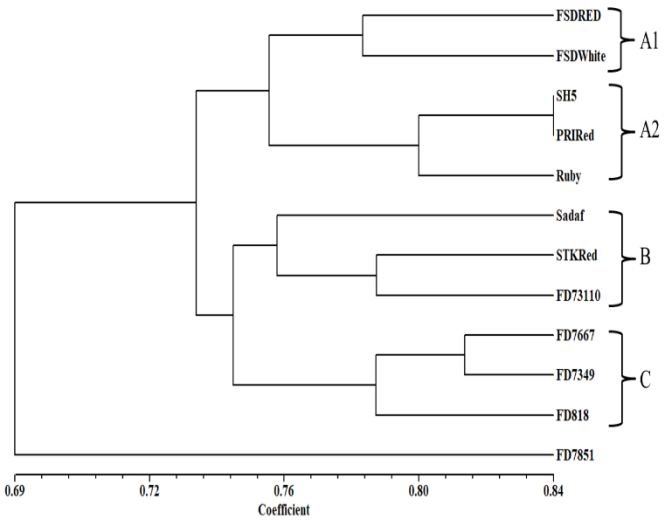


Fig 11. The UPGMA dendrogram analysis of 12 potato genotypes depicting the genetic similarity based on the binary data of the 217 SSR markers.

13. Effect of different nitrogen, potassium levels and crop rotation system on quantification of Cry1Ac protein

The objective of this work was to check expression of Bt endotoxin protein in different plant parts (leaves, square & bolls) at different nitrogen (0, 100, or 200 kg/ha), potassium (100 kg/ha as basal dose before planting &100 Kg/ha at the time of squaring) levels and crop rotation system (continues cotton field & field with cereal rotation). Sowing was completed. Over all protein expression for Cry1Ac was higher in continuous cotton crop field as compared to cereal rotation field. As N level increases, expression was also increased and more expression was observed at 200Kg/ha as shown in **Table 7**. Potassium application was not significantly affected Cry1Ac protein expression**.**

Table 7. The effect of Nitrogen and Potassium and crop rotation on Cry1Ac endotoxin expression. The effect was checked by quantification from various plant parts i.e. leaf, squares and bolls.

Nitrogen nutrition: (Cotton after Cotton)					
N applied Kg/ha	**Cry1Ac expression ug/g of fresh leaf sample)**				
	Leaf (Top)	**Leaf (Middle)**	**Leaf (Bottom)**	**Squares**	**Ten Days Bolls**
0	2.24	1.75	0.00	1.80	0.60
100	2.65	2.24	0.00	1.65	1.20
200	3.84	2.54	0.30	2.76	1.82
Nitrogen nutrition: (Cereal rotation)					
N applied Kg/ha	**Cry1Ac expression ug/g of fresh leaf sample)**				
	Leaf (Top)	**Leaf (Middle)**	**Leaf (Bottom)**	**Squares**	**Ten Days Bolls**
0	1.25	0.8	0.00	1.2	0.16
100	1.80	1.2	0.00	1.52	0.78
200	2.22	1.45	0.25	1.84	1.00

14. Validation of identified DNA marker for fiber quality and yield related traits in cotton

The objective of this experiment was the tagging of 21 cotton genotypes for fiber quality related traits using linked DNA markers. The linked DNA markers to different traits were

searched and DNA was extracted from 21 cotton genotypes obtained from different cotton research institute/stations. The 10 DNA markers linked to fiber quality traits were surveyed on 21 genotypes and results were compiled.

15. Lab accreditation under ISO-17025

The objective of this work was to continue the accredited activities of GMO testing lab as per instructions of ISO-17025 and to upgrade the lab according to 2017 version of standard. ISO/IEC 17025 accreditation is the single most important standard for calibration and testing laboratories around the world. ISO 17025 accredited laboratory's have demonstrated that they are technically proficient and able to produce precise and accurate testing data. This is a voluntary, third party-reviewed process that ensures a laboratory's quality management system is thoroughly evaluated on a regular basis to guarantee continued technical competence and compliance with ISO 17025. Laboratory accreditation bodies use the ISO 17025 standard specifically to assess factors relevant to a laboratory's ability to produce precise, accurate testing data. To ensure continued compliance, accredited laboratories are regularly re-assessed to check that they are maintaining their standard of technical expertise. These laboratories are also required to participate in regular proficiency testing (PT) programs as an ongoing demonstration of their competence. For customers, ISO/IEC 17025 accreditation helps you minimize risk by ensuring that you are choosing a technically competent lab that has a sound quality system in place. This also allows you to avoid expensive retesting, which enhances your confidence in our product by assuring that it has been thoroughly evaluated by an independent, competent testing laboratory that has been assessed by a third party. GMO testing lab of the institute accredited for the identification of 02 GM elements i.e. 35S promoter & NOS terminator. Lab has participated in international Proficiency Testing (PT) program with FAPAS with 100% correct results. First–surveillance visits of GMO testing lab was carried out by PNAC officials and total 15 NCs were raised. Corrective actions for NCs were taken and send to PNAC for evaluation. CAs were thoroughly evaluated by PNAC and found satisfactorily. GMO testing lab upgraded to new version of standards i.e. ISO-17025:2017. All the quality indicators like internal audit, MRMs, equipment calibrations, testing/re-testing, data recording etc. are carried out as per plan to maintain the quality of work. A total of 137 seed/leaf samples of

different crops were tested at molecular level for the presence of GM elements and reports submitted timely.

16. DNA markers based detection of quality related genes in spring wheat

Bread and durum wheats are the major food for much of the human population and are mainly consumed as processed products because of the unique functional properties they confer to the derived foods. Improving end-use quality has become of increasing importance to wheat breeders over the past few decades with an emphasis on developing cultivars of durum and bread wheat. The selections of superior breeding lines with improved quality characteristics, as well as the dissection of their complexity, are being greatly favored by the establishment and exploitation of biochemical and molecular markers. Molecular marker technology is playing an increasingly important role in the selection of wheat lines with improved quality attributes. Marker assisted selection (MAS) increased the efficiency of the breeding process, particularly when phenotyping requires laborious and time-consuming analyses, performed in advanced generations because of the relatively large amount of grain required. Moreover, the implementation of MAS allows the selection of individuals carrying the favorable alleles at the target loci, and also the pyramiding of favorable QTL alleles from different sources and for different traits. This research work was planned to find quality related genes in candidate spring wheat genotypes using MAS. 50 wheat genotypes were used for the identification of 5 optimized linked DNA markers for wheat quality. Total DNA was isolated from wheat leaves using modified CTAB methodologies and quantified. Working dilution with equal DNA concentrations were prepared for onward PCR analysis to track the quality related genes. It was found that marker Dx2,Dx5 was present in 47 genotypes, By8 was found in 18 genotypes, primer Xgwm537 was present in 41 lines, Xgwm577 was found 41 lines and Xcfa2019 was amplified in 33 wheat genotypes. 15 wheat lines showed maximum quality markers while no marker was amplified from line no. 41. Optimization of more quality related genes in progress.

Table.8 :Molecular screening data for quality related genes/linked DNA markers

Entry No.	V-Code	Dx2,Dx5	By8	Xgwm537	Xgwm577	Xcfa2019
1	NR 552	+	-	+	+	+
2	TWS 1637	+	-	-	-	-

3	V-16152	+	-	-	-	-
4	HYT 70-1	+	-	+	+	+
5	V-17259	+	-	-	+	+
6	18C124	+	-	+	-	-
7	PGMB 17-6	+	-	+	-	-
8	JOHAR-16	+	-	+	+	+
9	V-17262	+	-	-	+	+
10	HYT 70-16	+	-	+	+	+
11	18-1601	+	+	+	+	+
12	NW-9	+	-	+	+	-
13	TWS 16164	+	+	+	+	+
14	NR 549	-	-	+	+	+
15	BARANI-17	+	-	+	+	-
16	V-1821	+	-	+	+	+
17	HYT 20-4	+	-	+	+	+
18	16FJ17	+	-	+	+	+
19	NR 547	+	-	+	+	+
20	BF-1807	+	+	+	+	+
21	18C116	+	+	+	+	+
22	V-17055	+	+	+	+	+
23	V-17053	+	-	+	+	+
24	V-18103	+	-	+	+	-
25	V-16128	+	-	-	+	-
26	WV 128	**+**	**-**	**+**	**-**	**-**
27	15BT019	+	-	+	-	-
28	HYT 70-36	+	-	+	+	+
29	V-16144	-	-	-	-	+
30	V-18097	+	+	+	+	+
31	HYT 70-4	+	+	+	+	+
32	V-18106	+	+	+	+	+
33	NR 533	+	-	+	+	+

34	16FJ39	+	+	+	+	+
35	172149	+	+	+	+	+
36	PBG 10123	+	+	+	+	+
37	18C117	+	+	+	+	-
38	FSD-08	+	+	+	+	-
39	BF 1808	+	+	+	+	+
40	HYT 70-3	+	-	+	+	+
41	V-17086	-	-	-	-	-
42	HYT 70-20	+	-	+	+	-
43	HYT 70-40	+	+	-	+	-
44	V-18614	+	+	+	+	+
45	HYT 20-19	+	+	+	+	+
46	WV 1195	+	+	+	+	+
47	NR 5456	+	-	+	+	+
48	V-17065	+	-	+	+	+
49	V-18623	+	-	+	-	-
50	TWS 15110	+	-	-	+	-

17. Identification of rust resistance genes in advance lines of wheat

Wheat is an important cereal food crop providing key nutrients to humankind. Rusts are the most destructive pathogens of cereal crops, with the exception of rice, across the world and resistant cultivars have been widely employed to reduce the yield losses caused by them. Conventional breeding efforts have not been effective in quickly developing new varieties with durable and broad- spectrum resistance against the rapidly evolving rust pathogen races. In recent years, improved versions of biotechnological breeding methods such as genomic selection, genome editing technologies, cisgenesis and intragenesis, RNA- dependent DNA methylation (RdDM), agroinfiltration and reverse breeding are gaining popularity. These technologies provide a tremendous capability to manipulate crop plants more precisely than before and accelerate crop improvement efforts for sustained food production as well as overcoming safety concerns associated with food crops. The objective of this work was the molecular characterization of wheat genotypes to identify/tag

rust resistance genes in advanced lines of PUWYT for onward approval process and use in the breeding program. A total of 10 DNA markers linked to resistant genes for wheat rusts were surveyed to identify stem, yellow and leaf rust resistance genes on 50 wheat advance lines. Total DNA was isolated from wheat leaves using modified CTAB methodologies and quantified. Working dilutions with equal DNA concentrations were prepared for onward PCR analysis to track the rust resistant genes/markers. Out of 50 wheat advanced lines it was observed that 03 entries were resistant for Lr19, 02 resistant for Lr34/Yr18, 33 for Lr28, 31 for Lr46/Yr29, 22 for Lr67, 33 for Yr5, 46 for Yr15, 36 for Yr46. No line was positive for Sr-2 & Sr26. Maximum no. of rust resistance genes (07) was detected from entry no 35.

Table.9 Molecular screening data for rust resistant linked DNA marker

Entry No.	V-Code	Lr19	Lr28	Lr34	Lr46	Lr67	Yr5	Yr15	Yr46	Sr2	Sr26
1	NR 552	-	+	-	+	+	+	+	+	-	-
2	TWS 1637	-	-	-	-	-	-	+	+	-	-
3	V-16152	-	-	+	-	-	-	+	+	-	-
4	HYT 70-1	-	-	-	+	+	-	+	+	-	-
5	V-17259	-	-	-	-	-	+	+	-	-	-
6	18C124	-	+	-	-	-	+	+	+	-	-
7	PGMB 17-6	-	+	-	-	+	-	+	-	-	-
8	JOHAR-16	-	+	-	-	+	+	+	+	-	-
9	V-17262	-	-	-	-	+	+	+	+	-	-
10	HYT 70-16	-	-	-	-	+	+	+	+	-	-
11	18-1601	+	+	-	+	-	+	+	+	-	-
12	NW-9	-	-	-	-	-	+	+	+	-	-
13	TWS 16164	-	+	-	+	-	-	-	-	-	-
14	NR 549	-	+	-	+	-	-	+	-	-	-
15	BARANI-17	-	+	-	-	-	-	+	+	-	-
16	V-1821	-	-	-	-	-	+	+	+	-	-
17	HYT 20-4	-	-	-	-	-	-	+	+	-	-
18	16FJ17	-	+	-	-	-	-	+	+	-	-
19	NR 547	-	+	-	+	-	+	+	-	-	-
20	BF-1807	-	+	-	+	+	+	+	+	-	-
21	18C116	-	+	-	+	+	+	+	+	-	-
22	V-17055	-	+	-	+	+	+	+	-	-	-
23	V-17053	-	+	-	-	-	+	-	+	-	-
24	V-18103	-	-	-	-	-	+	+	-	-	-
25	V-16128	-	-	-	+	-	-	+	+	-	-
26	WV 128	+	+	-	+	-	+	-	+	-	-

27	15BT019	-	-	-	-	-	-	+	+	-	-
28	HYT 70-36	-	+	-	+	+	+	+	-	-	-
29	V-16144	-	-	-	-	-	-	+	-	-	-
30	V-18097	-	+	-	+	+	+	+	+	-	-
31	HYT 70-4	-	+	-	+	+	+	+	-	-	-
32	V-18106	-	+	-	+	+	+	+	-	-	-
33	NR 533	-	+	-	+	+	+	+	+	-	-
34	16FJ39	-	+	-	+	+	+	+	-	-	-
35	172149	+	+	-	+	+	+	+	+	-	-
36	PBG 10123	-	+	-	+	+	+	+	+	-	-
37	18C117	-	+	-	+	-	+	+	+	-	-
38	FSD-08	-	+	-	+	+	-	+	-	-	-
39	BF 1808	-	+	-	+	-	+	+	+	-	-
40	HYT 70-3	-	+	-	+	-	+	+	+	-	-
41	V-17086	-	-	-	+	-	+	+	-	-	-
42	HYT 70-20	-	+	-	+	+	-	+	-	-	-
43	HYT 70-40	-	-	+	+	+	+	+	+	-	-
44	V-18614	-	+	-	+	+	+	+	+	-	-
45	HYT 20-19	-	+	-	+	+	+	+	+	-	-
46	WV 1195	-	+	-	+	-	+	+	+	-	-
47	NR 5456	-	+	-	+	-	+	-	+	-	-
48	V-17065	-	+	-	+	-	+	+	+	-	-
49	V-18623	-	-	-	-	-	-	+	-	-	-
50	TWS 15110	-	-	-	-	-	+	+	-	-	-

18. Rust resistance gene pyramiding in wheat using linked DNA markers

Gene pyramiding, a breeding procedure of bringing together more than one resistant genes in one desirable genetic background is useful technology to combat rust infection. The pyramiding of effective genes through conventional techniques involving phenotype based selection criteria is of course time consuming and laborious but the availability of genetic information, reaction pattern against individual races/pathotypes of rusts are helpful and valuable techniques that facilitate gene pyramiding through conventional methods. With the advent of molecular marker technology it is now possible to tackle such complex problem. DNA-based molecular markers have several advantages over the traditional phenotype based selection especially when disease-escaped susceptible plants are likely to be confused for resistant plants. Molecular marker can be used for MAS to improve the efficiency of selection in plant breeding because the environment does not affect the expression of molecular markers. Keeping in view the significance of MAS, rust resistant wheat parental

lines were selected and molecular information was tagged using gene linked markers through PCR. Conventional breeding plane was designed on the basis of molecular data and maximum crosses were made between high yielding susceptible and resistant wheat genotypes. Molecular screening and other yield parameters were keenly noted on each stage of segregating population. The objective of this work was the accumulation of desirable rust resistance genes from different parents to get genotypes having different combination of various Leaf, Yellow and Stem rust resistance genes. 56 cross combination were selected from R1 (13), R-II (11), R-III (15), R-IV (05), R-V (03) & R-VI (09) and screened against Leaf and yellow leaf rust. Total DNA was isolated from wheat leaves using modified CTAB methodologies and quantified. Working dilutions with equal DNA concentrations were prepared for onward PCR analysis to track the rust resistant linked marker/genes. It was found that 03 crosses were carry resistant marker for Lr19, 36 crosses for Lr28, 12 homozygous and 08 crosses were heterozygous for Lr34/Yr18, 19 crosses were positive for Lr67, 39 crosses for Lr46/Yr29

26 crosses were found positive for Yr5, 34 crosses were found positive for Yr15, 19 crosses were found positive for Yr46. No cross was found positive for Sr2 & Sr26.Generation advancement for homozygous lines and development of more cross combination are continued.

Table.10 Rust resistant gene pyramiding results of wheat

Sr.#	ID	Generation	Parentage/pedigree	Lr19	Lr28	Lr34/Yr18	Lr46/ Yr29	Lr67/ Yr46	Yr5	Yr15	Yr46
1	12	R1	V-11153/Fsd-08	-	+	+	+	-	+	+	+
2	16	R1	C11022/Fsd-08	-	+	-	-	-	+	+	+
3	17	R1	NS-14/Fsd-08	-	+	-	+	-	-	+	+
4	23	R1	15C042/Galaxy-13	-	-	-	-	-	-	+	-
5	24	R1	C11022/Galaxy-13	-	-	-	-	-	-	-	-
6	35	R1	NNG3/Fsd-08	-	-	-	+	+	+	-	-
7	43	R1	15C042/Jauhar-16	-	+	+/-	-	-	-	+	-
8	45	R1	C11022/Jauhar-16	-	-	-	-	-	+	+	-
9	52	R1	15C042/Ujala-16	-	-	-	+	-	+	-	+
10	61	R1	NR381/Ujala-16	-	+	-	-	-	+	-	-
11	67	R1	V-11153/Ufaq-02	-	+	-	+	-	-	+	-
12	90	R1	V-11153/Zincole-16	-	+	+	+	+	-	+	-

13	93	R1	C11023/Zincole-16	-	-	+	-	+	-	-	-
14	10	R2	NR-378/Anaj-17	-	+	-	+	-	-	-	-
15	28	R2	NR-378/Fsd-08	-	+	-	+	+	+	-	+
16	29	R2	NR-388/Abaj-17	-	+	-	-	-	-	+	+
17	53	R2	NNG3/Koh/Anaj-17	-	+	-	+	-	+	-	-
18	55	R2	NR-388/Galaxy-13	-	+	-	-	-	-	-	+
19	59	R2	NR-378/Fsd-08	-	-	-	+	-	+	-	-
20	64	R2	NR-381/Galaxy-13	-	+	-	+	+	+	-	-
21	78	R2	PBW343*2/Khorabi/ Ujala-16	-	+	-	+	-	-	+	-
22	80	R2	PBW343*2/Chapio/ Zincol-16	-	+	-	+	+	-	+	-
23	96	R2	NR-378/Galaxy-13	-	-	-	+	+	-	+	-
24	103	R2	NR-378/Anaj-17	-	+	+	+	-	-	+	+
25	30	R3	Millat-11/CB331	-	+	+/-	-	-	+	+	+
26	38	R3	Ujala-16/Ufaq-2002	-	+	+/-	-	-	-	+	+
27	39	R3	Ujala-16/Seher-06	-	+	-	+	-	+	+	+
28	43	R3	Ujala-16/Inq-91	-	-	-	-	+	-	-	+
29	68	R3	Seher-06/Ufaq-2002	-	+	-	+	-	+	+	-
30	70	R3	Seher-06/Ujala-16	-	-	-	+	-	+	-	-
31	73	R3	Galaxy-13/Seher-06	-	-	-	+	-	+	-	-
32	87	R3	Millat-11/Fsd-08	-	+	+/-	-	-	-	+	-
33	88	R3	Millat-11/Seher-06	-	+	-	-	+	+	+	-
34	86	R3	Millat-11/Ufaq-2002	-	+	-	+	-	+	+	+
35	98	R3	Inq-91/Ufaq-2002	-	+	-	+	-	-	+	-
36	100	R3	Inq-91/Punjab-11	-	-	-	-	-	-	-	-
37	102	R3	Lasani-08/Inq-91	-	+	-	+	-	+	-	-
38	103	R3	Lasani-08/Ufaq-2002	-	+	-	+	-	-	+	-
39	105	R3	Lasani-08/Fsd-08	-	-	-	+	-	-	+	-
40	8	R4	NNG-3/SH-88/87093//MH-97	-	-	+/-	+	+	-	+	-
41	10	R4	NNG-3/Ufaq-02	-	-	+	+	-	-	+	-
42	18	R4	V-11153/AARI-11	-	+	-	+	+	-	+	-
43	19	R4	Watan/Fsd-85	-	+	+/-	+	+	+	+	-
44	22	R4	Millat-11/V-11153	-	+	+/-	+	-	+	+	+
45	11	R5	INQ-91/2*Tukuru//NR-750	+	-	-	-	-	+	-	-
46	22	R5	INQ-91/2*Tukuru//NR-393/NNG-3	+	+	-	+	-	-	-	+
47	30	R5	Cham4/NR-393/NNG-3	-	-	+/-	-	+	-	-	-
48	51	R6	11C023/9452	-	+	+	+	-	+	+	-

49	54	R6	11C022/V-11153			+	+	-	-	-	+
50	59	R6	NNG-3/ C11022	-	-	+	+	+	+	+	-
51	60	R6	11C023/NNG3	-	+	-	+	+	+	-	-
52	66	R6	9452/NR393	-	+	+	+	+	-	-	-
53	67	R6	9452/11C022	-	+	+	+	-	-	+	-
54	70	R6	9452/NNG3	-	-	+	+	+	+	+	+
55	72	R6	11C022/Fret-2/Tukuru	+	+	-	+	+	+	+	+
56	77	R6	NNG3/9452	-	+	+	+	+	-	+	+

19. Genetic Transformation for Herbicide Tolerant Gene in Cotton

Weeds are undesirable plants competing with the crop plants for the resources that are typically needed for proper growth and development, namely, direct sunlight, soil nutrients, water, and space for growth. Weeds also act as hosts and vectors for plant pathogens, giving them greater opportunity to infect and degrade the quality of the crop plants. To combat this problem an experiment was initiated by artificially synthesizing Glyphosate Tolerance (GT) gene and then integrating this synthetic gene in agrobacterium strain LBA4404 for plant transformation. For genetic engineering purposes two elite cotton varieties viz. FH-444 and FH-490 were grown in field (during cotton season) and pots (under glasshouse during off-season). Post pollination micro-injection of Agrobacterium culture was administrated through pollen tube pathway with intervals of 4, 8, 16 hours. A total of 1200 flowers of both genotypes were inoculated with LBA4404 agrobacterium culture having synthetic EPSPS gene using PTP transformation method. Surviving 32 putative cotton plantlets of T_1 generation were raised in seedling trays under wire house. At three leaf stage plants were sprayed with round-up herbicide with half the recommended dose. All plantlets showed susceptibility to herbicide and died. This experiment will continue into next year with optimizations in inoculation technique and herbicide dose levels.

In a parallel experiment with same objectives of developing herbicide tolerant transgenic cotton, immature meristems of newly grown plantlets of cotton were cultured on specialized nutrient media under various Plant Growth Regulators (PGRs) like BAP and NAA and later inoculated with Agrobacterium LBA-4404 strain harboring the EPSPS glyphosate tolerance gene. In total 460 meristems were isolated and cultured on PGR supplemented media and inoculated with Agrobacterium construct. Only 10% seeds were germinated on nutrient

media and out of these only 1% seeds survived due to severe fungal contamination. For sterilization of cotton seed various methods were tried to minimize the growth of fungus on media. Seeds were initially rinsed in tap water for 10 minutes. After that 10 minute washing was given in 20% bleach. The seed was then rinsed 3 times in autoclaved d2H2O and dried on sterilized filter paper before culturing. In n other method mercuric chloride was used instead of Sodium Hypo chloride. However the cotton seeds were heavily contaminated and damaged by pink bollworm attack. Further optimization of sterilization method is needed and this experiment will be continued next year with optimizations.

Fig 12: Various stage of cotton transformation through Pollen Tube Transformation Technique

20. Use of molecular markers for identification of Red rot disease resistance in sugarcane (*Saccharum* spp) genotypes

Sugarcane is a vital crop grown for sugar and sugary products. This crop suffers many diseases caused by bacteria, fungi, and viruses Redrot disease of sugarcane not only reduces the quality of sugarcane but also cause reduction in its yield. The red rot of sugarcane was first reported

in 1883 in the Indo-Pakistan region in 1906 (Butler 1906). Red Rot is mainly caused by a fungus: *Colletotrichum falcatum* also called *Glomerella tucumanesis*. The fungus is prevalent in almost all sugarcane growing countries of the world. The susceptible varieties show considerable losses due to secondary infection, intensive cultivation, and poor management practices. Yield losses due to red rot are around 18-31% in planted crops and 52-73 % in ratoon crops.

Finding the resistance genotypes at early filial generation is one of the main objective of sugarcane breeding program. A genomic study was designed to optimize the screening of red rot disease resistant sugarcane genotypes by using molecular markers for precise detection of disease resistant material. Seven disease resistant genotypes viz FD-18, US-54, UMC87/599, US-133, SP-302, CPF-247 and CPF-248 and 07 redrot susceptible sugarcane genotypes viz US-127, AUS-133, AUS-633, AUS-778, Co-1148, BF-162 and SPF-234 were obtained from Sugarcane Research Institute, AARI, Faisalabad. Four newly developed varieties CPF-250, CPF-251, CPF-252 and CPF-253 were added later. Twenty (20) SSR markers were applied for the differential amplification with respect to disease resistance and susceptibility. Two SSR markers SMC597CS and UGSM316 uniquely differentiated redrot resistant and susceptible genotypes. Marker SMC597CS associated a band size of 160bps with disease resistant lines whereas, UGSM316 associated a band sized of 1050bps with redrot susceptible genotypes. Both these SSR markers can be used in further studies for identification of redrot resistant material.

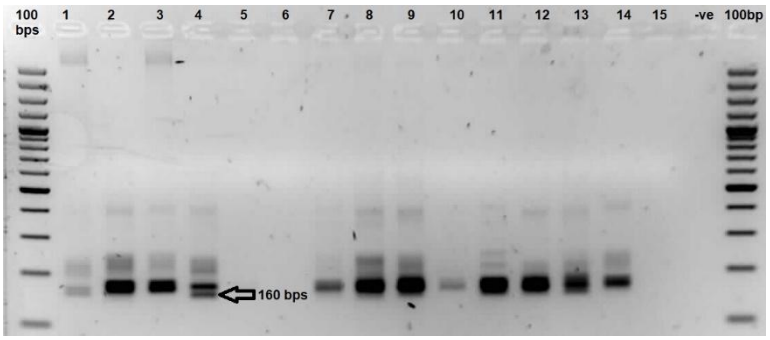


Fig 13: Amplification of unique band size with SSR marker SMC597CS identifying disease resistant sugarcane genotypes

21. Development of glyphosate herbicide tolerance in economically important crops (wheat) for the effective weeds control

Weeds are a major issue in wheat crop. The quality of produce from weed infested field is inferior due to mixing of weed seeds with wheat grains. Weeds also act as an obstacle in the cultural practices e.g. harvesting. Some weeds also help in the spread of diseases and act as multiplication places. Weeds cause losses up to 30% in wheat production. Weeds reduce crop yield, deteriorate quality of farm produce and hence decrease market value of wheat. In Pakistan, the annual losses in wheat crop amount to more than Rs. 28 b at national level. Herbicide is a chemical used to kill or inhibit the growth of weeds and other unwanted plant pests. Herbicide activity can be either selective or nonselective. Selective herbicides are used to kill weeds without significant damage to desirable plants. Nonselective herbicides kill or injure all plants present if applied at an adequate rate. Wheat is susceptible to all kind of nonselective herbicides including Glyphosate (Roundup). To thoroughly eradicate the weeds and to minimize the use of herbicides, an experiment was executed for the development of transgenic wheat harboring glyphosate resistance EPSPS under the control of constitutive promoter. A monocot specific hyper virulent Agrobacterium strain AGL1 was transformed with vector pB7WG2D/1 through electroporation process. Antibiotic resistant colonies were confirmed through colony PCR and utilized for transformation experiment. Eight (08) liter Callus Induction Media, three (03) Liter Agro inoculation Media (AIM) was prepared, sterilized and poured in Petri plates. About 1500 immature embryos of three wheat varieties viz. Akbar-19, Anaj-18 and Ujala-16 were isolated, sterilized and inoculated with synthetic EPSPS (GT) gene construct under AGL1 strain. After inoculation calli were placed on Callus inoculation media (CIM). Out of

these 670 calli were cultured on AIM media and experiment is in progress. After herbicide selection 90 putative transgenic plants of variety Akbar-2019 and 22 plantlets of Ujala-16 were shifted to pots and DNA was extracted. PCR with GP1 gene/promoter specific primer amplified 1250bps size fragment in 38 plants of Akbar Variety where no plant of Ujala-16 variety was found positive for EPSPS gene. The transgenic plants will be further analyzed for herbicide tolerance and homozygosity.

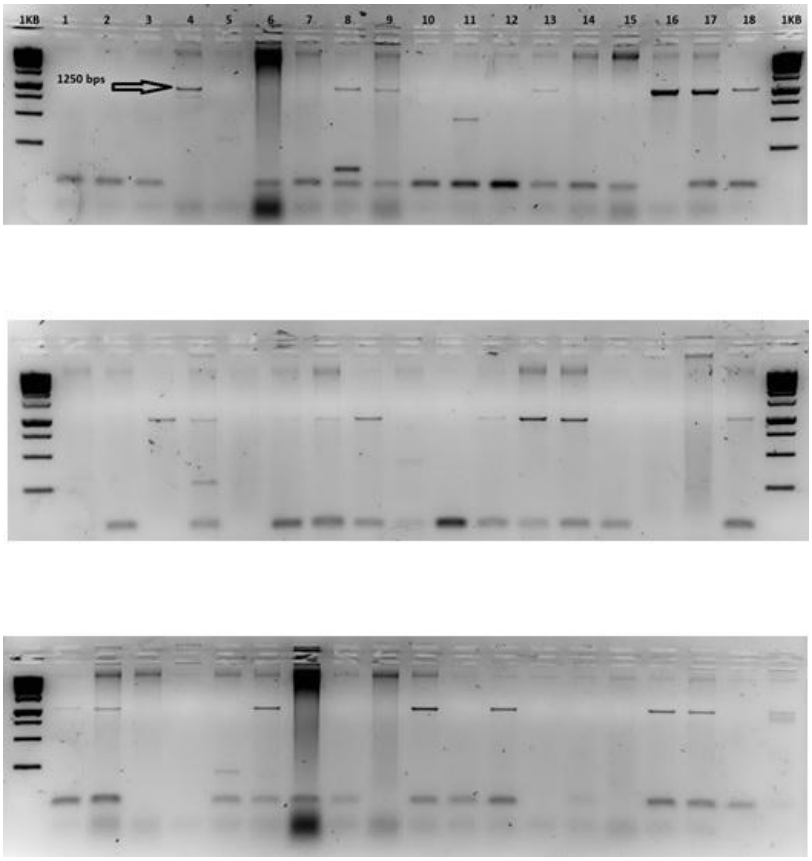


Fig 14: PCR analysis and confirmation of glyphosate herbicide tolerant transgenic events of wheat variety Akbar-19 through promoter-gene specific primers

22. Molecular detection of *Colletotrichum falcatum* causing red rot disease of sugarcane by using SCAR, URP and ISSR marker

Redrot is the single most destructive disease of sugarcane. Its causal pathogen is *Colletotrichum falcatum* having many races throughout the world. The pathogen initially enters to the plants through the soil and subsequently extends to the stalk by various ways, including borer, which makes the hole in the stem, as well as by other vectors in the field. Infection on leaves may not affect overall yield to a great extent but stalk infection with fungus is very severe as the sugar content is reduced after the infection. The pathogen *C. falcatum* normally resides in the soil as dormant spores and on decayed host plant parts as active saprophytes. Very little molecular identification work has been done to identify and understand the mechanism and spread and diversification of its races under different climatic conditions and in genetically different sugarcane genotypes. For this purpose an experiment was designed for the identification of *Colletotrichum falcatum* races and their pattern of spread through regions and varieties on molecular basis. Diverse molecular markers like SCAR, URP and ISSR were selected from latest international literature and primer sequences were synthesized from abroad. Redrot disease samples were collected from 6 susceptible genotypes of sugarcane and cultured on PDA media for colony development. After confirmation of purity of fungal colony, DNA was isolated from the fungus by using modified CTAB method. DNA was purified and quantified on Nanodrop Spectrophotometer. From fungal isolates the presence of *Colletotrichum falcatum* pathogen was confirmed through ITS1 (forward) and ITS4 (reverse) primers with the help of PCR machine. Marker ISSR-2 identified isolates Cf-234 and CF-375, Cf-gd and Cf-1a, Cf-2 and Cf-3 into three distinct strains. Fungal isolate Cf-285 was different from all other isolates. Whereas marker URP2R identified isolates Cf-234 and CF-375 as same strain of *C. falcatum*. However, on the basis of banding pattern all other strains were differentiated as separate strains. Marker URP2R was found to be most suitable for diversity analysis of *Colletotrichum falcatum* isolates under our local conditions.

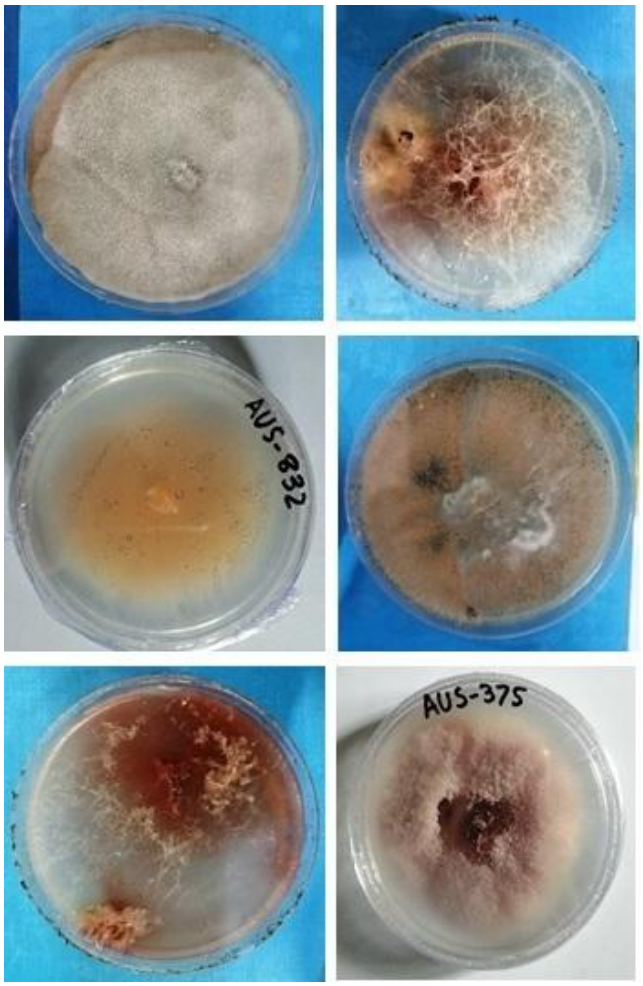


Fig 15: Colletotrichum falcatum fungal colonies isolated and cultured on PDA media

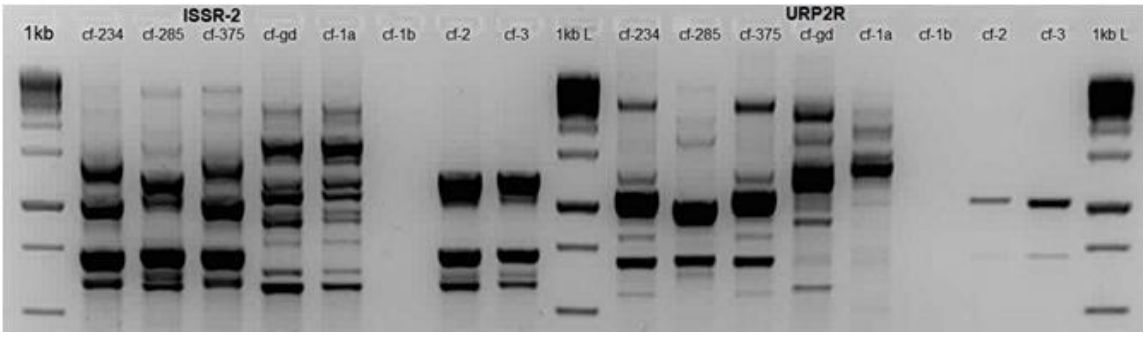


Fig 16: PCR analysis of various Colletotrichum falcatum isolates with two diverse molecular marker and identification of isolates on the basis of polymorphisum.

23. DEVELOPMENT OF DROUGHT RESISTANT WHEAT SOMACLONES THROUGH TISSUE CULTURE

Immature embryos and seeds of three wheat varieties (Galaxy, Ujala and Pasban-90) were surface sterilized with 10-20% clorex for 10-15 minutes followed by 70% ethanol and then rinsed three times in sterilized distill water in a laminar flow cabinet. Cultured tubes were placed in an incubation room at temperature 22-25C^0 in the dark at least 15-21 days. Callus of three wheat varieties were developed from mature seeds and immature embryos at two PEG levels (0.125 and 0.25mg/L) having 2, 4 D levels (2 and 5mg/L), respectively. Maximum callus induced in Pasban-90 and earlier shootings observed in Galaxy. Shoots initiation were observed maximum in MS medium having BAP (1.5mg/L) + Kin (0.5mg/L). Half strength (1/2 MS) medium was found best for maximum rooting. The plantlets were shifted into pots having peat and mud (1: 3), placed incubation room for hardening and then shifted into field. Total 53 somalclones were developed as mentioned below.

Table:11 Wheat Somaclone Developed Per Each Variety

Sr. No.	Name of variety	No. of somaclones
1	Galaxy	21
2	Ujala	19
3	Pasban-90	13

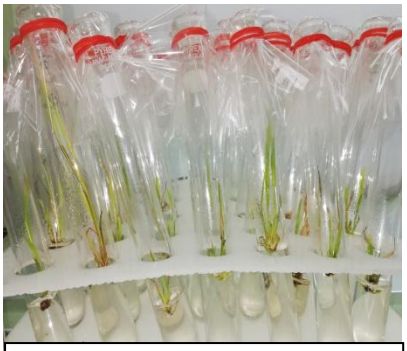


Figure: wheat shooting

Figure 17: wheat shooting in MS medium having BAP (1.5mg/L) + Kin (0.5mg/L)

24. OPTIMIZATION FOR MICROPROPOGATION PROTOCOAL IN DATE PALM

Two to three years aged suckers were detached from female date palm variety “Hilavi” present in ABRI field. Apical meristems portion separated from suckers and washed it with

running tap water for 1 hour. Phenolic compounds effects were controlled when apical meristem portion were dipped for 3 hours in ascorbic acid (1g/L) before surface sterilization. To control infection, apical portion washed with various levels of clorex (15, 20, and 25%) for 20 to 30 minutes followed by 70% ethanol then rinses three times in sterile distilled water. Best results obtained when washed 20% clorex for 30 minutes. Apical meristems cultured in different media using various levels and their combination of cytokinin (BAP and Kinetin). for shoots induction. Meristematic tissues of apicle meristem were also cultured for callus induction in MS medium having various levels of 2, 4 D (2, 3, 5 and10mg/L). Callus were developed less than 1% from meristematic tissues after four sub culturing in 2, 4 D (5mg/L) medium. The developed callus were shifted in regeneration media having MS with BAP (0.5 and 1 mg/L) + sucrose (30g/L). Some success of regeneration was observed from callus when cultured in MS + BAP (0.5mg/L) medium. Another technique was adopted by selecting female Inflorescence portion of date palm for callus induction at various levels of 2,4 D (0.5, 1, 2 and 3mg/L**)** and shooting in MS media having various phytho-hormones media (BAP and Kinetin) at various levels (0.5, 1, 1.5 and 2 mg/L), respectively. Inflorescence portion was sub-cultured for callus induction and shooting, however, this technique did not result significantly.

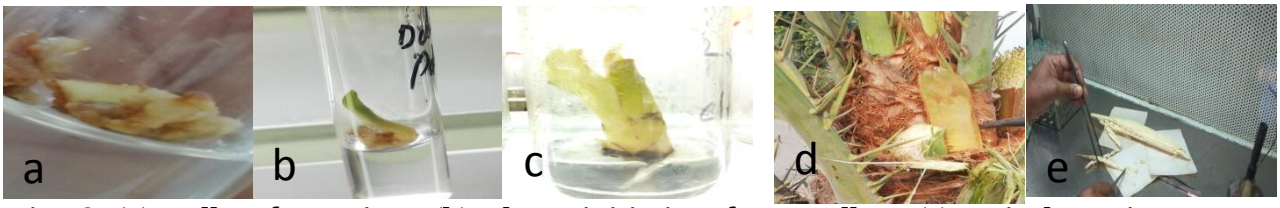


Fig:18 (a) callus formation, (b) shoot initiation from callus, (c) apical meristem regeneration, (d) female inflorence portion, (e) Inflorescence portion cultured

25. Maize-mediated doubled haploid production in wheat

Wheat segregated material (F_2) of the desirable cross was sown in field. Four maize varieties i.e. Malka-2016, Pearl, MMRI Yellow and Sadaf were obtained from Maize and Millet Research Institute, Yousafwala, and sown in tunnel with 8 days interval i.e. 8, 16, 24, 32, 40 and 48 days after 1st date of sowing (31 October, 2019) to ensure maize pollen availability. Two sets of maize varieties (Malka-2016 and Sadaf) also sown in glass house

with 15 days interval at ABRI. Wheat tillers were selected at booting stage for emasculation and cut from the base with appropriate length. All leaves were removed except flag leaf from the tiller and also detached central floret of each spikelet with the help of forceps to easily emasculate the lateral florets. One-third portion of lateral florets also cut from the top to accelerate the emasculation and pollination. Then anthers were removed with forceps and emasculated spikelets were covered with butter paper bag to avoid from outcrossing. Emasculated tillers were taged, mentioned date of emasculation/pollination, then kept in the tap water jar and placed in a growth room under controlled temperature 22-25C^0 and light 1500-2000 flux. After 3 days of emasculation, fresh maize pollens were collected in petri dishes and wheat spikelets were pollinated with the help of camel hair brush. Pollinated spikes were kept in pollinated media (40g/L Sucrose + 6% Sulfurous acid + 100mg/L 2, 4 D) for two days and these tillers were taken out from pollinated media and kept into growth media (40g/L Sucrose + 6% Sulfurous acid). Growth media changed every 72 hours for 10-12 days then haploid seeds were separated from spikes. Developed haploid seeds placed in a petri dish then seeds were surface sterilized with clorex, added two drops of tween-20 and placed for 15 minutes on water bath shaker. Haploid seeds were washed three times with autoclaved distilled water in laminar air flow cabinet. Haploid embryos was dissected under stereomicroscope, haploid embryo rescued and put into autoclaved half strength MS. The whole procedure was performed under a laminar flow cabinet. The pH of the half strength MS medium was adjusted at 5.8 by adding 1N HCl or 1N NaOH before autoclave. The media were then autoclaved at 15 psi for 20 min at 120EC. 134 haploid embryos rescued under stereo microscope and cultured in ½ MS media in laminar flow cabinet. 26 haploid plants were developed and treated with colchicine.

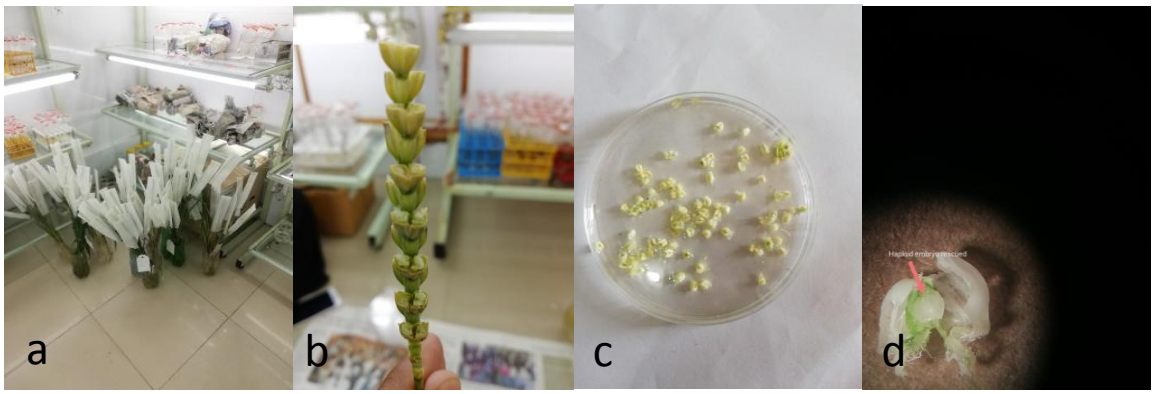


Fig: 19 Process of double haploid development in wheat
a) spikes in incubation room, b) haploid seed grow in spike, c) haploid seed, e) haploid embryo rescued

26. MAINTENANCE & UTILIZATION OF WHEAT GENEPOOL FOR CROSSING AND USE AS EXPLANT IN TISSUE CULTURE

The objective of study was to evaluate and maintain the germplasm available at the Institute for hybridization work and to use the desirable genotypes in various cross combinations for tissue culture to exploit somaclonal variation and gene pyramiding experiments. Gene pool available at this institute was maintained and evaluated for different cross combinations. 266 entries of the crossing block were sown twice with the interval of fifteen days to synchronize early and late varieties for successful crossing program. Each entry was sown in two rows of 2.5meter length. Data was recorded for various traits, like; Germination %age, Plant height, No. of tillers per plant, Days to heading & maturity, 1000 grain weight and disease scoring for leaf and yellow rusts etc. Two hundred sixty-six (266) entries were maintained and 114 fresh crosses were made.

Table.12 Morphological Data of Wheat Crossing Block

Trait	Minimum	Maximum
Germination (%)	90	100
Plant height (cm)	87	124
No. of tillers per plant		5
Days to heading	99	118
Days to maturity	137	154
1000 Grain weight (g)	38	58
Leaf rust	0	50S
Yellow rust	0	100S

27. EXPLOITATION OF SOMACLONAL VARIATION IN WHEAT FOR YIELD AND RUST RESISTANCE

The objective of this study was to develop of high yielding and disease free somaclones of wheat through callus culture derived from immature/mature embryo. There are three types

of medium are used for wheat tissue culture and explant source mature or immature embryos are used.
Ten to fifteen days after pollination immature seeds were obtained and sterilized with sodium hypochlorite solution. Excised immature embryo was cultured on MS medium supplemented with 2, 4 d. Calli were maintained by sub-culturing after about 30-40 days. Embryogenic calli after 4-5 sub-cultures were utilized for shoot formation. Shoots were separated and cultured on rooting medium for plantlets development. 560 embryo of different wheat varieties/crosses were cultured on medium for callogenesis and sub cultured the callus for organogenesis. Fifty wheat somaclones were developed and included in R1 generation.

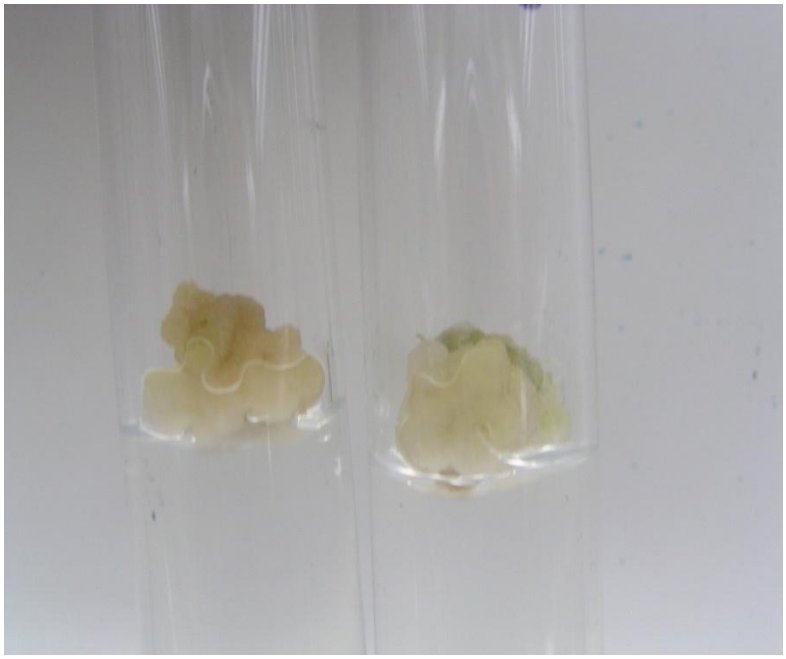


Fig.20 MS Medium supplemented with 2, 4 D

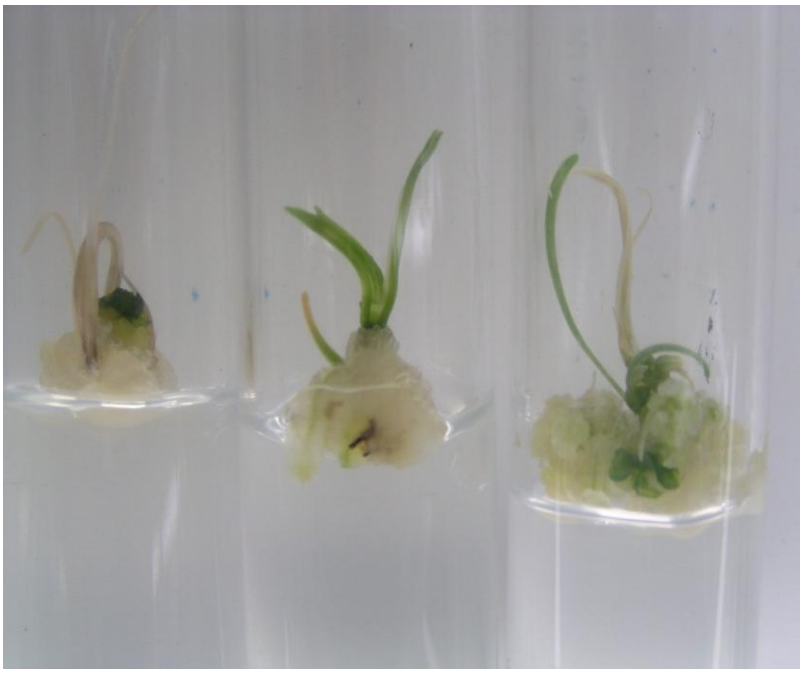


Fig.21 MS Medium supplemented with Kinetin

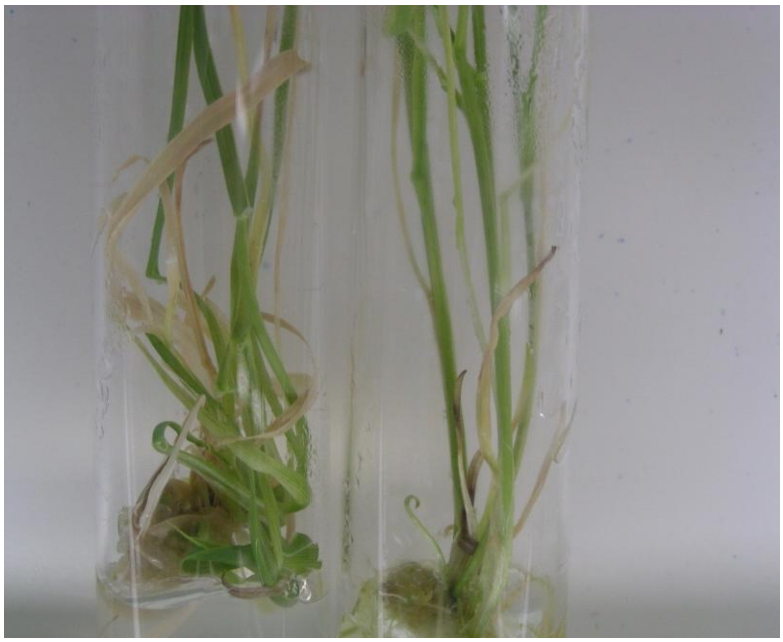


Fig.22 Wheat Somaclone Cultured on Rooting Medium Supplemented with NAA

Fig.23: Hardening of Wheat Somaclone in Pots

28. Somaclonal Variation studies in (R1-R6) generation on the basis of rust resistance and other morphological characters.

The objective of this study was to evaluate and selection of somaclones on the basis of rust resistance and other desirable traits. 447 entries of R1-R6 generation were sown for evaluation for rust resistance and other morphological parameters. Entries were sown in non replicated design. Plot size was kept in one to six rows of 2.5m length. 591 entries in various generations were studied and 320 entries were selected on the basis of rust resistance and morphological parameters.

Fig.24 Screening of Wheat Genotypes against rusts

Table 13: Selection of Somaclone on the basis of disease resistance

Sr. No	Somaclonal Generations	No of Somaclones/Entries studied	No of somaclones/Entries selected
1	R1	130	115
2	R2	152	62
3	R3	115	28
4	R4	60	33
5	R5	54	31
6	R6	80	51
	Total	591	320

29. PRELIMINARY WHEAT YIELD TRIALS (A-TRIALS)

This experiment was designed to study the yield performance of promising lines selected from advanced generations of wheat. Twenty-eight regenerated promising lines including two checks (Faisalabad-08 and Anaj-17) were sown in RCB design with three replications. At harvesting and threshing data on grain yield was recorded.

Table.14 List of Wheat Advance Lines with Grain yield

Sr. No	Type of Trial	Variety Code	Grain yield (Kg/ha)
1	A-I	19BT002	4750
2		19BT001	4527
3		19BT012	4500
	Check	Faisalbad-08	4111
	Check	Anaj-17	4277
			Cd=116
	A-II		
1		19BT020	4750
2		19BT016	4583
3		19BT022	4527
	Check	Faisalabad-08	4166
		Anaj-17	4333
			Cd=134

30. REGULAR WHEAT YIELD TRIAL (B-TRIALS)

Fourteen wheat advance lines including two checks (Faisalabad-08 and Anaj-17) selected from A-trials were tested in B-trial for yield performance and other morphological parameters. The experiment was sown in RCB design with three replications. Two advance lines i.e 18BT017 and 18BT026 were found high yielder than checks were sent to Punjab Uniform Wheat Yield Trial.

Table.15 List of wheat Advance Lines with Grain Yield

Sr. #	Type of Trial	Variety Code	Grain yield (Kg/ha)
1	B-Trial	18BT017	4833
2		18BT026	4416
	Check	Faisalabad-08	4194
	Check	Anaj-17	4333
			Cd=137

31. MICRO WHEAT YIELD TRIAL

The experiment was sown in RCB design with three replication keeping row to row distance 30 cm. Fifty entries received from Wheat Research Institute, Faisalabad were studied for yield performance. Three wheat advance lines i.e 16BT015, 15BT001 and 15BT019 were surpassed the check varieties were sent to NUWYT trial.

32. **Screening of Wheat Germplasm against Rusts**

Forty wheat advanced lines were screened out against rust at ABRI, Faisalabad in field. When crop was at tillering stage rust inoculums of leaf and yellow rusts were sprayed on the wheat crop and rusted leaves were also rubbed on each entry. After every ten lines morocco was also planted as a spreader. Field observation was recorded for rust initiation and development. Amongst Forty entries, 13 entries showed immune response to leaf rust and 5 entries to yellow rust, whereas 12 entries showed resistant response to leaf rust. 15 wheat entries showed resistant response to yellow rust, 10 and 8 entries showed moderately resistant response to leaf and yellow rust. 5 entries were found susceptible to leaf rust and 12 entries were found susceptible to yellow rust.

Table.16 Response of wheat Varieties/Lines against rusts

Total lines= 40		**Response**
LR	YR	
13	5	Immune
12	15	Resistant
10	8	Mod. Resistant
5	12	Susceptible

Fig.25 Wheat varieties/lines screening against Rusts

33. Screening of advanced wheat material against rusts at SARS, Kaghan

Forty-eight entries showing resistant disease reaction against rusts harvested from Kaghan were sown at ABRI, Faisalabad to record data of rust disease incidence. The data recorded showed that 26 and 20 entries showed immune response to leaf and yellow rust, 20 and 19 entries showed resistant response against leaf and yellow rusts, 2 and 9 entries were found susceptible response to leaf and yellow rusts.

Table 17: Wheat advanced lines screened against rust disease

Leaf Rust	Yellow Rust	Response
26	20	Immune
20	19	Resistant
2	9	Susceptible

Fig.26 Wheat varieties/lines screening at Kaghan

34. Use of Soma clonal variation for sugar cane improvement

The objective of this study was to develop the resistant soma clones against red rot and other desirable characters. Variation in sugarcane was induced for red rot and other desirable characters such as sucrose %, height, number of tillers, tonnage, and diameter etc. using callus culture. Callus of three sugarcane genotypes CPF-248 and SPF-234 were developed on MS medium having 3mg 2, 4-D, sucrose 30g, pH 5.7 for mutation. Meristem of sugarcane collected from Sugarcane Research institute, Faisalabad for callogenesis and sterilize them with ethanol under aseptic conditions in air laminar flow cabinet. Culture was done and incubated for 14 days under dark conditions and after formation of callus it was sub cultured four to five times and shifted to regeneration medium for shoot development. 190 plants of SPF-234 and CPF-248 were developed and shifted in field for further studies.

Table.18 Sugarcane somaclone shifted in Field

S. No.	Lines	Plants developed and shifted in field
1	SPF-234	140
2	CPF-248	50
	Total	190

35. Screening of wheat germplasm against Karnal Bunt

The objective of this experiment was screening against karnal bunt. Inoculum of karnal bunt was isolated from bunted seed on PDA media. The inoculum was prepared in distill water Bunted seed were taken and soaked in distill water and shaked well to get spores in the water after removing the seed and added 2 percent sodium hypochloroite solution and centrifuged at 3000 rpm two to three times repeatedly. Decanting the upper phase water took the pellet from falcon tube and washing the pellet with autoclave water two to three

times and finely sterilized 70 percent ethanol under sterilized conditions. Then take one micro litre and poured it on the PDA media and incubated at 20 degree centigrade for the growth of required spores of disease. Inoculum of karnal bunt was injected to the ten heads of 42 entries sown in A and B trial of wheat. 42 wheat lines in A-1, A-2 and B trials were tested against karnal bunt. Most of the wheat entries showed no disease reaction. 0.5 to 1 percent bunted grains were recorded in 19BT005, 19BT008, 19BT018, 19BT023, 18BT014 and 18BT020.

36.Screening of sugarcane regenerated lines against red rot under field conditions

The objective of this study was to select the resistant plants against red rot and other desirable character under field condition. 190 plants SPF-234 and CPF-248 were developed and planted in field for evaluation against red rot resistance and other economic characters. Median inter nod of six months old standing canes were inoculated by conidial suspension of red rot fungus (Colletotrichum falcatum) with the help of syringe. Inoculated canes were harvested after four months of inoculation and spread of disease was recorded on the basis of crossing of inter nodes. Somaclonal seed of SPF-234 developed at this Institute was handed over to Sugarcane Research Institute, Faisalabad for sowing at three locations SRI, Faisalabad, SRS, Khanpur and SRS, Sargodha to assess genetic resistant against red rot disease in sugarcane somaclone variety SPF-234.

37. IN-VITRO PRODUCTION AND QUANTIFICATION MICROBIAL MEDIATED BIO-TRANFORMATION OF 2-ACETYLE-1-PYRROLINE

Among aromatic rice cultivars basmati rice are one of the most important component of modern agriculture. Basmati rice are rich in 2-acetyl-1-pyrroline (2AP) a secondary metabolite, principally responsible for the aroma production. Several factors including genetics as well as edaphic governed the aroma production in basmati rice. Genome analysis showed that, aroma in rice is controlled by a recessive, monogenric inheritance and cytoplasmic indented gene. Studies suggested that an eight base pair deletion inexon 7 or a seven base pair deletion in exon 2 of *badh2* gene on chromosome 8 results in a loss of function of BADH2 results in 2AP accumulation in rice. However the non-functionality of gene (*badh2*) controls the accumulation of GABald/Δ1-pyrroline, the immediate precursor of 2AP Expression of four genes including betaine aldehyde dehydrogenase 2 *(badh2),* glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) triose phosphate isomerase *(TPI*) and Δ1-Pyrolline-5-carboxylic acid synthetase (P5CS) are mainly responsible for the enhancement of 2AP in aroma producing rice varieties. Non-functionality of gene (*badh2*) controls the accumulation of GABald/Δ1-pyrroline, the immediate precursor of 2AP (Deshmukh Khare and Patra 2016). The rate controlling factor in 2AP synthesis is the presence of GABald/Δ1-pyrroline that is further regulated by the lysis of polyamines and proline. Adams and Kimpe 2007). Therefore present study was carried out with the objective of detection and quantification of aroma producing 2AP from microbial origin Microbial isolates were collected from research rice belt. These isolates will be maintained on plate

count medium and tryptic soya agar (TSA) medium at 30ºC for 48 h. After colony development, **2-Acetyl-1-Pyrroline** were detected and quantified through **GC-MS.** Rhizosphere samples from rice belt were collected and purified.These isolates were supposed to be *Bacillus cereous* on the basis of biochemical testing.Furthermore, these were undergone biochemical testing for said compound i.e. 2-Acetyl-1-Pyroline on GC-MS in UVAS Lahore. 2AP contents of rice ranged from 64.40 to 79.67 ug/kg/ppb. Maximum 2 AP contents were recorded in Isolates Number 4 i.e. 79.67 ug/kg/ppb compared with control. While other values are 74.73 (Isolate 5), 71.36 (Isolate 3) 67.66 (Isolate 2). These isolates were preserved and multiplied for pot experiment. .

Table.19 Quantification of 2-Acetyl-1-Pyroline

Treatments	**2-Acetyl-1-Pyroline ug/kg/ppb**
Isolate 1	64.40 d
Isolate2	67.66cd
Isolate3	71.36bc
Isolate4	76.67a
Isolate5	74.73ab

38. MICROBIAL AIDD BIOSYNTHESIS OF 2 ACETYLE-1-PYRROLINE IN BASMQTI AND NON-BASMATI RICE

Rice (*Oryza sativa)* is second most consumed crop worldwide after wheat. People in Asia and Africa consume rice directly to fulfill their daily energy requirements. From nutritional point of view it ranks 2nd among staple food crops. In international trade market scented rice are of great demand because unlike most of the food, rice are consumed as whole without any postharvest processing. Therefore, sensory properties especially aroma is high desirable by the consumers and small changes in aroma and flavor may increase or decrease the market of rice. Among aromatic rice cultivars basmati rice are one of the most important component of modern agriculture. Basmati rice are rich in 2-acetyl-1-pyrroline (2AP) a secondary metabolite, principally responsible for the aroma production. Several factors including genetics as well as edaphic governed the aroma production in basmati rice. Genome analysis showed that, aroma in rice is controlled by a recessive, monogenric inheritance and cytoplasmic indented gene. Studies suggested that an eight base pair deletion inexon 7 or a seven base pair deletion in exon 2 of *badh2* gene on chromosome 8 results in a loss of function of BADH2 results in 2AP accumulation in rice. Non-functionality of gene (*badh2*) controls the accumulation of GABald/Δ1-pyrroline, the immediate precursor of 2AP.The mutualistic relationship between rhizosphere dwelling bacteria and host plants interactions play an essential role in sustainable plant growth and development.The biological means to modify plant metabolism in order to improve plant growth and aroma quality in rice seems reliable. The principal aroma producing compound

2AP was first reported by *B. cereus* (ATCC27522) from cocca bean fermentation boxex. The occurrence of 2AP has been reported in a range of living system from prokaryotes to higher plants. The biochemical pathway of 2AP synthesis showed that in non-aromatic rice functional *badh2* gene converts AB-ald to GABA instead of Δ1-pyrroline. In non-aromatic rice varieties, the expression of badh2 is 10 times less compared to aromatic. The concentration of 2AP in scented rice is rages between 0.04–0.09 ppm while in non-scented rice it is almost <0.006–0.008 ppm. So the present study was designed to produce and enhance characteristics aroma in basmati and non-basmati rice through microbial inoculation

Microbial isolates was collected from rice belt. These isolates were maintained on plate count medium and tryptic soya agar medium at 30ºC for 48 h. After colony development, isolates was multiplied on LB media in broth culture. For pot experiment, seed of locally available basmati and non-basmati cultivar or advance line were collected. Rice seedlings were inoculated with bacterial cultural. Transplanted seedlings will be inoculated with bacterial culture once per week till panicle initiation. Nutrient application was done through Hoagland's Media. Necessary cultural practices will be done. On maturity, **2-Acetyl-1-Pyrroline** analysis were performed through amino-acid analyzer/ GC-MS**.**

Isolates were undergone quantification of 2-Acetyle-1-Pyrolline. Five best isolates were selected for pot experiment, Rice variety both Basmati i.e. 515 and Non-Basmati i.e. 386 were collected from local market and nursery was grown. Three weeks old nursery was transplanted and inoculated with selected isolates once per week. Recommended dose of fertilizer i.e. micro and macro nutrients were applied. Crop was harvested at maturity.

Table 20: effect of microbial isolates in enhancing 2AP contents of Basmati and Non Basmati Rice

Sr. no	**Plant Height Cm**	**Grain Yield G**	**2 AP ug/kg**	**Root FW g**	**Root DW g**	**Shoot FW G**	**Shoot DW G**
Control Basmati	63.73 efg	7.18 fg	124.63 c	6.94 fg	1.46 h	29.93 ef	14.96 gh
T1 (Isolate)	67.78 b-e	7.72 def	135.17 bc	7.36 def	1.70 ef	32.67 de	16.34 efg
T2	69.57 bcd	8.10 cde	138.93 b	7.66 cd	1.80 de	34.26 bcd	17.28 de
T3	71.68 bc	8.42 bc	131.87 bc	7.92 bcd	1.99 c	37.00 ab	18.86 abc
T4	**78.62 a**	**9.28 a**	**153.83 a**	**8.76 a**	**2.56 a**	**39.42 a**	**20.13 a**

T5	73.05 b	8.74 abc	141.90 ab	8.28 abc	2.30 b	35.73 bc	18.23 bcd
Control Non Basmati	58.50 g	6.90 g	71.30 f	6.44 g	1.21 i	27.72 f	14.44 h
T1	62.08 fg	7.73 fg	73.26 ef	7.02 dfg	1.52 gh	30.25 ef	15.70 fgh
T2	64.06 def	7.64 ef	77.43 ef	7.36 def	1.64 fg	31.73 de	16.65 ef
T3	72.47 b	9.05 ab	75.16 ef	8.31 ab	2.17 b	36.47 b	19.39 ab
T4	66.30 c-f	8.31 cd	**80.73 a**	7.63 de	1.78 def	33.09 cd	17.49 cde
T5	67.57 b-f	8.61 bc	70.10 f	7.89 bcd	1.90 cd	34.26 bcd	18.13 bcd

39. BIOCHAR AND PHOSPHATE SOLUBILZING BACTERIA FAVOR P AVAILABILITY AND GRWTH IN MAIZE

Introduction

Plant, microbe and soil interactions play vital role in soil fertility and plant wellbeing. The success within the utilization of the useful microorganisms requires an excellent understanding of the complex interactions taking place between the distinctive components of the complex plant-soil microorganisms. Soil, plant and bacterial interactions are complex wonders. P as a mineral supplement in terms of quantitative plant prerequisite it is the second most critical component after nitrogen. In spite of the fact that it is copious in both organic and inorganic forms, its accessibility is confined to plants because it happens for the most part in insoluble forms. In this way, the discharge of these fixed and insoluble forms as soluble forms could be an exceptionally critical figure in expanding soil P accessibility. Soil microorganisms play a key part in soil P dynamics and ensuing accessibility of phosphate to plants. It is regularly a restricting mineral supplement for numerous agricultural crops since it is copious in numerous soils but in ineffectively assimilable forms. Plants utilize lesser amounts of applied phosphate fertilizers and the rest is quickly changed over to insoluble complexes. This leads to a lasting requirement for chemical inputs that create economic and ecological complications. In calcareous soils Calcium forming calcium phosphates (CaP), which is ineffectively soluble, is used to fix the soluble phosphates. Bacteria from *Pseudomonas* are among the foremost capable PSB. It is important to investigate and identify region specific microbial strains which can be utilized as potential plant growth promoters to attain higher yields under specific environmental and natural conditions. Phosphate solubilising Microbes (PSB) in soils make the insoluble forms of P into dissolvable forms through different mechanisms and make them accessible for plant uptake there by advancing its development. Bacteria efficiently help out in P solubilization than fungi. PSB are omnipresent and differ in shape and population in different soils. Chemical and physical properties, organic matter and P content of the soil have great impact on their

population. PSB have phosphate solubilizing capability and they can change over the insoluble phosphatic compounds into soluble forms in soil making them available for plants to take them in. Biochar is a carbon rich product and highly porous charcoal substance that is distinguished from other charcoals in its intended use as a soil amendment. It is produced after the pyrolysis process of any biomass feedstock. Additions of biochar to soil have shown definite increase in the availability of phosphorus. The immediate positive effects of biochar additions for nutrient availability may be due to higher P availability. Many studies have shown improved P uptake as result of biochar application. Various mechanisms, including: biochar as a source of available and exchangeable P; ameliorator of P complexing metals (Ca^{2+}, Al^{3+} and Fe^{3+2+}); modifier of soil pH and as a promoter of P mineralization and microbial activity. Biochar is a store of P bound to surface sites through its anion exchange capacity. Total reported P in biochars ranges from 0.27–48.0 %. The present work focused on evaluating the potential of biochar and PSB in maximizing P availability and plant growth Phosphate Solubilizing Bacteria belonging to *Pseudomonas* isolated from Rhizosphere of Rice, Wheat and Maize grown in Ayub Agriculture Research Institute, FaisalabadSoil samples from cereals like maize, wheat and rice, vegetable like Eggplant, legumes including mash bean, mung bean and cotton crop were collected. Microbial isolates were purified on LB, Nutrient agar and selective media for Bacillus and Pseudomonas. PCR based species identification was done. Wheat straw biochar was prepared at low temperature i.e. 350-400ºC. Soil was amended Biochar. At maturity all growth, yield and nutrient elemental analysis were done. For microbial community analysis Gram staining, Oxidase, Catalase tests, DNA isolation and PCR based detection were performed.

Table 21: Results: Isolation and characterization of Phosphate Solubilizing Bacteria

Isolate	Halo Zone Information	Solubilizing Index
PSB1	+++	2.93
PSB2	++	2.50
PSB3	+++	2.39
PSB4	+	1.79
PSB 5	++	2.25
PSB6	+	2.00
PSB7	++	2.05
PSB8	+++	2.22
PSB9	+	1.83
PSB10	++	2.12

Around 50 isolates were purified. Qualitatively, Halo Zone formation was considered positive for PSB. These isolates showed varying degree of Phosphate solubilization Index. Out of 50 isolates 10 were considered as best with PSI ranging from 1.73-2.93. Pot experiment was conducted using selected PSB on the basis of biochemical results. Treatments i.e. Biochar and PSB, were applied following treatment plan. Crop was harvested at maturity. Agronomic and yield parameters were recorded. A significant

increase in all growth parameters was observed. Besides, P contents of Grain and Olsen P were also significantly increased when biochar was applied with microbial inoculum.

Table 22. Effect of Biochar and PSB on P solubility and growth of Maize

Sr. no	RFW G	RDW g	SFW g	SDR g	Plant Height cm	Root P mg/kg	Shoot P mg/kg	Grain P mg/kg	Olsen P mg/kg
Control	4.43 g	1.27j	11.51h	2.80h	55.76j	2.41g	1.46 i	1.15d	7.54 f
PSB1	5.50f	1.56hi	12.11gh	3.11fgh	79.79cde	2.54g	1.62hi	1.31c	7.89def
PSB2	4.72fg	1.46i	12.76fg	2.89gh	63.66 i	2.68fg	1.75gh	1.38c	7.99c-f
PSB3	5.25ef	1.65gh	13.46 ef	3.25fg	66.72 hi	2.94ef	1.88fg	1.57b	8.15b-f
PSB4	6.28d	1.88ef	14.64de	3.68 e	73.68efg	3.47cd	2.36c	1.69b	8.13b-e
PSB5	5.68 e	1.73fg	12.74fg	3.36ef	69.04ghi	3.15 e	2.04 ef	1.93a	8.79ab
BC+PSB1	6.65d	1.80fg	14.51de	3.59e	72.61fgh	3.21de	2.09cd	1.27e	7.72 ef
BC+PSB2	7.83bc	2.15cd	14.97d	4.14c	80.03bcd	4.12b	2.43c	1.57a	8.16b-f
BC+PSB3	7.60c	2.01de	15.71cd	4.05d	77.87def	3.65c	2.29cd	1.69cd	8.13b-e
BC+PSB4	8.13bc	2.26bc	16.52bc	4.62bc	85.16abc	4.29b	2.62b	1.83bc	8.44a-d
BC+PSB5	9.01a	2.43a	17.89a	5.17a	91.30a	4.65a	3.06a	2.19a	9.07a
BC+ Cconsortia	8.43b	2.30ab	16.99ab	4.76b	87.60ab	4.38ab	2.75b	1.90b	8.61abc

40. POTENTIAL OF ENDOPHYTES FOR THE GROWTH PROMOTION OF SUNFLOWER AND RAYA

Endophytes colonize plants without causing any negative effects on them. They share a symbiotic relationship with their host and exert beneficial effects on them. They use various direct and indirect mechanisms to promote plant growth. A number of culture dependent and independent methods have been used to isolate and study endophytic bacteria. The endophyte communities isolated mainly belong to *Pseudomonas and Bacillus* Bacteria utilize certain traits to colonize plants. In this colonization process various compounds released by both plants as well as bacteria play a major role. The plant growth promoting effects of bacterial endophytes have been seen in numerous important crops and it has been observed that they confer protection from various environmental stress factors as well. The current study was designed to evaluate the efficiency of endophytic bacteria on growth and yield of sunflower and Raya.

Root and stem samples of different crops were collected. After sterilization endophytic bacteria capable of plant growth promotion were isolated and screened for P-solubilization, IAA production and other biochemical tests. Root-shoot elongation assay were conducted to check the growth promotion of crops in lab. Five isolates were tested for pot/field study. Trail was conducted in collaboration with Soil Bacteriology section AARI, Faisalabad. Yield data was recorded on maturity.

There was a significant increase in growth and yield of Raya under Endophyte inoculation compared with control. Maximum increase in yield was observed in T2 i.e. 1633. However, overall results were non-significant among treatments. A significant increase of 6.8% yield was recorded.

Table 23: Increase in growth and yield of Raya under Endophyte inoculation compared with control

Treatments	Yield Kg/ha
Control	1528ab
ENDO 1	1609ab
ENDO2	1633a
ENDO3	1626ab
ENDO4	1629ab
ENDO5	1619ab

There was a significant increase in growth and yield of Sunflower under Endophyte inoculation compared with control. Maximum increase in yield was observed in T4 i.e. 1746. However, overall results were non-significant among treatments. A significant increase of 7.9% yield was recorded.

Table 24: Growth and yield of Sunflower under Endophyte inoculation compared with control

Treatment	Yield kg/ha
Control	1618ab
ENDO 1	1719ab
ENDO2	1699ab
ENDO3	1732ab
ENDO4	1746a
ENDO5	1705ab

41. Testing of Bio-fertilizer/Bio-stimulants as reference lab for confirmation

Biofertilizers are “microbial inoculants” that stimulates soil biota hence improves pysico-chemical and biological properties of soil. Bio fertilizers are in-expensive renewable source of plant nutrients that supplements chemical fertilizer and enhance their effectiveness. Bio-fertilizer may contains only one type of microbes or consortia of PGPR. Mostly bio-fertilizers contain diaztrophs, phosphate solubilizes, cellulose degrader or mycorrhizae as a source material in order to prepare low cost medium for plant growth promotion. Institute of Agricultural Biotechnology Research Institute has been declared as Reference Lab and receives 30% fit samples of biofertilizer/ biostimulant for re-analysis. With the objective tofind out bacterial growth in bio-fertilizers/bio-stimulants. Standard methods were used to

analyze samples. Nine samples including 07 of Bio-stimulants and 2 of Bio-fertilizers were received and re-analyzed and results were sent to the concerned authority.

SOIL BACTERIOLOGY SECTION, AARI, FAISALABAD

LIST OF CONTENTS

LIST OF TABLES

INTRODUCTION

Soil is not a simple object providing nutrients and support to plants. It is diversified ecosystem accommodating micro and macro life including bacteria, fungi, protists, and animals. All this creation is in an unabated assortment of collaborative, competitive, antagonistic and mutualistic interfaces. Diversity of plants grown in the soil exerts numerous actions, reactions with these soil inhabitants. The whole components of this system collectively help food production. Since last century the efficiency of soil was increased with the help of chemical fertilizers which in turn posed threats to the environment and variations of biogeochemical cycles of the Sphere, through soil degradation, water pollution and greenhouse menace. Resultantly researchers have shifted their focus to the organic nutrient sources for crop production to feed the 750 billion people and to make good for environmental losses.

To enhance plant growth, microbes handle the hormonal signalling of plants, prevent or repent pathogenic microbial strains and increase the bioavailability of recalcitrant soil-borne nutrients. Most of the nutrients like N, P and S are bound or fixed with organic compounds and leas available to plants. Hence, microbes like bacteria and fungi make them available through their metabolic activities and mineralize or depolymerize organic bound N, P, and S including ionic species like NH_3^+, NO_3^{--}, PO_3^{--} and SO_4^{--} that are taken by plants. In natural system the transformations of these naturally occurring microbial nutrients, play main role in the plant growth.

Taking into consideration the role of microbial activities in soil and rhizosphere of plants, all research trials and other relevant activities were designed and conducted. Various operations including BNF, organic matter recycling, effect of anti-biological agents on microbial activity under various sets of conditions and transformation of inorganic and organic materials into their available forms for plants, were undertaken.

The sectional staff, during the year under report remained engaged in the selection of efficient strains of Rhizobia for different legumes such as berseem, lucerne, mung, mash, chickpea, vegetable pea and lentil etc. Studies on phosphor-bacterium (*Bacillus* spp.), P-solubilizing soil microbes and pesticides were also included in different experiments. Studies on associative N fixers like *Azospirillum* and *Azotobacter* were also carried out

under various sets of conditions. Artificial inocula of different N fixers and P solubilizers were also prepared for different crops to distribute among the farming community.

2. BUDGET

SR. #	DETAILS	ALLOCATION	EXPENDITURE
1	A011-1 Pay of officers	4,417,026/-	4,417,026/-
2	A011-2 Pay of establishment	2,376,210/-	2,531,570/-
3	A012-1 Regular allowances	6,882,790/-	6,998,853/-
4	A012-2 Other allowances	-	-
5	A03-Operating Expenditure. (Contingencies)	2,894,206/-	2,889,690/-
6	A13-Repair & Maintenance	659,690/-	659,611/-
7	A09470-Others	180,390/-	180,297/-
	Total:	**17,410,312/-**	**17,677,047/-**

3. RESEARCH WORK

3.1. SCREENING OF CROP SPECIFIC EXOPOLYSACCHRIDE PRODUCING PGPR FROM DROUGHT PRONE AREAS OF THE PUNJAB

INTRODUCTION

A group of bacteria that can be found in the rhizosphere, which is advantageous in improving the growth of plants, can be classified as plant growth-promoting rhizobacteria (PGPR). *Pseudomonas, Azospirillum, Azotobacter, Klebsiella, Enterobacter, Alcaligenes, Arthrobacter, Burkholderia, Bacillus,* and *Serratia* have been reported as PGPR able to promote plant growth. Bacteria belonging to the PGPR are one of high producing IAA. The PGPR are the potential IAA-producing bacteria. IAA-producing bacteria help some physiological processes of plants by inserting IAA to plants which induce growth of the plant. The effect on crops goes on changing IAA concentration thus helps in the formation of lateral roots, adventitious roots and primary root elongation. Condensed root can increase the nutrients quickly, thus increasing plant growth. Rhizosphere bacteria are also able to secrete siderophores which dissolve phosphate, and act as a bio control to protect crops from fungi, pathogens, and pests.

Drought is one of the main problem which limits agricultural productivity in the arid region. Limited water availability in the soil can inhibit the absorption of nutrients by the roots of plants. Drought can effect growth of microorganisms and plants as the water deficiency restrain the survival of plants and microorganisms. Adaptation of microbes to drought causes secretion of exopolysaccharides (EPS) in high quantities and also secretes high molecular weight compounds in the environment, known as the extracellular polymeric substances or EPS. These remain attached with the outer surface of bacterial cells and release during cell growth into its medium. The EPS material mainly comprises of polysaccharides but also contains non-sugar components such as proteins and nucleic acids.

Like many higher organisms, bacteria produce a variety of polysaccharides as part of their normal metabolic process. These bacterial polysaccharides can be divided into three main groups: (i) intracellular polysaccharides, whose primary function is carbon and energy storage within the cell, (ii) structural polysaccharides, which provide physical structure to the bacterial cell wall, and (iii) extracellular polysaccharides, which are secreted outside of the cells, and are the focus of this work. While polysaccharides are the major component of bacterial EPS material, the EPS matrix also contains such non-sugar components as proteins and nucleic acids. These bacterial exopolysaccharides are synthesized in two basic forms as capsular EPS and slime EPS that can be distinguished on the basis of degree of association of the cell surface.

Bacteria are able to survive under stress due to production of such compounds. EPS is a structural component of the extracellular matrix in biofilms that are synthesized by cells of microorganisms in response to physiological stress on the environment. EPS have important role for bacteria to protect against a broad range of environmental stress. Such as the Bacterium Pseudomonas sp. which increases the production of EPS during the dry season.

The role of microbial EPS in soil aggregation is well documented. The structurally diverse exopolysaccharides play different roles in soil and may get modified under biotic and abiotic stresses. Exopolysaccharides (EPS) have been reported to play a significant role in providing protection to the cell as a boundary, contributing to soil aggregation due to its gluing properties and binding heavy metals due to the presence of several active functional groups present it. The production of exopolysaccharides (EPSs) by bacterial populations in the rhizosphere has been demonstrated to contribute to water and nutrient uptake by plant

roots through the modification of the physical properties of rhizosphere soil. Amendment of soil with microbial EPS results in an increased soil aggregation.

MATERIALS AND METHODS

Isolations of exopolysaccharides producing bacteria were carried out in lab on specific medium. Characterization on the basis of different biochemical tests and IAA production was carried out. These isolates were further screened on the basis of root shoot elongation study carried out in lab on maize crop.

RESULTS

Out of 40 isolates, 24 isolates were positive for EPS production and this production was confirmed by ethanol test. Out of these 24 isolates, 9 isolates having high production of IAA, were selected and screened on the basis of root shoot elongation. Among these isolates PS-2 (A) showed maximum root (52.3 cm) and shoot (31.5 cm) elongation. CH-3 (A) and CH-1 (C) also showed increased root and shoot length as compared to control.

Table 01: Screening of EPS producing isolates in lab conditions

Isolates of different locations		With L-tryptophan	Without L-tryptophan	Methyl red Test	EPS Production	Ethanol Confirmation
		($\mu g\ mL^{-1}$)				
CH-1 (A)	EPS-1	7.74	5.20	+	++	++
CH-1 (B)	EPS-2	7.45	4.90	++	++	++
CH-1 (C)	EPS-3	10.45	6.03	-	+	+
CH-1 (D)	EPS-4	7.31	4.49	-	+	+
CH-1(E)	EPS-5	7.88	5.03	+	++	++
CH-1(F)	EPS-6	7.45	5.74	-	++	++
CH-2(A)	EPS-7	7.88	4.31	+	++	++
CH-2 (B)	EPS-8	10.02	5.88	+	++	++
CH-2 (C)	EPS-9	9.17	5.73	-	++	++
CH-2 (D)	EPS-0	7.15	3.79	++	+	+
CH-3 (A)	EPS-11	10.57	6.24	-	+	+
CH-3 (B)	EPS-12	7.21	4.17	+	++	++
CH-3 (C)	EPS-13	7.02	4.91	+	++	++
CH-4 (A)	EPS-14	8.45	5.21	+	++	++
CH- (B)	EPS-15	6.94	4.31	-	++	++
PM-1	EPS-16	7.11	4.01	++	++	++
PM-2	EPS-17	7.05	4.51	+	+	+
PM-3	EPS-18	9.02	5.27	-	+	+
PS-1(B)	EPS-19	7.02	4.27	+	++	++
PS-1 (C)	EPS-20	6.72	4.44	+	++	++
PS-2 (A)	EPS-21	9.45	5.86	+	++	++
PS-2 (B)	EPS-22	8.02	5.37	++	++	++
PS-2 (C)	EPS-23	7.17	4.87	+	++	++
PS-2 (D)	EPS-24	8.74	5.67	-	+	+

Table 02: Root-shoot elongation assay of EPS producing PGPR

Treatments	Shoot length (cm)	Root length (cm)	Fresh shoot mass (g)	Fresh root mass (g)
Control	19.6 F	27.8 H	8.9 H	4.0 G
CH-3(A)	28.9 B	48.6 B	15.0B	7.0 B
CH-1(C)	27.3 BC	45.2 C	14.3 BC	6.5 C
CH-2(B)	26.3 CD	41.9 DE	13.6 CD	5.7 E
PS-2(A)	31.5 A	52.3 A	16.2 A	7.5 A
CH-2(C)	23.6 E	31.6 FG	10.9 G	5.0 F
PM-3	25.1 CDE	39.1 E	13.0 DE	5.3 EF
PS-2(D)	26.9 BCD	42.3 CD	14.0 BCD	6.1 D
CH-4(A)	24.7 DE	29.8 GH	12.1 EF	5.3 EF
PS-2(B)	24.5 DE	33.6 F	11.8 FG	5.3 F

3.2. BIOCHEMICAL & MOLECULAR CHARACTERIZATION OF SOIL BENEFICIAL BACTERIA FOR THE SUPPRESSION OF PLANT PATHOGENS

The precise identification of bacteria is essential for the soil microbiological research. The identification of microbes can be carried out by various means. The phenotypic identification of microorganisms involves using apparent traits, including profiles of structural components such as lipids, sugars or amino acids, or storage compounds such as poly-β-hydroxybutyrate. An unidentified microbe can be identified from the mass spectrum produced when it is analyzed by matrix assisted laser desorption/ionization time of flight mass spectrometry. Microbes can be identified by determining their lipid compositions, using fatty acid methyl esters or phospholipid-derived fatty acids analysis. The study of accumulated proteins of microbes in solution culture can also be used for bacterial identification (Proteomic analysis). Glyco-proteins in the plasma membrane or cell wall structures can be used for microbial identification.

Soil microbial population plays vital role in soil properties and influences the rhizosphere processes. Microbial composition, variations and functions, amended the soil quality through decomposition of organic matter, recycling of nutrients, and biological control of plant pests. The identification of soil microbes may be translated into benefits for biotechnology, agricultural management, plant ecology, biodegradation of pollutants, and waste treatment systems, hence there is great need of scientists for isolation and characterization of variety of microbes. Soil microbial population plays key roles in nutrient cycles including nitrification, denitrification, sulfur and manganese oxidation, N_2-fixation

and carbon cycle. Besides many microbes releases chemicals or volatile compounds that are being explored in biotechnology. Soil bacteria are a potent source to produced biologically active substances and novel commercially important products.

Plant growth promoting rhizobacteria (PGPR) are the rhizosphere bacteria that may be utilized to enhance plant growth and suppress plant diseases. Rhizobacteria that benefit plants by stimulating growth and suppressing disease are referred as PGPR. PGPR have been tested as biocontrol agents for suppression of plant diseases and also act as disease resistance in plants. The widely known mechanisms of plant growth promotion by PGPR are production of phytohormones, fixation of nitrogen and phosphate solubilization. The mechanisms of biocontrol include competition with phytopathogens and production of inhibitory compounds and hydrolytic enzymes that are often active against a broad spectrum of phytopathogens. The main mechanisms adopted by PGPR to affect plant growth are phytohormone production, N_2-fixation, nutrient mobilization (P & Zn solubilization), protection of plants under abiotic stresses and biocontrol of plant pathogens. The bacterium possessing any one or more of these above mentioned mechanisms are termed as PGPR. Present study was designed to determine the effect of soil beneficial bacteria on the suppression of soil borne pathogens following the characterization (biochemical & molecular).

MATERIALS AND METHODS

The rhizosphere samples were collected from the two districts of the Punjab province having excessive usage of pesticides. Isolation of soil bacteria were carried out on nutrient agar and LB media from rhizosphere of different crops. Soil samples were collected from Sahiwal and Multan District. Isolations were carried out on LB and nutrient agar medium, the biochemical characterization of ten isolates were carried out for auxin biosynthesis, solubilization index, siderophore formation, and EPS production qualitatively. The remaining screening and testing is under progress. These ten isolates will also be assessed for plant growth promotion activities by bioassay (root-shoot elongation assay).

In the next phase, PGPR will be screened for production of secondary metabolites and characterized for their ability to synthesize HCN on TSA plates supplemented with glycine. Plant pathogens (*Fusarium sp.*) will be collected from Plant Pathology Section, Faisalabad on the respective medium. The antifungal activity of isolates will be assayed by the disc

diffusion method. Isolates exhibiting suppression in growth of pathogens will be used for further experimentation. Biochemical (biochemical tests) and molecular characterization (DNA Extraction, PCR analysis followed by sequencing) will be carried out to screen the efficient isolate. Promising / efficient isolates will be selected and screened for suppression of plant pathogens.

RESULTS

Out of 20 isolates, 10 isolates were screened for different biochemical tests viz. auxin biosynthesis, solubilization index, siderophore formation, and EPS production. The isolates showed variable response for IAA equivalents with and without L-TRP. The solubilization index (SI) was determined using Pikovskaya's medium and isolates showed variable response i.e. 2.93-3.39 while EPS production determined qualitatively. The CAS assay for siderophores halozone formation was also determined qualitatively. The Siderophore unit

Table 03: Some stats of biochemical screening of PGPR

District	Isolates	Auxin Biosynthesis ($\mu g\ mL^{-1}$)		SI*	Halo zone formation (CAS assay)	SU* (%)	EPS* Production
		L-TRP[+]	L-TRP[-]				
Multan	MT-1	5.154	2.846	3.32	+	24.4	++
	MT-2	5.538	3.000	3.34	+	31.6	++
	MT-3	5.769	2.923	3.09	++	38.9	+++
	MT-4	4.769	2.769	3.00	+	26.7	++
	MT-5	4.923	2.692	2.93	+	22.5	+
Sahiwal	SL-1	5.000	3.000	2.94	+	22.0	+++
	SL-2	6.000	2.846	2.97	++	10.2	++
	SL-3	5.769	2.769	3.19	++	22.2	++
	SL-4	6.538	3.462	3.31	+	36.6	+++
	SL-5	6.923	3.308	3.39	++	46.2	+++
SI: Solubilization Index**; SU:** Siderophore Unit; EPS: Exopolysaccharide							

3.3. MUTUALISTIC APPROACH OF *BRADYRHIZOBIUM*, PGPR AND *PIRIFORMASPORA INDICA* ON NODULATION AND YIELD OF SOYBEAN

INTRODUCTION

Nitrogen (N) and phosphorus (P) are among the most limiting nutrients for plant growth. Phosphorus is generally deficient in most of the soils due to its ready fixation. Inadequate P restricts root growth, the process of photosynthesis, translocation of sugars, and other such functions, which directly or indirectly influence nitrogen fixation by legume plants.

The replenishment of N and P nutrients is mostly done through application of inorganic fertilizer to the soil. However, prices of N and P fertilizers have increased, particularly in developing countries. Therefore, it is very challenging for farmers to supplement N and P fertilizers in the soil to avoid the nutrient deficiencies. Given the reported negative environmental impacts of chemical fertilizers and increasing costs, utilization of plant growth promoting rhizobacteria (PGPR) and rhizobia is advantageous for sustainable agricultural practices. Thus, one area of increasing interest is the use of microorganisms with the ability to solubilize mineral and organic P or to fix N. The association between PGPR and plant roots plays a key role in P nutrition in many agro-ecosystems, particularly in P-deficient soils.

The inoculation of plants with selected PGPR to increase native population can mobilize P from poorly available sources and therefore improve plant nutrition. Legumes growth and yields have been shown to increase with inoculation with Rhizobia. Co-inoculation of PGPR and *Rhizobium* stimulated plant growth more than their separate inoculations. Co-inoculation of *Bacillus* strains in soybean plants with *Brady rhizobium japonicum* provided the largest increases in nodule number, nodule weight, shoot weight, root weight, total biomass, total N and grain yield. Non-rhizobial plant growth promoting bacteria improves nodulation and grain yield of the legumes upon co-inoculation with crop specific rhizobia. Hence, endophytic plant growth promoting bacteria and N_2-fixing *Rhizobium* species synergistically promoted N_2-fixation efficiency in lentils.

The endophytic fungus *Piriformospora indica (P. indica)* is an arbuscular mycorrhiza like fungus, which colonizes the roots of many plants and improves plant growth, absorption of nutrients and plant tolerance to environmental stresses. Several studies have shown that P. indica can increase plant growth and tolerance to various stresses. The present study was planned to check the alone and combined effect of PGPR, *Bradyrhizobium* and *P. indica*.

MATERIALS AND METHODS

A field experiment was conducted at oilseed Research Institute, and pot trial at Soil Bacteriology Section AARI, Faisalabad to evaluate the mutualistic effect of *Bradyrhizobium*, PGPR and *P. indica* inoculation on growth and yield of soybean. Soil having pH 7.9, ECe 2.6, Organic matter 0.69% and available P 8.5 $mgkg^{-1}$ was used in field trial while in pot study soil characteristics were; pH 7.8, ECe 2.4, O.M. 0.67% and available

P 8.1 mgkg^{-1} . Treatments were control (T_1), *Bradyrhizobium* sp (T_2), PGPR inoculation (T_3) *P. indica* inoculation (T_4), T_2+T_3 (T_5), T_3+T_4 (T_6), T_2+T_4 (T_7) ,T_2+T_3+T_4(T_8). Crop was sown during Kharif 2019.

RESULTS

Results revealed that in field and pot trials, maximum biomass yield (7552 kg ha^{-1}, 37.7 g pot^{-1}) was observed in T_8 where combination of PGPR, *Bradyrhizobium* and *P. indica* was applied as compared to control. Highest plant height, gain yield was also observed in T_8 under both field and wire house conditions (70.0 59.0 cm, 3017 kg ha^{-1}, and 23.0 g pot^{-1}). Significant increase was observed in root length and IAA equivalents of all treated plants.

Table 04: Effect of *Bradyrhizobium*, PGPR and *P. indica* on growth and yield of soybean under field conditions

Treatments	Plant height (cm)	Biomass Yield (kg ha^{-1})	Grain Yield (kg ha^{-1})	IAA Equivalents (µg mL^{-1})
T_1: Control	51.00d	4666e	2073h	1.89
T_2: *Bradyrhizobium*	63.33c	5680c	2137g	2.94
T_3: PGPR	66.67b	5053d	2240f	2.42
T_4: *P. indica*	62.00c	5675c	2447e	3.84
T_5: *Bradyrhizobium* + PGPR	64.00bc	5866c	2687c	4.82
T_6: *P. Indica* + PGPR	62.33c	6048c	2533d	4.94
T_7: *Brady* + *P. indica*	62.00c	7003b	2837b	4.98
T_8: *Brady* +*P. indica*+ PGPR	70.00a	7552a	3017a	5.20
LSD	3.1541	375.73	49.481	5.67

3.4. EFFECT OF POTASSIUM SOLUBILIZING BACTERIA ON ENHANCING GROWTH AND YIELD OF WHEAT

INTRODUCTION

Feeding a growing world population, as projected to reach 9 billion by 2050, adopting more efficient and sustainable production methods, responding to increased concerns about managing the natural resources, and adapting to climate change and drought conditions are some of the significant challenges that agriculture will face in the 21st century. In order to feed the increasing world population, agriculture must be intensive and sustainable in the future. However, it is well known that the food production by agriculture cannot be generally sustained unless the nutrients removed from soil as a result of increased crop production are replaced. Many agricultural soils lack a sufficient amount of one or more of

essential plant nutrients so that plant growth is suboptimal. To avert this problem and obtain higher plant yields, farmers have become increasingly dependent on chemical sources of fertilizers. The chemical fertilizers in vogue may ensure the better plant grows but unluckily they do not help improve the properties of soil. It is an established fact that the incessant use of chemical fertilizers in single or integrated form of NPK fertilizers levies hazardous effects not only on the environment but also to the living creations of that environment. After N & P, the K is the most important plant nutrient that plays a key role in growth, metabolism, development and other numerous operations of plant's life cycle. In addition to increasing plant resistance to diseases, pests, and abiotic stresses, K is required to activate over 80 different enzymes responsible for plant and animal processes, e.g. energy metabolism, starch synthesis, nitrate reduction, photosynthesis, and sugar degradation. Potassium is the seventh most abundant element found in the earth's crust. Total K content in soils ranges from 0.04 % to 3.0 %. Although K is present as an abundant element in soil nevertheless, only 1 to 2% of this element, in solution form, is available to plants while rest of this major nutrient element binds with other minerals and hence transforms into unavailable forms for plants. Depending on soil type, from 90 to 98% of soil K is mineral K and most of this K is unavailable for plant uptake. It is necessary to find an alternative indigenous source of K and maintain K level in soils for sustainable crop production. It has been proven that microbial soil community is able to influence soil fertility through soil processes viz. decomposition, mineralization, and storage / release of nutrients. It has also been reported that some beneficial soil microorganisms such as a wide range of saprophytic bacteria, fungal strains and actinomycetes can help solubilizing the fixed or insoluble K from soils by various mechanisms. Some of these mechanisms include the production of inorganic and organic acids, acidolysis, polysaccharides, complexolysis, chelation, polysaccharides, and exchange reactions. Among these microorganisms, K solubilizing bacteria (KSB) have attracted the attention of agricultural researchers and other stake holders to exploit it as soil inoculum to promote the plant growth and yield. The KSB are effective in releasing K from inorganic and insoluble pools of total soil K through solubilization. It has been reported that inoculation with KSB produced beneficial effect on growth of different plants .The present study was design to evaluate the effect of K solubilizing bacteria on growth and yield of wheat.

MATERIALS AND METHODS

Isolation of K solubilizing bacteria is the first step to select best strains for crops. Hence, isolation was carried out in lab on nutrient agar medium. Further characterization and screening was carried out on Aleksandrove medium. IAA biosynthesis potential of isolates was also determined. Lab studies were conducted to check their effect on root shoot of wheat. Further isolations are under progress.

RESULTS

Among seven isolates, in case of halo zone formations, KS1, KS6, KS7, showed high potential of halo zone formation. Maximum solubilization zone (2.60) from the isolate KS6 was observed. KS4 had maximum potential to produce IAA. In lab bioassay maximum root, shoot length and biomass was observed in KS7 (14.17, 9.83 cm, 1.19, 1.29g)) respectively as compared to control.

Table 05: Isolation and characterization of K solubilizing bacteria (KSB)

Isolate	Halo Zone formation	Solubilization Index	IAA Equivalents ($\mu g\ mL^{-1}$)	
			With L-Try	Without L-Try
KS1	+++	2.50	5.83	2.42
KS2	++	2.30	6.75	4.08
KS3	+	2.29	3.83	1.25
KS4	+	2.24	7.42	4.42
KS5	++	2.33	6.83	3.83
KS6	+++	2.60	4.08	3.75
KS7	+++	2.55	6.08	3.58

Table 06: Effect of K solubilizing bacteria on wheat lab bioassay

Treatments	Root length (cm)	Shoot Length (cm)	Root mass (g)	Shoot mass (g)
T_1: Control	10.21	6.61	0.79	1.14
T_2: KS1	12.64	9.08	1.10	1.58
T_3: KS2	13.45	9.24	1.15	1.55
T_4: KS3	12.90	9.01	0.99	1.50
T_5: KS4	12.66	10.11	0.97	1.54
T_6: KS5	12.93	9.87	1.03	1.47
T_7: KS6	11.28	9.32	0.88	1.39
T_8: KS7	14.17	9.83	1.19	1.29

3.5. ISOLATION, CHARACTERIZATION AND SCREENING OF BIOFILM PRODUCING PGPR

INTRODUCTION

Currently, global population is over 7 billion and this is projected to reach 9 billion by 2050. One of the biggest challenges to overcome is to substantially increase the amount of food produced in a sustainable and environmentally friendly manner. In fact, studies have shown that in order to meet global food demand by 2050, we will need to increase food production by at least 70%. One of the limitations to increasing worldwide crop productivity is insufficient essential nutrient in most agricultural soils, resulting in suboptimal crop growth. To overcome these challenges, agricultural practices have depended on the use of chemical fertilizers, insecticides, fungicides and herbicides to increase crop yields. However, these approaches are costly and have numerous negative environmental impacts. In order to meet the increased food demands projected for 2050, a tremendous increase in the production, distribution and application of fertilizers will be required, which is neither economically nor environmentally desired.

Current trends in agriculture focus on alternative sustainable and environment friendly approaches that may improve soil quality and crop production. One of these alternatives is the use of biological agents, such as microorganisms (bacteria and fungi). Previous studies have proved that use of planktonic-PGPR (P-PGPR) offers promising prospects in *in vitro,* growth chamber and greenhouse trials, demonstrating the potential to diminish the use of chemicals. However, planktonic inoculants found inconsistent under field conditions probably due to inability of planktonic cells to compete with the indigenous micro flora.

To address this issue, scientists have begun to investigate the use of biofilm PGPR (B-PGPR) as alternative inoculum. Biofilms are defined as dense colonies of single or multi-species microbial cells adherent to either a biotic or abiotic surface encased in a matrix of extracellular polymeric substances (EPS). Interestingly, in natural environments microbial cells are predominantly found as biofilms, as opposed to their planktonic equivalents, which may prove to be a more suitable and durable option compared to the traditional application of P-PGPR inoculums. In fact, the formation of biofilms enhances the survival of the bacterial cells, which may allow the bacterial inoculants to survive the initial application into natural soils and allow them to thrive in the long term. Although relatively new, initial investigations have provided encouraging results in regards to utilizing B-PGPR. Therefore

the goal of this research experiment was to assess the effect of biofilmed PGPR on growth and yield of maize and wheat.

MATERIALS AND METHODS

Isolations, characterization, and screening of biofilm producing PGPR was carried out in Lab. A pot experiment was conducted at Soil Bacteriology Section, AARI, Faisalabad to evaluate the effect of biofilm producing PGPR on growth and yield of maize and wheat. Characteristics of soil were as: pH 7.9, ECe 2.2, Organic matter 0.69% and available P 8.3 $mgkg^{-1}$ for maize and pH 7.8, ECe 2.3, Organic matter 0.67% and available P 8.4 $mgkg^{-1}$ for wheat crop. Treatments were control (T_1), PGPR1 (T_2), PGPR2 (T_3), PGPR3 (T_4), PGPR4 (T_5) PGPR5 (T_6). Recommended dose of chemical fertilizer (wheat 160-114-60 kg NPK ha^{-1}, maize 100-60 NP kg ha^{-1}) was used. All P and K and half dose of N was applied at the time of sowing. Remaining N was applied with first irrigation. Completely randomized block design was used. Maize crop was sown during Kharif 2019 and wheat was sown in Rabi 2019-2020.

RESULTS

Isolates that had high potential of biofilm formation, EPS production, IAA biosynthesis were used in experiment. Data revealed that maximum fodder yield (264.3 g pot^{-1}) of maize was obtained in treatment where PGPR-5 was applied following by Treatment T_2 (261.7 g pot^{-1}) both treatments are statistically at par but significant increase was observes as compared to control. In case of plant height and shoot dry biomass maximum response was observed in T_6 (126.7cm, 52.9 g pot^{-1}) as compared to control i.e. 98.7cm, 37.9 g pot^{-1} respectively. Highest root length (78.7cm) was observed in T_4 while maximum root mass was observed in T_3 (203.3 g pot^{-1}) as compared to control. Data regarding wheat crop was revealed that maximum grain yield, shoot dry biomass, no of tillers $plant^{-1}$, root length, root biomass 28.6 g pot^{-1}, 64.75 g pot^{-1}, 15.0, 56.87 cm, 80.25 g pot^{-1} , respectively were observed in T_3 treatment as compared to control.

Table 07: Characterization of biofilm producing PGPR

Isolates	Biofilm formation (595nm)	EPS Production	IAA Equivalents (µg mL^{-1})	
			With L-Tryptophan	Without L-Tryptophan
PGPR-1	0.4	++	6.7	4.8
PGPR-2	0.5	+++	8.2	6.5
PGPR-3	0.7	+	7.2	5.0
PGPR-4	0.9	++	8.7	6.5

PGPR-5	0.8	+++	9.4	6.3

Table 08: Effect of biofilm forming PGPR on growth and yield of maize

Treatments	Fodder Yield (g pot^{-1})	Plant height (cm)	Shoot dry biomass (g pot^{-1})	Root Length (cm)	Root biomass (g pot^{-1})
T_1: Control	189.3e	98.7d	37.9e	62.0d	142.3c
T_2: PGPR-1	252.0b	110.7c	50.4b	74.3bc	192.7b
T_3: PGPR-2	261.7a	112.7c	52.3a	75.7ab	203.3a
T_4: PGPR-3	245.0c	117.3bc	49.0c	78.7a	197.0ab
T_5: PGPR-4	222.7d	121.7ab	44.5d	71.0c	192.7b
T_6: PGPR-5	264.3a	126.7a	52.9a	72.0c	193.3b
LSD	4.7809	7.9227	2.432	3.6314	7.1406

Table 09: Effect of biofilm forming PGPR on growth and yield of wheat

Treatments	Grain Yield (g pot^{-1})	Plant height cm)	Shoot dry biomass (g pot^{-1})	No of tillers plant^{-1}	Root Length (cm)	Root biomass (g pot^{-1})
T_1: Control	17.6f	71.25e	51.50e	8.70e	44.25e	63.50e
T_2: PGPR-1	21.2e	74.50d	56.00d	11.00d	51.50c	67.25d
T_3: PGPR-2	28.6a	76.75bc	64.75a	15.00a	56.87a	80.25a
T_4: PGPR-3	24.5c	79.75a	63.75ab	12.50bc	53.87b	73.21c
T_5: PGPR-4	26.1b	77.25b	61.0bc	13.50b	48.50d	75.20bc
T_6: PGPR-5	23.4d	75.23cd	59.75c	12.00cd	54.57b	76.75b
LSD	1.0258	1.9417	2.8715	1.0358	1.9304	2.4448

3.6. ISOLATION & CHARACTERIZATION OF SIDEROPHORE PRODUCING BACTERIA FOR THE GROWTH PROMOTION OF MAIZE & WHEAT

INTRODUCTION

Iron is among the bulk minerals present on the surface of the earth, yet it is unavailable for plants. It is found in nature as Fe^{3+}, a highly insoluble form. To solve this problem, PGPR secrete siderophore, a low molecular weight Fe binding protein compound chelating ferric iron (Fe^{3+}) from the environment and hence provides the plants with Fe for better growth. These molecules act as solubilizing agents for Fe from minerals or organic compounds under conditions of Fe limitation. Siderophores, generally form 1:1 complexes with Fe^{3+}, which are then taken up by the cell membrane of bacteria, where the Fe^{3+} is reduced to Fe^{2+} and released from the siderophore into the plant's cell.

PGPR enhance plant-growth by producing very efficient extracellular siderophores which control several plant diseases by depriving the pathogens of Fe nutrition. In addition, Fe siderophores form stable complexes with other metals of environmental concern, such as Al, Cd, Cu, Ga, In, Pb and Zn. The presence of heavy metals induces bacterial siderophore production. Paradoxically, plants grown in metal-contaminated soils are often Fe deficient and the bacteria may help plants to obtain its sufficient quantity. Microbial siderophores are used as metal chelating agents that regulate the availability of Fe in plant rhizosphere. This in turn helps plants to alleviate the toxicity of metals as reported for arsenic uptake by several plants. Bio-fortification designates the natural enrichment of plants with nutrients and health promoting factors during their growth. Bio-fortification focuses on generating and breeding major staple food crops that would produce edible products enriched in bioavailable amounts of micronutrients, provitamin-A, carotenoids or several other known components that enhance nutrient use efficiency and are beneficial to human health.

MATERIAL AND METHODS

Soil samples were collected from the different locations of Punjab. Isolates were screened out for siderophore producing bacteria by spread plate technique. These isolates were characterized for biochemical tests. The Chrome Azurol S (CAS) assay was used for detection of siderophore production. The Chrome Azurol S (CAS) dye contains agar in blue color, hence on inoculation the organisms secrete siderophore and Fe is stripped from the dye, causing the change of color of media from blue to orange or yellow. Therefore, the

presence of siderophore is indicated by coloration surrounding the inoculation site. The screened siderophore producing isolates were used for iron biofortification in wheat and maize.

Field experiment was conducted at the Soil Bacteriology Section, Faisalabad to check the efficiency of siderophore producing bacteria in improving growth and yield of wheat and maize. Trials were carried out in sandy clay loam soil having pH: 8.5, EC: 2.8 dS m^{-1}, available P: 7.5 mg kg^{-1} and organic matter 0.86%. Recommended dose of fertilizer @ 100-60 kg NP ha^{-1} was applied to maize.

TREATMENTS

T_1: Control

T_2: SPP-1 (Siderophore, Rawal Pindi)

T_3: SPP-2

T_4: SPP-3

T_5: SPP-4

T_6: SPP-5

T_7: SPS-9 (Siderophore, Sargodha)

T_8: SPS-10

Note: Recommended dose of fertilizer was applied to maize and wheat.

RESULTS

Results clearly indicated that SPP-3 and SPP-5 gave highest (72.33 and 74.00 t ha^{-1}) grain yield as compared to control (62.3 t ha^{-1}). Results regarding wheat trial clearly indicated that SPP-4 and SPS-9 produced the highest (3383 and 3470 kg ha^{-1}) wheat grain yield as compares to control (2917 kg ha^{-1}).

Table 10: Effect of siderophore producing bacteria on the yield attributes of maize

Treatments	Fodder yield (kg ha^{-1})	Shoot dry weight (g $plant^{-1}$)	Plant height (cm)	Leaf length (cm)
T_1: Control	62.3 B	0.36 B	192 C	60.5 C
T_2: SPP-1	63.0 B	0.41 B	201 ABC	63.5 ABC
T_3: SPP-2	69.0 AB	0.39 B	202 ABC	66.6 ABC
T_4: SPP-3	72.3 A	0.51 A	206 ABC	68.4 AB
T_5: SPP-4	70.0 AB	0.41 B	200 BC	69.3 AB
T_6: SPP-5	74.0 A	0.54 A	215 A	69.7 A
T_7: SPS-9	66.7 AB	0.43 B	208 AB	62.0 C
T_8: SPS-10	69.0 AB	0.38 B	205 ABC	63.3 BC
LSD	8.27	0.07	14.26	6.34

Table 11: Effect of siderophore producing bacteria on the yield attributes of wheat

Treatments	Grain yield (kg ha^{-1})	1000 grain weight (g)	Plant height (cm)	Spike length (cm)
T_1: Control	2917 E	27.5 D	95.0 B	13.8 AB
T_2: SPP-1	3110 D	28.3 D	96.3 B	14.7 A
T_3: SPP-2	3143 D	27.5 D	100.3 AB	13.3 B
T_4: SPP-3	3193 CD	30.8 C	103.7 A	14.3 AB
T_5: SPP-4	3383 B	30.9 C	105.3 A	14.0 AB
T_6: SPP-5	3167 D	32.2 BC	104.7 A	13.0 B
T_7: SPS-9	3470 A	34.3 A	105.3 A	13.3 AB
T_8: SPS-10	3253 C	33.2 AB	101.3 AB	14.8 A
LSD	84.68	2.15	6.38	1.55

3.7. BIOFERT-PLUS: PRECURSOR ENRICHED NOVEL MICROBIAL FORMULATIONS

INTRODUCTION

Plant hormones play vital role in controlling plant growth and development. Under sub-optimal growth conditions, plants may not have the capacity to synthesize sufficient endogenous plant hormones for optimal growth and development. Plant hormones applied exogenously affected plant growth by altering the balance of endogenous levels of hormones, modified the growth and development of plants.

Plants are capable to synthesize auxins, yet they respond to exogenously applied auxins. L-tryptophan (L-TRP) is considered an efficient physiological precursor of auxins. L-TRP is responsible for the microbial biosynthesis of auxins. Exogenous application of L-TRP to soils has also been shown to stimulate synthesis of auxins, influencing plant growth and development positively. Exogenous application of L-TRP increased the growth and yield of legumes and non-legumes. L-TRP applied exogenously to plants produced higher values of auxins and exerted positive effects on plants. Auxin biosynthesis with L-TRP can be carried out by many pathways and usually through by indole-3-acetaldehyde via indole-3-pyruvate or by tryptamine etc. Tryptamine is an amino acid and is byproduct during auxin biosynthesis and might exert its effect on the plants.

Cytokinin applied exogenously improves the plant growth by delaying senescence and by preventing degradation of chlorophyll and photosynthetic proteins. Cytokinins are also produced by plant growth promoting rhizobacteria and exert their effect to plants. The application of different cytokinin precursors if applied exogenously may results to overcome

sub-optimal levels of cytokinin in plants. Adenine, the precursor of cytokinins enhances the cell division and exogenous application of cytokinins and its precursors like adenine improved the growth and yield of crops.

Gibberellic acid is an important growth regulator that may have many uses to modify the growth, and yield contributing characters of plant**.** Application of GA_3 stimulates root-shoot growth and thus promotes plant growth. Studies revealed that the physiological precursors exert their profound effect on the growth of crops. The present study is planned to access the role of different physiological precursors on the growth promotion of maize.

Rhizobium sp have received worldwide attention as they have shown significant increase in growth and yield of legumes. Rhizobium sp promote plant growth by producing plant hormones, fixing nutrients, mobilizing or solubilizing nutrients, inducing systemic resistance and suppressing plant pathogens. These hormones enhance nutrients availability through fixation and mobilization. They also reduce harmful effects of pathogens on plants by different mechanisms for plant growth promotion. Rhizobium sp in combination with plant hormones or their precursors promote plant growth and development and also exerts beneficial effects on crop growth and yields.

The role of precursors has more beneficial than plant hormones due to certain factors. The precursors are water soluble, inexpensive, non-photosensitive and continuous source of hormones to plants. Precursor-inoculum interaction offered continuous source of hormones to plants and enhanced the plant-microbe interactions results in better plant growth. It was reported that precursor-inoculum interaction affected the yield of crops. Present studies were carried out on wheat, rice and maize to exploit the physiological precursors to improve the crop growth and yield in a cost effective and sustained way.

MATERIALS AND METHODS

Efficient Rhizobium species of chickpea, lentil and mung bean were screened and characterized and after multiplication, their inocula were enriched with various precursors / PGR's as per treatments. Isolates were screened for their auxin biosynthesis potential in lab conditions. Precursor enriched inoculum were applied at recommended dose of NPK. Fertilizer NP was applied as basal. Layout was RCBD with three repeats. The pre-sowing soil analysis for field study conducted at Pulses Research Institute, Faisalabad showed that soil was medium texture soil having pH 7.90, EC 1.40 dS m^{-1}, soil N 0.035%, organic matter

0.65% and available P 7.40 mg kg^{-1} soil. Field studies were planned to assert the new formulations and to check their response on chickpea, mungbean and lentil growth and yield.

TREATMENTS

T_1: Control

T_2: *Rhizobium* Inoculation

T_3: *Rhizobium* Inoculum enriched with L-Tryptophan @ 10^{-5}M

T_4: *Rhizobium* Inoculum enriched with Kinetin @ 10^{-5}M

T_5: *Rhizobium* Inoculum enriched with L-Methionine @ 10^{-5}M

T_6: *Rhizobium* Inoculum enriched with GA_3 @ 10^{-5}M

Note: Specific *Rhizobium* sp of chickpea, lentil and mung bean was applied.

RESULTS

Data regarding mungbean yield parameters of field trials conductedat Pulses Research Institute (PRI), Faisalabad, revealed that *Rhizobium* inoculum enriched with GA_3 produced the highest grain yield, biomass yield, No. of nodules $plant^{-1}$, nodular mass i.e. 1563.3 kg ha^{-1} , 3083.3 kg ha^{-1} , 21.3 and 0.135 g $plant^{-1}$, respectively. Similar trend was also noted in lentil trials and results revealed that *Rhizobium* inoculum enriched with GA_3 produced the highest lentil grain yield, biomass yield, no. of nodules $plant^{-1}$, nodular mass i.e. 1466.7 kg ha^{-1} , 4433.3 kg ha^{-1} , 16.3 and 0.067 g $plant^{-1}$, respectively. Chickpea trial also showed similar tendency and it was found that *Rhizobium* inoculum enriched with GA_3produced the highest chickpea grain yield, biomass yield, No. of nodules $plant^{-1}$, nodular mass i.e. 1636.7 kg ha^{-1} , 6350 kg ha^{-1} , 26.7 and 0.1490 g $plant^{-1}$, respectively.

CONCLUSIONS

- Precursor enriched microbial inoculant improved the growth and yield of chickpea.
- Maximum effect recorded withGA_3enrichedinoculants and followed by L-TRP.

Table 12: Mung bean yield parameters at Pulses Research Institute, AARI, Fsd. (Field Study)

Treatments	Grain Yield (kg ha^{-1})	Biomass Yield (kg ha^{-1})	Nodules $plant^{-1}$	Nodular dry mass (g $plant^{-1}$)
T_1: Control	1263.3 C	2193.3 B	12.3 C	0.115 D
T_2: *Rhizobium* Inoculation	1413.3 B	2700.0 AB	16.3 BC	0.123 C
T_3: *Rhizobium*+ L-TRP @ 10^{-5}M	1546.7 A	2800.0 AB	20.3 AB	0.134 AB
T_4: *Rhizobium*+ Kinetin @ 10^{-5}M	1506.7 AB	2566.7 AB	16.7 B	0.129 BC
T_5: *Rhizobium*+ L-MET @ 10^{-5}M	1430.0 B	2650.0 AB	17.0 B	0.131 AB

T_6: *Rhizobium* + GA_3 @ 10^{-5}M	1563.3 A	3083.3 A	21.3 A	0.135 A
LSD	113.69	656.04	4.24	0.006
L-TRP: L-Tryptophan; L-MET: L-Methionine; GA_3: Gibberellic acid				

Table 13: Lentil yield parameters at Pulses Research Institute, AARI, Fsd. (Field Study)

Treatments	**Grain Yield (kg ha^{-1})**	**Biomass Yield (kg ha^{-1})**	**Nodules plant^{-1}**	**Nodule dry mass (g plant^{-1})**
T_1: Control	1073.3 C	3720.0 D	10.0 E	0.047 B
T_2: *Rhizobium* Inoculation	1283.3 B	3806.7 CD	12.3 D	0.053 AB
T_3: *Rhizobium*+ L-TRP @ 10^{-5}M	1390.0 AB	3926.7 CD	14.0 C	0.057 AB
T_4: *Rhizobium*+ Kinetin @ 10^{-5}M	1426.7 A	4333.3 AB	16.0 A	0.060 AB
T_5: *Rhizobium*+ L-MET @ 10^{-5}M	1390.0 AB	4066.7 BC	15.0 B	0.063 AB
T_6: *Rhizobium* + GA_3 @ 10^{-5}M	1466.7 A	4433.3 A	16.3 A	0.067 A
LSD	111.01	313.68	0.9197	0.0187
L-TRP: L-Tryptophan; L-MET: L-Methionine; GA_3: Gibberellic acid				

Table 14: Chickpea yield parameters at Pulses Research Institute, AARI, Fsd. (Field Study)

Treatments	**Grain Yield (kg ha^{-1})**	**Biomass Yield (kg ha^{-1})**	**Nodules plant^{-1}**	**Nodular dry mass (g plant^{-1})**
T_1: Control	1286.7 C	4833.3 C	11.0 E	0.0997 C
T_2: *Rhizobium* Inoculation	1466.7 B	5633.3 B	16.0 D	0.1110 B
T_3: *Rhizobium*+ L-TRP @ 10^{-5}M	1550.0 AB	5733.3 B	20.0 C	0.1407 A
T_4: *Rhizobium*+ Kinetin @ 10^{-5}M	1600.0 AB	6000.0 AB	24.7 AB	0.1430 A
T_5: *Rhizobium*+ L-MET @ 10^{-5}M	1500.0 AB	6250.0 A	23.0 B	0.1403 A
T_6: *Rhizobium* + GA_3 @ 10^{-5}M	1636.7 A	6350.0 A	26.7 A	0.1490 A
LSD	165.91	372.47	2.65	0.0110
L-TRP: L-Tryptophan; L-MET: L-Methionine; GA_3: Gibberellic acid				

3.8. BIOFERT-PLUS: NOVEL MICROBIAL FORMULATIONS ENRICHED WITH PRECURSORS AND PGR'S FOR CEREALS

INTRODUCTION

Plant hormones play vital role in controlling plant growth and development. Under sub-optimal growth conditions, plants may not have the capacity to synthesize sufficient endogenous plant hormones for optimal growth and development. Plant hormones applied exogenously affected plant growth by altering the balance of endogenous levels of hormones, modified the growth and development of plants.

Plants are capable to synthesize auxins, yet they respond to exogenously applied auxins. L-tryptophan (L-TRP) is considered as an efficient physiological precursor of auxins. L-TRP

is responsible for the microbial biosynthesis of auxins. Exogenous application of L-TRP to soils has also been shown to stimulate synthesis of auxins, influencing plant growth and development positively. Exogenous application of L-TRP increased the growth and yield of legumes and non-legumes. L-TRP applied exogenously to plants produced higher values of auxins and exerted positive effects on plants. Auxin biosynthesis with L-TRP can be carried out by many pathways and usually through by indole-3- acetaldehyde via indole-3-pyruvate or by tryptamine etc. Tryptamine is an amino acid and is byproduct during auxin biosynthesis and might exert its effect on the plants.

Cytokines applied exogenously improves the plant growth by delaying senescence and by preventing degradation of chlorophyll and photosynthetic proteins. Cytokinins are also produced by PGPR and exert their effect to plants. The application of different cytokinin precursors if applied exogenously may results to overcome sub-optimal levels of cytokinin in plants. Adenine, the precursor of cytokinins enhances the cell division and exogenous application of cytokinins and its precursors like adenine improved the yield of crops.

Gibberellic acid is an important growth regulator that may have many uses to modify the growth, and yield contributing characters of plant**.** Application of GA_3 stimulates root-shoot growth and thus promotes plant growth. Studies revealed that the physiological precursors exert their profound effect on the growth of crops. The present study was planned to access the role of different physiological precursors on the growth promotion of maize.

PGPR have received worldwide attention as they have shown significant increase in growth and yield of crops. PGPR promote growth by producing plant hormones, fixing nutrients, mobilizing or solubilizing nutrients, inducing systemic resistance and suppressing plant pathogens. These hormones enhance nutrients availability through fixation and mobilization. They also reduce harmful effects of pathogens on plants by different mechanisms for plant growth promotion. PGPR in combination with plant hormones or their precursors exerts beneficial effects on crop growth and yields.

The role of precursors has more beneficial than plant hormones due to certain factors. The precursors are water soluble, inexpensive, non-photosensitive and continuous source of hormones to plants. Precursor-inoculum interaction offered continuous source of hormones to plants and enhanced the plant-microbe interactions results in better plant growth. It was reported that precursor-inoculum interaction affected the yield of crops. Present studies were

carried out on wheat, rice and maize to exploit the physiological precursors to improve the crop growth and yield in a cost effective and sustained way.

MATERIALS AND METHODS

Efficient PGPR isolates available at Soil Bacteriology Section were used for commercial inocula preparation and were enriched with various precursors / hormones as per treatments. Isolates were screened for their auxin biosynthesis potential in lab conditions. Precursor / hormones enriched inoculum were applied along with recommended dose of NPK. All P, K and half N was applied at sowing and remaining half N at tillering stage. Layout was CRD / RCBD with three repeats. Field studies were planned to assert the new formulations and to check their response on wheat and rice growth.

TREATMENTS

T_1: Control

T_2: PGPR Inoculation

T_3: PGPR Inoculum enriched with L-Tryptophan @ 10^{-5} M

T_4: PGPR Inoculum enriched with Kinetin @ 10^{-5} M

T_5: PGPR Inoculum enriched with L-Methionine @ 10^{-5}M

T_6: PGPR Inoculum enriched with GA_3 @ 10^{-5} M

Table 15: The pre-sowing soil analysis of different locations

Parameters	**Soil Bacteriology Faisalabad**	**WRI, Faisalabad**	**ARS, Farooqabad**	**SSRI, Pindi Bhattian**
pH	7.90	8.20	8.30	8.50
EC_e(dS m^{-1})	1.40	1.45	1.45	3.50
Soil N (%)	0.035	0.032	0.036	0.026
Organic Matter (%)	0.70	0.65	0.66	0.60
Available P (mg kg^{-1})	7.90	7.28	7.64	6.50

RESULTS

Field studies were conducted to exploit the plant hormone or physiological precursors to improve the crop growth and yield in a cost effective and sustained way. Treatment includes control, PGPR, PGPR enriched L-tryptophan, Kinetin, L-Methionine and GA_3 each at 10^{-5} M. Field studies were conducted at Agronomic Research Station, Farooqabad and Soil Salinity Research Institute, Pindi Bhattian and uniform recommended fertilizer was applied to each trial. Results revealed that plant hormone or physiological precursors enriched inoculants improved the growth and yield at each location, significantly. Results of the rice trials conducted at ARS, Farooqabad and SSRI, Pindi Bhattian revealed that PGPR enriched with GA_3 produced the highest paddy yield i.e. 4400, 2817 followed by Kinetin enriched bacterial inoculants as compared to control i.e. 3867, 2280 kg ha^{-1}, respectively (Fig.3). Results of maize trial at Soil Bacteriology Section revealed that PGPR enriched with GA_3 produced the highest maize fodder and dry matter yield i.e. 74.2, 15.6 as compared to control i.e. 58.3 and 11.0 t ha^{-1}, respectively (Fig.4). Results of wheat trials at different locations (SSRI, Pindi Bhattian, Soil Bacteriology, WRI, Faisalabad and ARS, Farooqabad) revealed that PGPR enriched with GA_3 produced the highest wheat grain yield i.e. 2243, 3706, 3217 and 3660 kg ha^{-1}), respectively (Fig.5).

Results of rice trial at SSRI, Pindi Bhattian revealed that PGPR enriched with GA_3 produced the highest rice plant height, tillers m^{-2}, biomass and paddy yield i.e. 91.7 cm, 264, 14077 and 2816.7 kg ha^{-1}, respectively. Results of rice trial at ARS, Farooqabad revealed that PGPR enriched with GA_3 produced the highest rice plant height, tillers m^{-2}, biomass and paddy yield i.e. 104.7 cm, 376, 15747 and 4433.3 kg ha^{-1}, respectively. Results of wheat trial at SSRI, Pindi Bhattian revealed that PGPR enriched with GA_3 produced the highest wheat plant height, tillers m^{-2}, biomass and paddy yield i.e. 84.7 cm, 301, 9100 and 2243.3 kg ha^{-1}, respectively. Results of wheat trial at Soil Bacteriology Section, Faisalabad revealed that PGPR enriched with GA_3 produced the highest wheat plant height, tillers m^{-2}; biomass and paddy yield i.e. 106 cm, 356, 11267 and 3620 kg ha^{-1}, respectively. Results of wheat trial at WRI, Faisalabad revealed that PGPR enriched with GA_3 produced the highest wheat plant height, tillers m^{-2}; biomass and paddy yield i.e. 107 cm, 376, 12583 kg ha^{-1} and 3216.7 kg ha^{-1}, respectively. Results of wheat trial at ARS, Farooqabad revealed that PGPR enriched with GA_3 produced the highest wheat plant height, tillers m^{-2}; biomass and paddy yield i.e. 111.3 cm, 370, 12500 and 3660 kg ha^{-1}, respectively. Results of maize trial, at Soil

Bacteriology Section, revealed that PGPR enriched with GA_3 produced the highest (209.3 cm) maize plant height; fresh fodder yield (74.2 t ha^{-1}) and dry matter yield (15.58 t ha^{-1}).

CONCLUSIONS

- Enriched microbial inoculant improved growth, yield of rice and wheat crop at each location of the trial
- The effect was found maximum when inoculant was enriched with GA3 and followed by the effect of L-TRP.

Table 16: Rice yield parameters at SSRI, Pindi Bhattian (Field Study)

Treatments	**Plant Height (cm)**	**Tillers m^{-2}**	**Biomass Yield (kg ha^{-1})**	**Grain Yield (kg ha^{-1})**
T_1: Control	78.7	208.0 C	10827 B	2280.0 C
T_2: PGPR Inoculation	82.0	216.0 C	11383 B	2446.7 BC
T_3: PGPR+ L-TRP @ 10^{-5}M	85.7	242.7 B	13593 A	2580.0 AB
T_4: PGPR + Kinetin @ 10^{-5}M	90.7	250.7 AB	13780 A	2673.3 AB
T_5: PGPR + L-MET @ 10^{-5}M	89.7	248.0 AB	12993 A	2600.0 AB
T_6: PGPR+ GA_3 @ 10^{-5}M	91.7	264.0 A	14077 A	2816.7 A
LSD	13.08	20.18	1542.4	265.34
L-TRP: L-Tryptophan; L-MET: L-Methionine; GA_3: Gibberellic acid				

Table 17: Rice yield parameters yield at ARS, Farooqabad (Field Study)

Treatments	Plant Height (cm)	Tillers m^{-2}	Biomass Yield (kg ha^{-1})	Grain Yield (kg ha^{-1})
T_1: Control	99.3	301.3 D	13267 B	3866.7 B
T_2: PGPR Inoculation	101.7	328.0 C	14343 AB	4026.7 AB
T_3: PGPR+ L-TRP @ 10^{-5}M	103.0	344.0 BC	14867 A	4173.3 AB
T_4: PGPR + Kinetin @ 10^{-5}M	104.0	368.0 AB	15213 A	4260.0 AB
T_5: PGPR + L-MET @ 10^{-5}M	102.0	357.3 AB	14600 AB	4140.0 AB
T_6: PGPR+ GA_3 @ 10^{-5}M	104.7	376.0 A	15747 A	4433.3 A
LSD	6.12	24.01	1442.4	478.07
L-TRP: L-Tryptophan; L-MET: L-Methionine; GA_3: Gibberellic acid				

Table 18: Wheat yield parameters at SSRI, Pindi Bhattian (Field Study)

Treatments	Plant Height (cm)	Tillers m^{-2}	Biomass Yield (kg ha^{-1})	Grain Yield (kg ha^{-1})
T_1: Control	75.3 B	275.3 C	7383 D	1643.3 C
T_2: PGPR Inoculation	81.0 AB	280.3 BC	7883 CD	1723.3 BC
T_3: PGPR+ L-TRP @ 10^{-5}M	83.7 A	292.0 ABC	8250 BC	2040.0 AB
T_4: PGPR + Kinetin @ 10^{-5}M	83.3 A	296.7 AB	8433 B	2180.0 A
T_5: PGPR + L-MET @ 10^{-5}M	82.7 A	282.3 BC	8650 AB	2080.0 A
T_6: PGPR+ GA_3 @ 10^{-5}M	84.7 A	301.0 A	9100 A	2243.3 A
LSD	6.506	18.61	548.55	350.46
L-TRP: L-Tryptophan; L-MET: L-Methionine; GA_3: Gibberellic acid				

Table 19: Wheat yield parameters at Soil Bacteriology Section, Faisalabad (Field Study)

Treatments	Plant Height (cm)	Tillers m^{-2}	Biomass Yield (kg ha^{-1})	Grain Yield (kg ha^{-1})
T_1: Control	100.0	309.7 D	10217 D	3203 B
T_2: PGPR Inoculation	101.7	322.3 CD	10883 CD	3496 A
T_3: PGPR+ L-TRP @ 10^{-5}M	102.0	333.3 BC	11100 C	3540 A
T_4: PGPR + Kinetin @ 10^{-5}M	104.7	348.3 AB	12100 B	3653 A
T_5: PGPR + L-MET @ 10^{-5}M	102.7	340.7 ABC	11267 C	3620 A
T_6: PGPR+ GA_3 @ 10^{-5}M	106.0	356.0 A	13017 A	3706 A
LSD	7.389	21.57	713.48	278.97
L-TRP: L-Tryptophan; L-MET: L-Methionine; GA_3: Gibberellic acid				

Table 20: Wheat yield Parameters at WRI, Faisalabad (Field Study)

Treatments	Plant	Tillers	Biomass	Grain Yield

	Height (cm)	m^{-2}	Yield (kg ha^{-1})	(kg ha^{-1})
T_1: Control	98.3 C	291.7 E	11067 D	2433.3 D
T_2: PGPR Inoculation	100.3 C	328.3 D	11583 C	2550.0 CD
T_3: PGPR+ L-TRP @ 10^{-5}M	102.3 C	355.0 BC	12167 AB	2756.7 BC
T_4: PGPR + Kinetin @ 10^{-5}M	106.0 AB	371.7 AB	12233 AB	3083.3 A
T_5: PGPR + L-MET @ 10^{-5}M	105.0 AB	349.3 C	12117 B	2966.7 AB
T_6: PGPR+ GA_3 @ 10^{-5}M	107.0 AB	376.0 A	12583 A	3216.7 A
LSD	4.368	19.308	462.63	252.56
L-TRP: L-Tryptophan; L-MET: L-Methionine; GA_3: Gibberellic acid				

Table 21: Wheat yield parameters at ARS, Farooqabad (Field Study)

Treatments	Plant Height (cm)	Tillers m^{-2}	Biomass Yield (kg ha^{-1})	Grain Yield (kg ha^{-1})
T_1: Control	103.0 C	246.7 C	10283 C	2933.3 D
T_2: PGPR Inoculation	106.3 BC	313.3 C	10350 C	3083.3 CD
T_3: PGPR+ L-TRP @ 10^{-5}M	108.7 AB	341.0 B	10600 C	3316.7 BC
T_4: PGPR + Kinetin @ 10^{-5}M	110.0 AB	368.3 A	12033 AB	3466.7 AB
T_5: PGPR + L-MET @ 10^{-5}M	109.7 AB	358.0 A	11667 B	3516.7 AB
T_6: PGPR+ GA_3 @ 10^{-5}M	111.3 A	370.0 A	12500 A	3660.0 A
LSD	4.7557	14.40	545.4	302.36
L-TRP: L-Tryptophan; L-MET: L-Methionine; GA_3: Gibberellic acid				

Table 22: Maize fodder yield at Soil Bacteriology Section, Faisalabad (Field Study)

Treatments	Plant Height (cm)	Fresh Fodder Yield (t ha^{-1})	Dry Matter Yield (t ha^{-1})
T_1: Control	190.7 C	58.3 D	10.97 E
T_2: PGPR Inoculation	194.0 BC	63.3 C	12.10 D
T_3: PGPR+ L-TRP @ 10^{-5}M	204.0 AB	68.3 B	13.54 C
T_4: PGPR + Kinetin @ 10^{-5}M	200.0 ABC	72.5 A	14.50 B
T_5: PGPR + L-MET @ 10^{-5}M	203.0 AB	66.7 BC	13.56 C
T_6: PGPR+ GA_3 @ 10^{-5}M	209.3 A	74.2 A	15.58 A
LSD	10.21	3.49	0.7891
L-TRP: L-Tryptophan; L-MET: L-Methionine; GA_3: Gibberellic acid			

3.9. RESPONSE OF LENTIL AND MASHBEAN TO RHIZOBIUM AND PSM IN COMBINATION WITH PLANT GROWTH REGULATORS

INTRODUCTION

Biological N fixation provides an economic and ecological way by reducing cost of mineral nitrogen. PGPR have received worldwide attention as they have shown significant increase in growth and yield of crops. They induce plant growth and development directly or indirectly by releasing plant hormones. These hormones enhance nutrients availability through fixation and mobilization. They also reduce harmful effects of pathogenic microorganisms on plants by multiple mechanisms for plant growth promotion. In legumes, PGPR have beneficial effect of releasing plant hormones, and thus present more niches for the *Rhizobium* to form nodules and chemical signaling of plant and *Rhizobium*. Therefore, the co-inoculation of *Rhizobium* and PGPR influence growth, yield, nodulation and N-fixation in plants. Several mechanisms have been suggested by which PGPR can promote plant growth during co-inoculation with *Rhizobium*, including plant hormone production, N-fixation, subsiding pathogens and stimulating nutrient uptake. Biofertilizers improve crop growth and quality by fixation of atmospheric nitrogen and also by dissolving insoluble forms of phosphorus.

Phosphate solubilizing bacteria (PSB) possesses the ability to convert insoluble phosphorus into soluble forms in the soil by secreting organic acids. PGPR follow numerous mechanisms of action which affect plant growth, directly or indirectly. These mechanisms may work sequentially or simultaneously at different stages of plant growth. For instance, i) increased mineral nutrient solubilization and N_2-fixation, making nutrients available for the plant; (ii) repression of soil borne pathogens by the production of hydrogen cyanide, siderophore, antibiotics, and/or competition for nutrients; (iii) improving tolerance against drought, salinity, and metal toxicity; and (vi) production of phytohormones such as indole-3-acetic acid (IAA). Moreover, some PGPR have the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase which hydrolyses ACC, the immediate precursor of ethylene in plants. Field experiment was conducted at Pulses Research Institute, Faisalabad to check the efficacy of *Rhizobium sp.* and PSM in the presence of precursor L-tryptophan (L-TRP) on the growth and yield of lentil and mash bean.

MATERIALS AND METHODS

Rhizobium sp and PGPR was screened out in lab for their growth hormone production potential. *Rhizobium* species of lentil and mash bean will be isolated and screened in the lab before application. PSM will be screened on the basis of auxin production and P-solubilization. Recommended fertilizer dose will be applied to all treatments in RCBD arrangement. All P and N will be applied at sowing. Study was conducted on medium texture soil having pH 7.94, EC 1.44 dS m^{-1}, soil N 0.030%, organic matter 0.62% and available P 7.60 mg kg^{-1}. The treatment details were as under,

TREATMENTS

T_1: Control

T_2: L-TRP (10^{-5} M)

T_3: Rhizobium Inoculation

T_4: PSM Inoculation

T_5: Rhizobium + PSM Inoculation

T_6: Rhizobium Inoculation + L-TRP (10^{-5} M)

T_7: PSM Inoculation + L-TRP (10^{-5} M)

T_8: Rhizobium + PSM + L-TRP (10^{-5} M)

RESULTS

Data regarding lentil response to following set of treatments revealed that co-inoculation influenced the nodulation positively as compared to their separate application and the effect was more pronounced with L-TRP application. Co-inoculation of Rhizobium and PSM with L-TRP application exhibited highest no. of nodules 15, nodular mass 0.077 g pot^{-1}, grain yield 1513.3 kg ha^{-1} and biomass yield 4506.7 kg ha^{-1} as compared to control.

Data regarding mash bean response revealed that co-inoculation of Rhizobium and PSM influenced the nodulation positively as compared to their separate application and the effect was more pronounced with L-TRP application. Co-inoculation of Rhizobium and PSM with L-TRP application exhibited highest no. of nodules 14, nodular mass 0.063 g pot^{-1}, grain yield 1493.3 kg ha^{-1} and biomass yield 4820.0 kg ha^{-1} as compared to control.

CONCLUSION

Co-inoculation of *Rhizobium* and PSM improve the growth of lentil and mash bean as compared to their separate application and the effect was more pronounced with L-TRP application.

Table 23: Response of lentil to different set of treatments

Treatments	Grain Yield (kg ha^{-1})	Biomass Yield (kg ha^{-1})	Nodules plant^{-1}	Nodular mass (g plant^{-1})
T_1: Control	1086.7 d	3566.7 b	10.0 e	0.047 e
T_2: L-TRP (10^{-5} M)	1153.3 cd	3773.3 ab	11.0 e	0.050 de
T_3: Rhizobium Inoculation	1296.7 bc	3923.3 ab	13.0 cd	0.063 bc
T_4: PSM Inoculation	1210.0 cd	3800.0 ab	10.7 e	0.053 cde
T_5: Rhizobium + PSM Inoculation	1386.7 ab	4100.0 ab	14.0 bc	0.057 bcde
T_6: Rhizobium Inoculation + L-TRP	1493.3 a	4273.3 ab	15.0 ab	0.067 ab
T_7: PSM Inoculation + L-TRP	1426.7 ab	4086.7 ab	12.7 d	0.060 bcd
T_8: Rhizobium + PSM + L-TRP	1513.3 a	4506.7 a	15.3 a	0.077 a
LSD	153.17	921.9	1.231	0.0127

Table 24: Response of Mashbean to different set of treatments

Treatments	Grain Yield (kg ha^{-1})	Biomass Yield (kg ha^{-1})	Nodules plant^{-1}	Nodular mass (g plant^{-1})
T_1: Control	1140.3 C	3660.0 E	11.7 C	0.050 C
T_2: L-TRP (10^{-5} M)	1216.7 BC	3730.0 E	13.3 BC	0.053 BC
T_3: Rhizobium Inoculation	1273.3 B	4096.7 CD	13.7 ABC	0.057 BC
T_4: PSM Inoculation	1243.3 BC	3910.0 DE	15.0 AB	0.053 BC
T_5: Rhizobium + PSM Inoculation	1306.7 B	4313.3 BC	14.7 AB	0.060 ABC
T_6: Rhizobium Inoculation + L-TRP	1493.3 A	4580.0 AB	15.7 AB	0.063 AB
T_7: PSM Inoculation + L-TRP	1451.7 A	4683.3 A	14.3 AB	0.063 AB
T_8: Rhizobium + PSM + L-TRP	1493.3 A	4820.0 A	16.0 A	0.070 A
LSD	126.69	337.75	2.4949	0.0111

3.10. GROWTH AND YIELD RESPONSE OF OIL SEED CROPS TO PGPR AND MICROBIALLY SYNTHESIZED METABOLITES

INTRODUCTION

Plant Growth Promoting Rhizobacteria (PGPB) is considered to promote plant growth directly or indirectly. PGPB can exhibit a variety of characteristics responsible for influencing plant growth. The common traits include production of plant growth regulators (auxin, gibberellins, ethylene etc.), siderophores, HCN and antibiotics. Indole acetic acid (IAA) is one of the most physiologically active auxins. IAA is a common product of L-tryptophan metabolism by several microorganisms including PGPR. Microorganisms

inhabiting rhizospheres of various plants are likely to synthesize and release auxin as secondary metabolite because of the rich supplies of substrates exuded from the roots, compared with non-rhizospheric soils. Plant morphogenic effects may also be a result of different ratios of plant hormones produced by roots as well as by rhizosphere bacteria. Diverse soil microorganisms including bacteria, fungi and algae are capable of producing physiologically active quantities of auxins, which may exert pronounced effects on plant growth and establishment.

Plants as primary producers synthesize tremendous amounts of organic compounds while consuming CO_2 and light energy. The spectrum of synthesized compounds is dependent on the plant species and physiological and environmental conditions. Some of the synthesized compounds are released into the rhizosphere, the soil directly surrounding roots which is affected by those released chemicals. Plants deposit approximately 11% of fixed carbon into the rhizosphere. The released carbon may appear to represent a significant energy loss for the plant, however; it may actually be beneficial due to the stimulation of biological activity in the rhizosphere, including stimulation of rhizosphere bacteria, which provide the plant with increased nutrient solubility, fix nitrogen and/or competitive suppression of pathogens, as well as plant growth promoting molecules. Exuded compounds can further change the properties of the surrounding soil and are important for obtaining nutrients, mediating biological interactions, or decreasing the toxicity of pollutants. Plant exudates and decomposing litter contain secondary plant metabolites (SPMEs) besides other compounds. Beyond their role in mediating plant–microbe interactions, it is hypothesized that SPMEs can stimulate microbial metabolism of pollutants present in the environment, which is termed the "secondary compound hypothesis". In a contaminated environment, indigenous microflora usually contain genetic determinants enabling the synthesis of degradative enzymes, however, environmental conditions can also often limit natural decontamination processes.

Plant metabolites can also enter the rhizosphere through decomposition of deposited litter and below-surface root turnover. Plants annually support the growth of fine roots during spring and summer; however, 40% to 70% of these fine roots die off in autumn as the plant prepares for winter, and these decaying fine roots provide rhizosphere microflora with nutrients and SPMEs. In addition, the decaying roots release air channels in the soil, which

allow the increased O_2 flow necessary for most enzymatic activities and can preferentially be used by new roots in subsequent growing seasons.

MATERIALS AND METHODS

A field experiment on raya was conducted at Agri. Biotech Research Institute, AARI, Faisalabad and on sesame was conducted at Oilseed Research Institute, AARI, Faisalabad to evaluate the effect of PGPR and their metabolites on growth and yield of raya and sesame. Soil having pH 8.04, ECe 2.3 and organic matter 0.59% was used in field trial of sesame while soil having pH 7.7, ECe 2.4 and organic matter 0.61% was used in the field trial of raya. Treatments were control (T_1), Azotobacter (T_2), Pseudomonas (T_3), Metabolite spray (T_4), T_2+T_4 = (T_5) and T_3+T_4 = (T_6). Sesame crop was sown during Kharif, 2019 and raya was sown during Rabi 2020.

RESULTS

Results revealed that in case of sesame and raya crop, maximum (1053 kg ha^{-1} and 3366 kg ha^{-1} respectively) grain yield was observed in T_6 where seeds were inoculated with Pseudomonas and their metabolites were sprayed as compared to control. Highest plant height, biomass yield was also observed in T_4 in both crops.

Table 25: Effect of PGPR and microbially synthesized metabolites on growth of sesame (Field Study)

Treatments	Grain Yield (kg ha^{-1})	Plant Height (cm)	Dry biomass (kg ha^{-1})	No. of pods ($plant^{-1}$)
T_1: Control	876.3 C	146.3 E	25533 D	763 D
T_2: Azotobacter	901.7 BC	170.0 D	27100 CD	971 B
T_3: Pseudomonas	928.0 BC	188.7 BC	28267 BCD	983 B
T_4: Metabolite spray	896.3 BC	178.3 CD	29687 BC	961 B
T_5: Azotobacter + Metabolite spray	967.3 B	193.0 AB	31033 AB	859 C
T_6: Pseudomonas + Metabolite spray	1053.3 A	204.0 A	33667 A	1102 A
LSD	80.52	12.400	3853.6	24.171

Table 26: Effect of PGPR and microbially synthesized metabolites on growth of Raya (Field Study)

Treatments	Grain Yield (kg ha^{-1})	Plant Height (cm)	Dry biomass (kg ha^{-1})	No. of pods $plant^{-1}$

T_1: Control	2886.7 C	252 E	12500 A	841 E
T_2: Azotobacter	3016.7 BC	280 BC	13000 A	988 CD
T_3: Pseudomonas	3316.7 AB	283 AB	16000 A	1091 BC
T_4: Metabolite spray	3100.0 ABC	265 D	13000 A	883 DE
T_5: Azotobacter + Metabolite spray	3200.0 AB	275 C	15500 A	1158 B
T_6: Pseudomonas + Metabolite spray	3366.7 A	289 A	17167 A	1389 A
LSD	326.15	6.3689	5479.7	135.36

3.11. PRECURSOR-INOCULUM INTERACTION FOR IMPROVING GROWTH AND YIELD OF BEANS (MUNGBEAN AND SOYBEAN)

INTRODUCION

Mungbean is one of the important pulse crops of our country especially in Punjab while soybean is also fetching the attraction of people due to its oil and protein contents. Both being legumes; take most of their nitrogen through symbiosis with *Rhizobium*. Efficiency of Rhizobium-legume symbiosis depends upon a number of factors. Among them the most important one is nodule causing bacteria. Plant hormones are of chemical nature and are the substances which are naturally produced by the plants. They may alter the growth of a plant or plant part. These hormones control many plant functions such as root growth, fruit setting and drop, growth and other development processes. One of such hormones, the auxins, usually affects other processes in addition to cell elongation of stem cells but this characteristic is considered critical of all auxins and thus helps to define the hormone. An exogenously applied of auxins may affect the endogenous hormonal pattern of the plant. L-Tryptophan is an essential amino acid as the physiological precursor of auxins in higher plants and microbial biosynthesis. A variety of soil microorganisms are actively involved in the production of auxins in pure culture and in soil amended with IAA and L-Tryptophan. The formation of nodules on legumes is associated with auxin forming Rhizobium. This study was planned to assess the potential of different *Rhizobium* species along with physiological precursor to improve the crop growth and yield of Mungbean and Soybean crops.

MATERIAL AND METHODS

A field experiments were conducted at Pulses Research Institute and Oil Seed Research Institute, AARI, Faisalabad during the year 2019-20.

TREATMENTS

T_1: Control

T_2: *Rhizobium* inoculation-1

T_3: *Rhizobium* inoculation-2

T_4: *Rhizobium* inoculation-3

T_5: L-Tryptophan@ 10^{-5} M

T_6: *Rhizobium*-1 + L-TRP@ 10^{-5} M

T_7: *Rhizobium*-2 + L-TRP@ 10^{-5} M

T8: *Rhizobium*-3 + L-TRP@ 10^{-5} M

METHODOLOGY

Rhizobium species were isolated from Mungbean and Soybean nodules and their pre-auxin level and without L-Tryptophan was checked in the lab study. Efficient *Rhizobium* isolates were used for inocula preparation. Crop specific precursor enriched inocula and recommended NPK were used for trials. All the P, K and half N were applied at sowing, while rest of half N was applied after 30-35 days. Layout was RCBD with three repeats.

RESULTS

Data regarding grain yield, no. of nodules plant^{-1} as well as nodular mass plant^{-1} of mungbean crop was presented in table. Rhizobial inoculation produced significantly higher (1506 kg ha^{-1}) grain yield i.e. with L-Tryptophan (T_8) followed by 1473 kg ha^{-}as compared to control (1136kg ha^{-1}). The combined effect of the L-Tryptophan and Rhizobium species produced the higher grain yield than the respective control. As concerned the nodulation the data presented in table. Rhizobia inoculation along with different concentrations of L-tryptophan produced higher number of nodules and nodular mass as compare to control.

Data regarding grain yield, no. of nodules plant^{-1} as well as nodular mass per plan^{-1}t of mungbean crop was presented in table. Rhizobial inoculation produced significantly higher (2423 kg ha^{-1}) grain yield with L-Tryptophan followed by 2207 kg ha-1 as compared to control (1510kg ha^{-1}). The integrated effect of the L-Tryptophan and *Rhizobium* species produced the higher grain yield than the respective control. Data regarding nodulation is

presented in the table. Rhizobia inoculation along with different concentrations of L-tryptophan produced higher number of nodules and nodular mass as compare to control.

CONCLUSION

Rhizobium inoculum along with L-Tryptophan significantly increased the yield and other growth parameters as compared to control.

Table 27: Effect of *Rhizobium* species and L-Tryptophan on the yield parameters of Mungbean

Treatments	**Mungbean**		
	Grain yield (kg ha^{-1})	**Nodules Plant^{-1}**	**Nodular Mass plant^{-1}**
T_1: Control	1136	6.0	0.039
T_2: *Rhizobium* inoculation-1	1246	7.0	0.045
T_3: *Rhizobium* inoculation-2	1343	8.3	0.050
T_4: *Rhizobium* inoculation-3	1366	8.0	0.057
T_5: L-Tryptophan@ 10^{-5} M	1393	8.7	0.063
T_6: *Rhizobium*-1 +L-TRP@ 10^{-5} M	1436	9.0	0.083
T_7: *Rhizobium*-2 +L-TRP@ 10^{-5} M	1473	9.7	0.088
T_8: *Rhizobium*-3 + L-TRP@ 10^{-5} M	1506	11.0	0.094
LSD	53.496	1.6043	0.006

Table 28: Effect of *Rhizobium* species and L-Tryptophan on the yield parameters of Soybean

Treatments	**Soybean**		
	Grain yield (kg ha^{-1})	**Nodules Plant^{-1}**	**Nodular Mass plant^{-1}**
T_1: Control	1510	7.7	0.040
T_2: *Rhizobium* inoculation-1	1640	8.0	0.040
T_3: *Rhizobium* inoculation-2	1830	8.3	0.043
T_4: *Rhizobium* inoculation-3	1882	8.3	0.035
T_5: L-Tryptophan@ 10^{-5} M	1923	8.0	0.045
T_6: *Rhizobium*-1 +L-TRP@ 10^{-5} M	1963	8.7	0.046
T_7: *Rhizobium*-2 +L-TRP@ 10^{-5} M	2207	9.0	0.048
T_8: *Rhizobium*-3 + L-TRP@ 10^{-5} M	2423	9.7	0.048
LSD	138.23	1.5523	0.00318

3.12. CO-INOCULATION OF *BRADYRHIZOBIUM* AND PHOSPHATE SOLUBILIZING MICROBESON ON GROWTH PROMOTION OF GROUNDNUT UNDER RAIN-FED CONDITIONS

Providing P to plants through biological means is an eco-friendly and viable alternative. Among heterogeneously distributed soil microflora, a group of micro-organisms commonly referred to as phosphate-solubilizing microorganisms (PSM) including bacteria (PSB), fungi (PSF), and actinomycetes, have been found active in conversion of insoluble P to soluble forms and making it accessible to plants. Different PGPR solubilize insoluble P, the conversion of organic and inorganic P, providing P to plants through biological means is an eco-friendly and viable alternative.

Conversion of organic and inorganic P by rhizobia to available P has dual advantages; besides P solubilization, they can provide other essential nutrient, for example, N to plants and also have the ability to improve legume growth synergistically with other PGPR and arbuscular mycorrhizal fungi. Accordingly, it is reported that the phosphate-solubilizing bacteria (PSB) when applied with PGPR could reduce P fertilizer application by 50% without any significant reduction in crop yields suggesting that PSB as inoculant/biofertilizers hold greater promise for sustaining crop production with optimized P fertilization. However, under certain real soil situations, the use of PSB to augment crop productivity has been limited largely due to the variability and inconsistency of results observed under laboratory, greenhouse and field trials. Such variation in the performance of PSB has been attributed to many factors including nutrient status of soils, plant genotypes, root exudates etc. Despite all these factors, increase in crop yields following PSB applications in the growth chambers and field trials have been observed. P-solubilizing microorganism endowed solely with P-solubilizing activity or multiple growth-promoting activities improve legume productivity in different agro-ecosystems.

Infection of legumes by rhizobia may occur by immediate intercellular penetration of root cells (crack entry) as an alternative mode to the more elaborate infection through infection threads.

Groundnuts are a popular source of food throughout the world, including Pakistan. In many countries groundnuts are consumed as peanut butter or crushed and used for the groundnut oil or simply consumed as a confectionary snack roasted, salted or in sweets. In other parts of the world, they are boiled, either in the shell or unshelled. Groundnuts are produced in the tropical and subtropical regions of the world, on sandy soils. The production practices vary from highly sophisticated commercial ventures in the western world to more traditional

cropping practices in third world countries. Yields vary from about 400 kg to several tons per hectare depending on production system and production area.

MATERIALS AND METHODS

For this purpose, groundnut rhizosphere samples were collected from different locations and *Bradyrhizobium* spp. were isolated in laboratory. Characterization of *Bradyrhizobium* and phosphate solubilizing bacteria were done in lab before their application as seed coating. Three efficient *Bradyrhizobium* isolates were selected for checking their effect on groundnut crop separately as well as in combination with PSM.

Field trial was conducted at the Soil & Water Conservation Research Institute (SAWCRI), Chakwal, using normal soil. Treatments were control (T_1), *Bradyrhizobium* isolate-1 (T_2), *Bradyrhizobium* isolate-2 (T_3), *Bradyrhizobium* isolate-3(T_4), Phosphate solubilizing microbe (PSM) (T_5), T_2 + PSM (T_6), T_3 + PSM (T_7), T_4 + PSM (T_8). Parameters recorded were plant height, fresh weight, no. of pods plant^{-1}, pods fresh weight, pods dry weight. Recommended dose of NPK (25:80:25) was applied as basal dose. Trial was designed following CRBD. Groundnut crop was sown during Kharif 2019.

RESULTS

Data revealed that maximum groundnut pod yield (2428 kg/ha) was obtained in treatment where inoculation with bacterial isolate-2 as compared to control. In case of plant height and shoot dry biomass maximum response was observed in T_7 (59.4 cm, 4733 kg ha^{-1}) as compared to control i.e. 40.5cm, 3156.7 kg ha^{-1} respectively.

Table 29: Co-inoculation of *Bradyrhizobium* and Phosphate solubilizing microbes for growth promotion of groundnut under rain-fed conditions

Treatments	Plant Height (cm)	No. of Pods plant^{-1}	Grain Yield (kg ha^{-1})	Biomass Yield (kg ha^{-1})	No. of Nodules
T_1: Control	40.5 ab	15.0 e	1234.6 d	3156.7 c	8.6 d
T_2: Brady-1	46.1 ab	23.7 de	1687.0 d	3251.3 bc	14.5 cd
T_3: Brady-2	54.4 ab	25.4 bcd	1786.0 c	3687.3 bc	16.7 ab
T_4: Brady-3	49.7 ab	21.8 cde	1444.0 d	3429.8 bc	14.4 cd
T_5: PSM Inoculation	53.4 b	27.0 abc	1585.0 d	4273.1 bc	12.3 cd
T_6: Brady-1 +PSM	53.5 ab	28..8 ab	1650.0 bc	4438.3 b	15.4 bc
T_7: Brady-2+ PSM	59.4 a	31.4 a	2428.0 a	4733.0 a	25.2 a
T_8: Brady-3+ PSM	54.1 ab	28.7 ab	1987.0 b	4176.3 bc	19.9 bc
LSD	5.28	4.76	312.18	690.48	5.1074

3.13. BIOCONTROL OF FUSARIUM WILT IN CHICKPEA BY CO-INOCULATION WITH RHIZOBIUM AND PGPR

INTRODUCTION

Chickpea (*Cicer arietinum L.*) is an important legume crop in semi-arid tropics of the world. In poor countries, it is an important food source because of its great wealth of seeds and leaves in vegetal proteins. It is generally grown as first-rate preceding crop to cereals. Chickpea shows excellent response to PGPR application. Use of PGPR is steadily increasing in agriculture since it offers an attractive way to replace chemical fertilizers, pesticides, and supplements. Preparations of live microorganisms (bacteria, fungi), generally referred as biofertilizers or microbial inoculant, are utilized for improving plant growth and crop productivity. Fusarium wilt, caused by *Fusarium oxysporumf*.sp.*ciceris* (Foc), is a major constraint to chickpea production worldwide. Different mechanisms may be involved in the biological control of fusarium wilt by non-host *F. oxysporum* isolates. These mechanisms include saprophytic competition for nutrients; parasitic competition for infection sites; and induced or enhanced resistance within the host. These mechanisms are not necessarily exclusive of one another and several mechanisms may be responsible for disease suppression by many bio control agents. Chickpea crop is grown in arid areas and it has sufficient ability to adopt in different biotic and abiotic environmental stress conditions. Through symbiotic N- fixation chickpea fulfills 70% of its N requirement. Plants absorb most of N in the form of nitrate and ammonia. Plants are unable to utilize N in its elemental form (N_2) for their growth, therefore, there is need to convert unavailable organic N to available inorganic N, carried out according to following pathways:

1- Symbiotic micro-organisms, especially in the roots of legumes
2- Fixation by free-living micro-organisms in the soil
3- Fixation through various industrial processes such as Haber-Bosch Process

In Biological N-fixation, rhizobia symbiotically fix atmospheric N into form which is available for plants in the presence of nitrogenase enzyme.

MATERIAL AND METHODS

Field experiment was conducted at the Pulses Research Institute, Faisalabad to check the antifungal response of microbes against *fusarium* wilt and enhancing the yield of chickpea

in diseased soil. Trial was carried out in normal and *fusarium* borne sick soil having sandy clay loam texture, pH: 8.5, EC: 2.5 dS m^{-1}, available P: 7.6 mg kg^{-1} and organic matter 0.76 %. Recommended fertilizer dose @ 30-60 kg NP ha^{-1} was applied to all treatments.

Isolation of PGPR from the rhizosphere and *Rhizobium* from nodules of chickpea was carried out. In the lab study, microbes were characterized for disease suppression. Virulence of *Fusarium sp.* was checked. Disease suppression capability of microbes was tested by growing the fungus and bacteria on the same petri plate. All NP was applied at sowing. Peat based inoculum was applied as seed coating. Trial was raised in field with three repeats following RCBD.

TREATMENTS

T_1: Control (Normal soil)

T_2: Control (*Fusarium* infested soil)

T_3: T_1 + *Rhizobium* inoculation

T_4: T_2 + *Rhizobium* inoculation

T_5: T_1 + PGPR inoculation

T_6: T_2 + PGPR inoculation

T_7: T_1 + Co-inoculation

T_8: T_2 + Co-inoculation

Note: Fertilizer @ 30-60 kg NP ha^{-1} was applied to all treatments.

RESULTS

In normal soils co-inoculation gives more response as compare to fusarium sick soil. But in fusarium sick soil co-inoculation gave more response as compared to control and other treatments. Results clearly indicated that co-inoculation gave highest (1210 kg ha^{-1}) grain yield in normal soil as well as in sick soil (1190 kg ha^{-1}) as compared to their respective control (1100 kg $ha^{-1}$and 1126 kg ha^{-1}).

Table 30: Bio-control of fusarium wilts in chickpea by co-inoculation with *Rhizobium* and PGPR

Treatments	Grain yield	100 grain	Nodules $plant^{-1}$	Plant height	No. of Dead Plants	
					After	After

	(kg ha^{-1})	weight (g)		(cm)	Germination	one month
T_1: Control (Normal soil)	1100.3 F	11.3 B	14.7 BC	44.3 C	1.0 AB	0.7 A
T_2:Control (Fusarium infested soil)	1126.0 EF	9.0 D	10.7 D	43.3 C	2.7 A	1.3 A
T_3: T_1 + *Rhizobium*	1138.7 CDE	11.5 B	15.7 BC	53.0 A	0.3 B	0.3 A
T_4: T_2 + *Rhizobium*	1126.7 DEF	9.7 CD	13.3 CD	47.3 BC	0.7 AB	0.7 A
T_5: T_1 + PGPR inoculation	1164.0 BCD	14.3 A	17.3 AB	53.7 A	0.3 B	0.00 A
T_6: T_2 + PGPR inoculation	1165.7 BC	10.5BC	15.7 BC	52.3 AB	1.0 AB	0.7 A
T_7: T_1 + Co-inoculation	1210.3 A	14.9 A	20.3 A	56.3 A	1.7 AB	0.7 A
T_8: T_2 + Co-inoculation	1190.7 AB	11.0 BC	18.3 AB	56.7 A	2.3 AB	1.3 A
LSD	37.87	1.21	3.77	5.13	2.08	1.48

3.14. INFLUENCE OF L- METHIONINE AND *RHIZOBIUM SP* ON NODULATION, GROWTH AND YIELD OF MUNG BEAN

Mungbean forms symbiotic associations with effective *Rhizobium* strains. Under favorable conditions, symbiotic N fixation can provide up to 85% of the N required by a mungbean crop. Inoculation of legumes is necessary in the absence of compatible *Rhizobium* or where indigenous Rhizobial populations are low or inefficient in fixing nitrogen. In rain fed areas, greater number of nodules in inoculated treatments may be observed compared with non-inoculated ones that relied on indigenous rhizobia. Several studies have demonstrated the effects of inoculating mungbean with *Rhizobium* on soil fertility improvement as well as growth and yield. Plant growth regulators (PGRs) are the organic molecules other than the vitamins and essential plant nutrients, which play role in the development and growth of plants even at very low concentration. Environment depending factors such as soil pH, soil moisture, organic matter, native strains and soil temperature have definite influence on the indigenous microbial population. Therefore, it is imperative that the effect of *Rhizobium* inoculation on the productivity of a crop be assessed in the target environment. PGRs are grouped into auxins, cytokinins, ethylene, gibberellins, abscisic acids, salicylic acids and jasmonic acids, which are synthesized within plant bodies and are also released by microbes living in the rhizosphere. Around the globe, application of these PGRs is considered as an essential practice and the use of these PGRs has been increased rapidly over the period of

last few years. It has been studied that production of phytohormones can be enhanced many times by the provision of suitable precursors to the microbes residing in soil. Microbes present in the rhizosphere utilize these precursors and convert them into phytohormones and thus provide a continuous source of these active substances for the uptake of plants. It has been clearly found that exogenous application of precursors enhances the production of PGRs many fold in soil and culture. Ethylene (C_2H_4) is an important gaseous plant hormone that affects almost all stages of growth and developmental processes. It plays a vital role in embryogenesis, germination, senescence, leaf abscission, flowers induction and fruit maturity. It also promotes the transcription and translation of ripening related genes involved in cell wall breakdown and carotenoid biosynthesis. Concentration of C_2H_4 in the soil depends upon soil properties, substrate nature and native micro biota. L-MET is an established precursor of C_2H_4 which is known to induce early onset of flowering and ripening of fruits in different vegetables and agronomic crops. Application of L-MET not only increases growth but also increases the level of C_2H_4 in the soil. Exogenous application of L-MET induces early flowering in different vegetables and agronomic crops. C_2H_4 accumulation increases in the soil atmosphere by the addition of L-MET. C_2H_4production in soil is highly dependent on availability of substrate. The addition of L-MET in soil stimulates C_2H_4production and a significant growth and yield response in soybean was noticed by the application of L-MET to soil. The present study was designed to evaluate the effect of different levels of L-MET alone and in combination on growth, and yield of mungbean.

MATERIALS AND METHODS

A field study was conducted to test the precursor L-methionine and *Rhizobium* effect to improve the mungbean growth and yield at Pulse Research Institute, AARI, Faisalabad by using normal having pH 7.8, ECe 2.4, Organic matter 0.67% and available P 8.1 $mgkg^{-1}$. Treatments were control (T_1), *Rhizobium* sp (T_2), L-methionine ($5mgL^{-1}$) (T_3) L-methionine ($10mgL^{-1}$) (T_4), L-methionine ($15mgL^{-1}$) (T_5), *Rhizobium* sp + L-methionine ($5mgL^{-1}$) (T_6), *Rhizobium* sp + L-methionine ($10\ mgL^{-1}$) (T_7), *Rhizobium* sp + L-methionine ($15\ mgL^{-1}$) (T_8). Crop was sown during Kharif 2019. Recommended dose of fertilizer @ 30-60 kg NP ha^{-1} was applied.

RESULTS

Results revealed that exogenous application of L-Methionine and Rhizobium has positive effect on growth and yield of mung bean. Maximum grain yield and dry biomass (1300, 2364 kg ha^{-1}) was observed in treatment T_6 where *Rhizobium* was applied along with L-Methionine @ 5mgL^{-1}, following by T_7 (1176, 2194 kg ha^{-1}) where *Rhizobium* was applied with L-Methionine @10mgL^{-1} as compared to control i.e. 963,1732 kg ha^{-1} respectively. Maximum plant height and nodule $plant^{-1}$ was observed in T_7, T_8 treated plants i.e. 67, 65cm , 22.0 respectively, as compared to control. Significant effect was observed in IAA biosynthesis potential of all treated plants as compared to control.

Table 31: Influence of L- methionine and *Rhizobium sp* on nodulation, and yield of mungbean

Treatments	**Grain Yield (kgha^{-1})**	**Dry biomass (kgha^{-1})**	**Plant height (cm)**	**Nodule $plant^{-1}$**	**IAA (µg mL^{-1})**		
					15 Days	**30 Days**	**45 Days**
T_1:Control	963e	1732f	48.0 e	12.0 d	0.8 e	1.1 f	1.5 f
T_2: Rhizobium	1010de	1877de	59.0 bc	18.0 ab	1.5 abc	1.8 c	2.1 de
T_3: L-MET @ 5mgL^{-1}	1087c	1899d	60.0 b	16.0 b	1.1d	1.5 e	1.8 ef
T_4: L-MET @10mgL^{-1}	1057cd	1885de	56.0 d	14.0 c	1.3 bc	1.7 cd	2.2 cde
T_5: L-MET @ 15 mg L^{-1}	1030cd	1843e	57.0 c	16.0 b	1.2 cd	1.6 d	2.3 bcd
T_6:T_2+ T_3	1300a	2364a	62.0 ab	20.0 ab	1.6 ab	2.5a	2.9a
T_7: T_2+T_4	1176b	2194b	67.0 a	22.0 a	1.9 a	2.2 b	2.5bc
T_8: T_2+T_5	1220b	2053c	65.0 a	22.0 a	1.7 ab	2.1bc	2.6ab
LSD	63.695	50.955	3.4077	3.067	0.255	0.282	0.411
L-MET: L-Methionine							

3.15. ISOLATION, CHARACTERIZATION OF SULPHUR OXIDIZING BACTERIA FOR ACIDIFICATION OF COMPOST

INTRODUCTION

Sulphur is now considered the fourth major plant nutrient after N, P and K, and is one of the sixteen nutrient elements which are essential for the growth and development of plants, especially in the agricultural crop production. This is mainly because of its wide-spread deficiency in the soil world over. The importance of sulphur is equal to that of nitrogen in terms of protein synthesis, and in terms of crop uptake it exceeds even that of phosphorus. The majority of sulphur taken up by plant roots is in the form of sulphate (SO_4), which undergoes a series of transformations prior to its incorporation into the original com-pounds. The soil microbial biomass is the key driving force behind all sulphur transformation. The biomass acts as both a source and sink for inorganic sulphate. The latter make sulphate

available from element sulphur or any reduced forms of sulphur through its oxidation process in the soil. The role of chemolithotrophic bacteria of the genus *Thiobacillus* in this process is essential. The objective of this study was to isolate and characterize the sulphur oxidizing bacteria from various sources.

The major problem related to the P in soil is its very low solubility and availability which is directly related to the soil pH and binding capacity of clay lattice. In alkaline soils, availability of P, especially those with excess $CaCO_3$, is relatively low. Calcareous nature of a soil leads to the low recovery of Olsen's phosphorous. Although, it was investigated the phosphorous transformation dynamics, release and fixation pattern in soil. However, actual amount of labile phosphorous to be made available in a growing season is still in question. Physico-chemical properties of the soil are greatly influenced by organic amendments. Conventionally, fertility status of the soil has been maintained by using farm manure as an organic amendment but in recent years compost has become an important ingredient of the farming system. Production of compost does not require any sophisticated setup and can be easily prepared from local left overs of agriculture products with little or no expense. Multiple benefits of compost have been reported i.e. increase in moisture holding capacity, increase in porosity, flocculation and stabilization of pH. Elemental sulphur was used for slow and steady soil acidification of leaf and yard pruning's compost. Under aerobic condition, biological oxidation of elemental sulphur yields sulphuric acid that produces acidity:

$$S^0 + 1.5\ O_2 + H_2O \rightarrow H_2SO_4$$

MATERIALS AND METHODS

The trial was planned to isolate the efficient strains of sulphur oxidizing bacteria to reduce pH of organic source. For this purpose, rhizosphere soil samples collected from different areas. Isolation was carried out on Thiobacillus agar, Starkey broth and Beijerinck media by using dilution plate technique. The isolates will be further purified by repeated streaking. Purified isolates were checked for reduction of pH in compost. Four purified isolates were checked for microbial count, auxin biosynthesis and reduction of pH in compost. Fifteen isolates of *Thiobacillus sp* were isolated on Beijerinck media. Four out of fifteen isolates were efficient in significant pH reduction. These four isolates were also tested on Starkey broth. Isolates decreased pH of the media in the following order (Table).Isolates efficient in pH reduction were tested in pot experiment using compost for estimation of pH reduction.

RESULTS

Results revealed that in pots at wire house conditions, maximum (3.97) pH reduction was observed in T_4 where isolate no.4 (Thio-4) was applied as compared to control. Highest auxin biosynthesis and microbial population was also observed in T_4 under wire house conditions (3.156 $\mu g mL^{-1}$, 8.92 @ 10^6 CFU g^{-1}).

Table 32: Isolation, characterization of sulphur oxidizing bacteria for acidification of compost

Isolates	pH				Microbial Population @ 10^6 g^{-1}			IAA Equivalents (μgmL^{-1})
	Initial pH	10 days	20 days	30 days	10 days	20 days	30 days	
Thio-1	8.00	7.75	5.59	4.69	4.98	6.87	7.65	2.031
Thio-2	8.00	7.18	6.74	4.45	4.50	6.35	7.96	2.712
Thio-3	8.00	6.83	4.55	3.99	5.26	6.66	8.28	2.864
Thio-4	8.00	5.54	4.45	3.97	5.43	7.90	8.92	3.156

3.16. PLANT MICROBE INTERACTIONS FOR THE GROWTH AND YIELD IMPROVEMENT OF BERSEEM FODDER

INTRODUCTION

Plant growth promoting rhizobacteria (PGPR) form an association with Rhizobia and colonize through roots. PGPR improve N-fixation and nodulation process in leguminous crops. *Rhizobium* and PGPR co-inoculation have both positive and negative impact on plant growth. It caused a decrease in growth due to the competition for attachment sites on root

surfaces and by the production of antibiotics. *Rhizobium* and PGPR co-inoculation enhances growth by the reduction in ethylene level. Increase in phosphate solubilization and number of *Rhizobium* colonization sites, pathogens are contained due to antibiotics production and hormone-induced amplification of the root system which results in enhanced nutrient uptake. Bacterial and fungal-inoculation increased the stomatal conductance, water use efficiency (WUE) and photosynthetic rate and the relative water content of plants. Plants inoculated with *Rhizobium* and PGPR possessing ACC deaminase activity bears longer roots that help the plants to uptake more water from soil under stress.

Berseem (*Trifolium alexandrinum* L.) is one of the most important leguminous forages in the Middle-East and the Mediterranean region. It is important winter forage because of its nutritional value and contains more than 20% and 70% crude protein and dry matter digestibility, respectively. In Pakistan, it is also cultivated as Rabi (winter) fodder crop in irrigated areas. It grows better on alkaline and high moisture soils but slightly less drought-resistant. Miscawi and Kahdrawiare are the berseem cultivars having higher productive potential with branching types of growth attributes. It is an erect, sparsely hairy, 30 to 80 cm in height and annual forage legume. Flowers are yellowish-white and form elliptical, dense clustered heads that are 2 cm in diameter. The fruit is a pod with one white to purplish-red seed, shallow taproot system, hollow stems, base branching, alternate leaves, with 2-3 cm broad × 4-5 cm long leaflets. The seeds are abundant under favorable conditions due to its comparable feed value, and it is compared to alfalfa; however, unlike alfalfa, it never showed bloat symptoms. Berseem clover is also used as a green manure crop. Berseem can also be made into silage with oats or be fed chaffed and mixed with chopped straw.

Tryptamine, a monoamine alkaloid with one indole ring, is similar in structure to the amino acid tryptophan. Tryptamine is found in many plants in small amounts. It influences plant microbiome and growth by acting as a feedstock for the metabolic pathways. It is found as a possible intermediate in the indole-3-acetic acid biosynthetic pathway. Total four tryptophan-dependent pathways occurred, 1): IPA Pathway (indole-3-pyrovate), 2): Tryptamine Pathway, 3): IAN Pathway (Indole-3-Acetonitrile), 4): IAM Pathway (Indole-3-Acetamide). The decarboxylation of tryptophan occurred, and then Tryptamine produced which convert it into indole-3-acetaldehyde with deamination. It has been demonstrated that

Trp-dependent auxin biosynthesis is essential for embryogenesis, seedling growth, flower development, vascular pattern formation, and other developmental processes.

However, considering the above-mentioned information, there is a need to conduct systematic work in Punjab province (Pakistan) on the factors limiting growth and fodder yield of Berseem with co-inoculation of PGPR and Rhizobia along with the foliar application of Tryptamine to enhance growth and fodder yield of berseem in the presence and absence of Tryptamine.

MATERIALS AND METHODS

Efficient isolates of *Rhizobium* and PGPR species available at Soil Bacteriology Section were used for inocula preparation. Seed treatment of respective bacteria was used as seed coating while Tryptamine foliar sprays after each cutting. All NPK were applied before sowing. Field trial was conducted with three repeats in RCBD layout. Field study was conducted at the Fodder Sub-Section, AARI, Faisalabad using normal soil. Treatments were control (T_1), *Rhizobium Inoculation* (T_2), *PGPR* Inoculation(T_3), *Co-inoculation*(T_4), Control + Tryptamine @ 10^{-5} M (foliar spray) (T_5), Rhizobium + Tryptamine @ 10^{-5} M (foliar spray) (T_6), PGPR + Tryptamine @ 10^{-5} M (foliar spray) (T_7), Co-inoculation + Tryptamine @ 10^{-5} M (foliar spray) (T8).Parameters recorded were fresh fodder yield, dry matter, plant height, nodule plant^{-1}, nodule mass. Result showed that T8 which is co-inoculation (*Rhizobium* + PGPR) along with foliar spray of tryptamine gave highest fresh fodder yield 44.6 t ha^{-1} that is significant increase in yield as compared to control.

RESULTS

Parameters recorded were fresh fodder yield, dry matter, plant height, nodule plant^{-1} and nodule mass. Result showed that T_8 which is Co-inoculation (*Rhizobium* + PGPR) along with foliar spray of tryptamine gave highest fresh fodder yield 44.6 t ha^{-1} that is significant increase in yield as compared to control. All remaining parameters were also increased maximum at treatment T_8 which is Co-inoculation (*Rhizobium* + PGPR) along with foliar spray of tryptamine.

Table 33: Growth and yield response of berseem to co-inoculation of Rhizobium and PGPR with and without Tryptamine

Treatments	Fresh Fodder Yield (t ha^{-1})	Dry Matter (t ha^{-1})	Plant Height (cm)	Nodules $plant^{-1}$	Nodule mass (g $plant^{-1}$)
T_1: Control	27.6 E	4.56 E	76.29 F	9.6 E	0.077 D
T_2: Rhizobium Inoculation	37.1 BCD	7.14 CD	90.38 DE	19.3 C	0.130 C
T_3: PGPR Inoculation	34.3 DE	6.65 D	86.19 CD	16.4 D	0.123 BC
T_4: Co-Inoculation	38.2 BC	6.99 AB	97.10 BC	20.8.3 B	0.148 B
T_5:Control + TRY*@ 10^{-5}M	32.1 CD	5.40 D	82.18 EF	16.4 CD	0.023 E
T_6: Rhizobium + TRY@ 10^{-5} M	41.4 AB	8.98 BC	99.34BCD	22.8 B	0.139 BC
T_7: PGPR +TRY @ 10^{-5} M	39.6 B	7.76 CD	95.76 B	19.5 B	0.131 B
T_8: Co-Inoculation + TRY@10^{-5} M	44.6 A	9.29 A	101.38 A	26.5 A	0.234 A
LSD	0.370	0.441	5.580	0.653	0.0180
*TRY: Tryptamine					

3.17. ISOLATION, CHARACTERIZATION AND SCREENING OF PGPR CAPABLE OF PROVIDING RELIEF IN ABIOTIC STRESSES

INTRODUCTION

Soil salinization is a major factor contributing to the loss of productivity of cultivated soils. Although it is difficult to accurately estimate, but the area of salinized soils is increasing and the phenomenon is especially intense in irrigated soils. It was estimated that about 20% (45 million ha) of irrigated land, producing one-third of the world's food, is salt-affected. Salinity is one of the major abiotic stresses that adversely affect modern agriculture and constitutes a problem everywhere in the world. More than 6% of the world's total land area is salt-affected and mainly this salt-affected land has arisen from natural causes and the accumulation of salts over long periods of time in arid and semiarid zones.

World is facing huge losses of agriculture productivity due to salt stress. Salinity is considered one of the major factors limiting plant growth and productivity, especially, in semi-arid and arid regions. About 831 mha of land is salt-affected that is 6.5% of the total land of the world.

Agriculture is the major contributing factor in Pakistan's economy. The sustainability of irrigated agriculture in Pakistan is confronted with the twin menace of water-logging and salinity, therefore; productive lands are continuously going out of cultivation. Hyper-ionic and hyper-osmotic stresses leading to plant death are the main effects caused by salinity. In

semi-arid and arid regions, the major contributing factors are scarcity of rainfall, high evapotranspiration rate and temperature. Molecular and physiological behavior of plants to salt stress has been studied extensively but the principal mechanisms are still not well understood. All the major plant processes like protein synthesis, energy, lipid metabolism and photosynthesis are badly affected during the development of salt stress in a plant.

Salinity affects plant growth and metabolism through reduced uptake, nutritional imbalance and toxic effect of some ions, e.g. uptake and translocation of Na^+ and K^+ is affected under salinity stress. Plant's mineral nutrient status plays a critical role in increasing abiotic stress factor tolerance. The increase of plant Na^+ level and decreased K^+ nutrition is a major distinction of plants facing salinity stress, thus K^+/Na^+ ratio and their uptake rate in stressed plants is considered good measure of salt tolerance. A plant's prolonged exposure to NaCl resulted in Na^+ translocation from the roots to transpiring leaves, where it can attain toxic levels. Many of the mechanisms are related to the maintenance of low Na^+ in shoots that enables plants to tolerate high soil salinity.

Soils that have excessive amounts of salts (i.e., EC > 4 dS/m) are classified as saline soils. Soil salinity stresses plants in two ways: high concentrations of salts in the soil make it harder for roots to extract water and high concentrations of salts within the plant can be toxic. Salts on the outside of the roots have an immediate effect on cell growth and associated metabolism; toxic concentrations of salts take time to accumulate inside plants, before they affect plant function.

One of the approaches to solve the salt stress problem is the use of plant growth-promoting bacteria (PGPB). Many gram-positive and gram-negative PGPB have been reported to colonize the plant rhizosphere and confer beneficial effects by various direct and indirect mechanisms, which can be correlated with their ability to form biofilms, chemo taxis, and the production of exopolysaccharides, indole-3-acetic acids (IAA) and aminocyclopropane-1- carboxylate (ACC) deaminase. Investigations on the interaction of PGPB with other microbes and their effect on the physiological response of crop plants under different soil salinity regimes are still at an embryonic stage. Alleviation of salt stress by PGPB inoculants has been shown in rice, wheat, maize, cotton, lettuce, tomato and pepper.

MATERIALS AND METHODS

A field experiment on wheat and rice was conducted at Soil Salinity Research Institute, Pindi Bhattian to evaluate the effect of salt tolerant PGPR on growth and yield of both crops. Soil having pH 8.58, ECe 6.8 d Sm^{-1} and SAR 26 was used in field trial. Treatments were control (T_1), KH-1 (T_2), Kh-2 (T_3), KH-3 (T_4), PGPR Azotobacter (T_5) and PGPR Pseudomonas (T_6). Rice crop was sown during Kharif 2019 and wheat crop was sown during Rabi 2020.

RESULTS

Results revealed that in case of wheat crop maximum (1942 kg ha^{-1}) grain yield was observed in T_3 where seeds were inoculated with KH-2 as compared to control. In case of rice crop, maximum (2983 kg ha^{-1}) paddy yield was also observed in T_3 where seeds were inoculated with KH-2 as compared to control. The other parameters were also significantly higher in KH-2 as compared to control.

Table 34: Effect of salt tolerant isolates and rhizobacteria on wheat (SSRI, Pindi Bhattian)

Treatments	Plant Height (cm)	Tillers m^{-2}	Biomass Yield ($kgha^{-1}$)	Grain Yield ($kgha^{-1}$)
T1: Control	72.0 C	170.7 B	5940 B	1572.7 D
T2: KH-1	74.3 BC	173.0 B	6123 B	1757.7 C
T3: KH-2	78.7 AB	190.3 A	6691 A	1942.7 A
T4: KH-3	80.3 A	180.7 AB	6453 AB	1832.0 ABC
T5: PGPR-Azotobacter	78.0 AB	176.0 AB	6911 A	1887.0 AB
T6: PGPR-Pseudomonas	77.7 AB	182.7 AB	6146 A	1813.3 BC
LSD	5.199	15.51	542.25	114.38

Table 35: Effect of salt tolerant isolates and rhizobacteria on rice (SSRI, Pindi Bhattian)

Treatments	Plant Height (cm)	Straw yield ($kg\ ha^{-1}$)	Tillers m^{-2}	Paddy Yield ($kg\ ha^{-1}$)
T1: Control	80 C	9433 C	277 B	2517 D
T2: KH-1	93 AB	10967 B	395 A	2627 CD
T3: KH-2	99 A	11733 A	400 A	2983 A
T4: KH-3	90 AB	1130 AB	350 AB	2773 BC
T5: PGPR-Azotobacter	84 B	11267 AB	323 AB	2877 AB
T6: PGPR-Pseudomonas	86 BC	11000 B	310 B	2780 BC
LSD	9.486	567.58	82.458	170.78

3.18. WHEAT AND RICE RESPONSE TO BIOSTIMULANTS UNDER FIELD CONDITIONS

INTRODUCTION

A plant biostimulant is a microorganism or an organic substance, applied to the plant, seeds or the root environment, soil or any other substrate with the intention to stimulate natural processes of the plants to benefit their nutrient use efficiency. Actually these are secondary metabolites and their quantities cannot be specified. The composition cannot be as definite as in case of mineral fertilizer. These are the products which contain a mixture of many things which further complicates analytical procedures of these products. The biostimulant products enhance plant's ability to assimilate nutrients and proper growth. As concerned for functions of the biostimulants, they improve water use efficiency, strengthen stress tolerance and recovery. They enhance quality, color, sugar content and shelf life of crops. As concerned for active ingredients of the biostimulants, there are 37% weeds, 12% microbes, chitin, plant extracts and 51% humic acid, fulvic acid. Regarding the agricultural uses of biostimulants, two main types should be considered with in taxonomic, functional and ecological diversity, one is mutualistic endosymbionts of the type Rhizobium, second is mutualistic rhizospheric PGPR. These biostimulants may be called as bioactivators, phytostimulants, plant strengtheners, soil, yield and crop and plant growth enhancer. There is need for a legal and harmonized definition of biostimulant but there is no legal or regulatory definition of plant biostimulants anywhere in the world. Following study was conducted to assess the role of different biostimulants on growth and yield of wheat and rice crop at Wheat Research Institute, AARI, Faisalabad and Agronomic Research Station (ARS), Farooqabad, district Sheikhupura, respectively. Different biostimulants were applied according to their dose and time of application. Uniform recommended dose of fertilizer specific for the crop was applied. All the agronomic practices were carried out well in time for obtaining maximum grain yield.

MATERIALS AND METHODS

Agri-business companies were taken on-board for ownership of the results. Products were applied at different growth stages according to their dose mentioned in their respective protocol. Crop stand of wheat and rice was remained normal throughout its growing season. Irrigation was applied with respect to growing stages of the wheat crop like tillering, booting and tasseling stages etc. to mature the crop at its proper time. All the cultural practices were carried out well in time throughout the growing season of wheat crop. Crop was harvested at maturity. Required physical parameters like grain yield, number of tillers

per meter square and average plant height were recorded. Regarding rice crop, the parameters like grain yield, number of tillers per plant, biomass yield as well as dry matter yield were given. Layout was RCBD with three repeats.

TREATMENTS

T_1: Control

T_2: Gibbrex

T_3: Osley

T_4: Galore

T_5: Sonata

T_6: Nature Time

RESULTS AND DISCUSSION

To assess the biostimulatory effect, the field trial was conducted at Agronomic Research Station (ARS), Farooqabad, District Sheikhupura. Data recorded revealed that all the biostimulant products tested for their response, performed better as compared to control i.e. 3933kg ha^{-1}. Maximum (4629kg ha^{-1}) paddy yield i.e. with Galore followed by 4587 kg ha^{-1} with Nature Time was obtained. While other physical parameters like plant height, number of tillers per meter square, biomass yield and dry matter yield also showed distinguished response as compared to control. Recorded data exhibited the effect of various biostimulant products on different parameters of wheat crop at two different locations. All the biostimulant products expressed positive response as compared to control. The highest (3220 and 3250 kg ha^{-1}) wheat grain yield at WRI, Faisalabad and ARS, Farooqabad, respectively, was obtained with Galore i.e. followed by Nature Time compared to control i.e. 2470 and 2260 kg ha^{-1}.

CONCLUSION

The commercial biostimulant products employed for the trial, Galore & Nature Time, showed distinguished effect on different growth parameters of wheat crop as compared to control and Sonata, Gibrex and Osley also performed better with respect to control.

Table 36: Effect of biostimulants on yield parameters of rice (ARS, Farooqabad)

Treatments	Paddy Yield (kg ha^{-1})	Plant height (cm)	Biomass Yield (kgha^{-1})	No. of Tillers (m^{-2})	Dry matter yield (Kg ha^{-1})

T_1: Control	3933	98	11658	333	8658
T_2:Gibbrex	4166	103	13692	357	10692
T_3: Osley	3993	100	13067	350	10066
T_4: Galore	4629	105	14249	390	11249
T_5: Sonata	4317	104	13917	360	10917
T_6: Nature Time	4587	104	14045	390	11045
LSD	237.45	4.69	815.35	38.40	-

Table 37: Effect of biostimulants on yield parameters of wheat (ARS, Farooqabad)

Treatments	Grain Yield (kg ha^{-1})	Plant height (cm)	Biomass Yield ($kgha^{-1}$)	No. of Tillers (m^{-2})
T_1: Control	2260	103	9323	319
T_2:Gibbrex	2450	106	9460	335
T_3: Osley	2980	109	9993	359
T_4: Galore	3250	112	10523	384
T_5: Sonata	3000	107	9593	355
T_6: Nature Time	3236	110	10426	377
LSD	306.91	3.53	171.57	14.87

Table 38: Effect of biostimulants on yield parameters of wheat (WRI, AARI, Faisalabad)

Treatments	Grain Yield (kg ha^{-1})	Plant height (cm)	Biomass Yield ($kgha^{-1}$)	No. of Tillers (m^{-2})
T_1: Control	2470	95	7720	241
T_2:Gibbrex	2580	96	7980	248
T_3: Osley	2850	97	8100	270
T_4: Galore	3220	101	8380	294
T_5: Sonata	2740	96	8270	264
T_6: Nature Time	3140	100	8210	288
LSD	142.98	5.366	261.72	13.005

3.19. GROWTH AND YIELD RESPONSE OF RICE TO PGPR AND DIFFERENT LEVELS OF KINETIN

INTRODUCTION

Hormones play a critical role in regulating the plant growth and responding to drought stress. Kinetin regulates several plant growth aspects and developmental processes including cell division, apical dominance, chloroplast biogenesis, nutrient mobilization, leaf senescence, vascular differentiation, photomorphogenic development, shoot differentiation and anthocyanin production. Seed enhancement (seed priming) with kinetin is reported to increase tolerance level of plants against salt stress. Kinetin receptor genes of other species

are regulated by changes in the osmotic conditions as well indicating that their function in the osmotic stress response might be common although mechanically not well understood. Seed germination and stand establishment in wheat farms is very often poor due to high level of salinity of irrigation water. Therefore, the objective to design this experiment was to study the effect of seed pre-sowing treatment with kinetin and ABA on germination and seedling growth of a wheat cultivar under salinity stress conditions. Cytokinin (CK) prevents the leaf from dying, so its presence prevents the plant from dying and its leaves from falling. Kinetin regulates cell division and differentiation in plants. Plant Growth Promoting Rhizobacteria plays an important role in enhancing plant growth through a wide variety of mechanisms. The mode of action of PGPR that promotes plant growth includes (i) abiotic stress tolerance in plants (ii) nutrient fixation for easy uptake by plants (iii) plant growth regulators (iv) the production of siderophores (v) the production of volatile organic compounds and (vi) the production of protectiolytic enzymes such as chitinase, glucanase, and ACC-deaminase for the prevention of plant diseases. Nutrient availability for plant uptake PGPR has the ability to increase the availability of nutrient concentration in the rhizosphere by fixing nutrients, thus preventing them from leaching out. As an example, nitrogen, which is needed for the synthesis of amino acids and proteins, is the most limiting nutrient for plants. The mechanisms by which atmospheric-N is added into organic forms, assimilated by plants, are exclusive to prokaryotes. Rice is one of the main food crops as a source of carbohydrates, protein, and vitamins. As a source of vitamins, rice is essentially a source of niacin, riboflavin with vitamin content ranging from 27-32%. An environmentally friendly technology approach in rice production can be carried out with the use of bio-ameliorant through the application of useful organic manures and beneficial microorganisms that can increase growth and health of plants. The use of bacteria, the Plant Growth Promotion Rhizobacteria (PGPR), can increase plant growth through N-fixation activities, nutrient solubilization, antibiotic production and production of growth regulators.

MATERIAL AND METHODS

Field experiment was conducted at the Soil Chemistry Section, Faisalabad to check the best levels of Kinetin along with PGPR to improve growth and yield of rice crop. Trial was

carried out in sandy clay loam soil having pH: 8.0, EC: 2.5 dS m^{-1}, available P: 7.0 mg kg^{-1} and organic matter 0.76%.

Efficient isolates of PGPR were used for wheat and rice inoculation at Soil Bacteriology Section. Different levels of Kinetin solution were applied after 2 weeks of seedling emergence as per treatment. Recommended dose of NPK was applied. Layout was CRD / RCBD with three repeats.

TREATMENTS

T_1: Control

T_2: PGPR Inoculation

T_3: Kinetin @ 10^{-3} M

T_4: Kinetin @ 10^{-4} M

T_5: Kinetin @ 10^{-5} M

T_6: PGPR + Kinetin @ 10^{-3} M

T_7: PGPR + Kinetin @ 10^{-4} M

T_8: PGPR + Kinetin @ 10^{-5} M

Note: Fertilizer @ 120-100-70 kg NPK ha^{-1} was applied to all treatments.

RESULTS

Results clearly indicated that PGPR in combination with Kinetin @ 10^{-5} M produced highest (4246 kg ha^{-1}) grain yield as compare to control (3803 kg ha^{-1}).

Table 39: Growth and yield response of rice to PGPR and different levels of Kinetin

Treatments	Paddy yield (kg ha^{-1})	1000 grain weight (g)	Plant height (cm)	Biomass (t ha^{-1})
T_1: Control	3803 C	12.7 D	109.7 C	17.9 B
T_2: PGPR inoculation	3933 B	15.7 BCD	111.0 C	21.4 AB
T_3: Kinetin@ 10^{-3} M	3760 C	13.5 CD	112.3 BC	17.9 B
T_4: Kinetin@ 10^{-4} M	3747 C	19.3 ABCD	122.0 A	22.3 AB
T_5: Kinetin@ 10^{-5} M	3820 C	20.2 ABC	116.3 ABC	21.2 AB
T_6: PGPR + Kinetin@ 10^{-3} M	3933 B	16.6 ABCD	118.0 ABC	20.2 AB
T_7: PGPR + Kinetin@ 10^{-4} M	4203 A	21.0AB	120.0 AB	19.2 AB
T_8: PGPR + Kinetin@ 10^{-5} M	4246 A	22.6 A	120.0 AB	23.6 A
LSD	108	7.40	8.43	4.60

3.20. SOIL BACTERIOLOGY LAB FUNCTIONING AS BIOFERTILIZER TESTING LAB, GOVT OF THE PUNJAB.

INTRODUCTION

Biofertilizers might be defined as a substance that comprised of living microbes colonizes the rhizosphere or the interior of the plant and promotes growth by increasing the supply or availability of primary nutrient and stimulates growth of target crop. Biofertilizers / biostimulants are referred as environmental friendly microbial interventions for better growth of crops and soil health. Biofertilizers or microbial inoculants improved the growth of crops by various means. The quality of biofertilizer is one of the most important factors resulting in their success or failure and acceptance or rejection by end-user, the farmers. Basically, quality is meaning the number of selected microbes in the active form per gram or milliliter of biofertilizer. The parameters of quality might be type of carrier, moisture holding capacity of carrier, pH of biofertilizer, microbial count / number in per gram / mL of product, no contamination i.e. free from other unwanted microbes and ability to improve the growth of plants. Certain types of biofertilizers improve the nutrient mobilization such as phosphate solubilization, zinc solubilization etc. So, quality of biofertilizer having phosphate solubilizers means that the sample has the capability to solubilize the insoluble phosphates etc.

Similarly, biostimulants are the products that improve plant growth contain synthetic growth hormones / amino acids / combination of hormones / amino acids or microbially synthesized hormones. Specific concentration of biostimulants is very much important to make up the efficient product of biostimulants. Specific biostimulants caused growth promotion to specific plants. The quality control of biostimulants is the need of time and each biostimulants require specific protocol. Soil Bacteriology Section is involved in research on different aspects of Soil Microbiology and at the same time work as Biofertilizer Testing Lab. Testing of biofertilizers / biostimulants is mandatory from Govt. of the Punjab under FCO for registration of biological formulations and quality control.

MATERIALS AND METHODS

For registration products are received from directorate of Soil Fertility (regulatory body under FCO) and for quality control from Agri. Inspectors / District officers Agri. Extension. Lab / pot studies are carried out for testing of different parameters (pH, microbial count, moisture percentage, P-solubilization, N-fixation, bioavailable P, root-shoot elongation,

bioassays, contamination and pathogenicity) using PSQCA approved and standard published methods. Soil Bacteriologist being analyst also play its role in formulating / reviewing the standards / specifications in PSQCA and Punjab fertilizer Standardization Committee for biological nature products.

PREVIOUS YEAR'S RESULTS

Table 40: The biofertilizer / biostimulant samples analyzed during 2019-20.

S. No.	Nature of product	Sample analyzed	Fit	Unfit
1.	Biofertilizer / Biostimulant	73	62	11

3.21. ISOLATION, PURIFICATION AND MAINTENANCE OF PGPR, PSM AND RHIZOBIUM SPECIES

This continuous nature of the part of research is to isolate and maintain the most suitable and efficient strains of rhizobia of different leguminous crops and to prepare commercial inocula for their supply to the farmers for seed inoculation in order to introduce a cost-effective crop production technology in the country. Various rhizobium species were isolated, purified, screened and maintained during this year and their culture packets were supplied to interested farmers. Most suitable and efficient strains of rhizobacteria of different crops were isolated, maintained and their commercial inocula were prepared to supply to the interested farmers. Different growth promoting rhizobacteria were isolated, purified, screened and maintained during this year and their culture packets were also provided to farmers.

The staff at Soil Bacteriology Section isolates and maintains the most suitable and efficient strains of phosphate solubilizer microorganisms (PSM) from different crops to prepare commercial inocula for their supply to the interested farmers. Variety of growth promoting P-solubilizers were isolated, purified, screened and maintained during this year and culture packets were supplied to interested farmers during the year. Total 12,180 miscellaneous inocula were supplied to the interested farmers.

Table 41: Isolates maintained and preserved during the year 2019-2020

Microbial species	No. of Isolates	IAA Equivalents ($\mu g\ mL^{-1}$)
Rhizobium sp	125	1.50-2.25
PGPR	200	1.70-2.90
Phosphate Solubilizers	210	1.65-2.90
Zinc Solubilizers	40	1.90-2.60

PGPR form salt affected areas	20	1.80-2.50
PGPR form drought areas	15	1.70-2.30
Bradyrhizobium sp (Groundnut)	12	1.00-1.95
Bradyrhizobium sp (Soybean)	12	1.90-2.40
Endophytes	25	1.60-2.40
Acetobacter sp	10	1.30-2.65
Siderophore producing bacteria	15	1.50-2.65

3.22. EFFECT OF VARIOUS CROP RESIDUES MANAGEMENT STRATEGIES ON SOIL HEALTH IN RICE WHEAT CROPPING SYSTEM

INTRODUCTION

Crop residues are a major source of organic matter to the soil. Before introduction of mineral fertilizer, crops had been grown organically from thousands of years using crop residues, animal dung and human excreta as nutrition source for soil and ecosystem sustainability.

Green revolution and switching to mono-cropping increased crop intensity and developed chemical fertilizer responsive varieties as tool to grow more food. But biological sources of soil health improvement like FYM, agro-industry wastes and incorporation of crop residues have been neglected. Sole dependence on chemical fertilizer for plant nutrition degraded soil health, reduced soil organic matter and posed environmental threats.

Open field burning of crop residues practice is opted generally to clean crop harvested fields to facilitate soil tillage, control insects and emergence of invasive weeds. But this crop residue burning practice has been questioned for several reasons, such as loss of soil organic matter, soil health degradation, economic loss, environmental degradation and even for adverse human health impacts. All this may contribute to the overall deterioration of agriculture soil productivity.

Evaluation of soil quality is based on physical, chemical and biological characteristics, which vary as a function of the applied management, such as tillage, crop rotation, the handling of crop residues. Crop residues are important in the maintenance of soil productivity and the returning of crop residues to the soil associated with some other agronomic practices such as minimum tillage, crop rotation and relay cropping, may improve soil quality and productivity and may also be useful for the environment.

In Pakistan farmers are in the habit of crop residues burning especially of rice and wheat, the two major cereals of the country. Concerns regarding the effect of field burning crop

residues have been in the country during last decade. Therefore the agricultural scientists, civil society and many organizations are resisting this practice for the environmental quality and protection. Farmers of rice-wheat growing areas burn crop residues, after harvesting the crop, for easy seed bed preparation. The purpose of this research is to assess agronomic, economic and environmental losses of crop residues burning and to generate local data on the issue to convince the farming community about the ill effects of this practice.

MATERIALS AND METHODS

Samples from burnt / unburnt wheat and rice fields were collected by soil fertility field staff, after rice and wheat harvest, from various districts of the Punjab and processed and analyzed at Soil Bacteriology Lab microbial count using dilution plate technique and soil organic carbon as per ICARDA manual.

RESULTS

Results of total 840 burnt vs unburnt soil samples, after rice and wheat harvest, showed significant reduction in microbial population expressed as CFU 10^7 $gram^{-1}$ of soil. Measurable reduction in total organic carbon (TOC), because of residue burning was also observed. On an average at all locations 35-40% reduction in microbial count and TOC / organic matter content was observed.

Table 42: Summary of the burnt vs. unburnt soil samples analyzed during 2019-2020

District	Year	Sample analyzed after rice harvest	Sample analyzed after wheat harvest
Chiniot, Faisalabad, Jhang, Sargodha, Khushab	2019-2020	720	120

3.23. RESPONSE OF MAIZE TO SLOW RELEASING PHOSPHATIC FERTILIZERS IN COMPARISON WITH OTHER FERTILIZR SOURCES

INTRODUCTION

It is the crying need of the day to optimize the available resources. In agriculture, highly productive agricultural systems are based on technological innovations to multiply the competence of in hand inputs. Since old days farmers had been using organic substances and materials like farm yard manure (FYM) and miscellaneous domestic organic wastes for agricultural crop production. This help enriched peat moss abundant in soil bacteria, fungi,

earthworms, and other bio-organisms to supplement the soil to produce safe, nutritious and ample crops.

Phosphate fertilizers management plays pivotal role for better crop production. On the other hand P application, from micro to macro levels, involves pre-sowing practices either through drills or mostly applying by broadcast technique. In calcareous soils, like of Pakistan, it may result into large scale fixation of applied P, especially, in case of high solubility P fertilizers which render the least availability of P to the target plant.

In this perspective of P sorption processes, the main focus of researchers is to prolong the contact time of P with soil components. Previous studies have revealed that 75% of P adsorption takes place within 30 minutes of applied P contact in highly weathered soils with higher P adsorption capacity and more recently it was concluded that the first minute of contact with P solution is more decisive.

Hence, researchers are eagerly switching over to the technologies to supply plants with P in a slower pace to protract the time of release from the applied P source. Hence, the following study was meant to investigate commercial product available in indigenous market. There is a mushrooming industry of Slow Releasing P Fertilizers, having numerous names with galaxy of products. Many of them have got registered with government for their commercial production and sale. Some of them were selected on their better performance record in the BTL analysis. These products were tested on maize and wheat and the results are given as under;

MATERIAL AND METHODS

A field trial was conducted at the area of Soil Bacteriology Section on a sandy clay loam soil having pH: 8.5, EC: 2.8 dS m^{-1}, available P: 7.5 mg kg^{-1} and organic matter 0.86%, to compare Slow Releasing Phosphatic Fertilizers with traditional Diammonium Phosphate and powdered Rock Phosphate + Compost and PSM. Recommended dose of NPK was applied at sowing, considering P contents of all phosphatic fertilizers except control where only recommended N & K were applied. Maize and wheat seeds were coated with P solubilizing bacteria for sowing for the respective treatment. RCBD was followed for layout plan.

TREATMENTS

T_1: Control (Recommended N&K)

T_2: DAP (Recommended dose)

T_3: Nutraful (Fortified DAP)

T_4: Marathon (BOP)

T_5: Charger (BOP)

T_6: Super Kissan (BOP)

T_7: Rock phosphate and Compost (70:30) + PSM

Note: Except Control, all treatments were given the recommended dose of P following respective treatment

RESULTS

All treatments showed significant increase in shoot fresh weight of maize and biomass and grain yield of wheat.

Table 43: Growth & yield response of maize& wheat to slow release P- fertilizers

Treatments	**Maize Shoot Fresh wt. (kg ha^{-1})**	**Wheat Biomass Yield (tons ha^{-1})**	**Wheat Grain yield (tons ha^{-1})**
T_1: Control (Recommended P&K)	27250	6.40 D	3.26 B
T_2: DAP (Recommended dose)	42500	9.20 AB	4.68 A
T_3: Fortified DAP (Nutraful)	46500	9.60 A	4.88 A
T_4: BOP (Marathon)	28000	8.52 BC	4.50 A
T_5: BOP (Charger)	34250	8.57 BC	4.40 A
T_6: BOP (Super Kissan)	29750	8.60 BC	4.27 A
T_7: Rock phosphate & Compost(70:30) + PSM	31750	8.30 C	4.35 A
LSD	3995.5	0.2463	0.1903

3.24. LAB ACCREDITATION UNDER ISO/IEC 17025:2017

INTRODUCTION

ISO/IEC 17025:2017 is the main international standard for general requirements for the competence of testing laboratories. ISO/IEC 17025:2017 is the most recent version of ISO/IEC 17025:2017, updated from 2005. ISO/IEC 17025:2017 is the standard that most labs hold accreditation in order to be deemed technically competent. There are many commonalities with the ISO 9001 standard, but ISO/IEC 17025:2017 is more specific in requirements for competence, and applies directly to those organizations that produce testing results and is based upon more technical principles producing many benefits for labs.

The standard ISO/IEC 17025:2017 is applicable to laboratories regardless of extent of the scope of testing practices. The labs used this standard to develop their management system for quality and technical operations.

The labs use ISO/IEC 17025 to implement a quality management system, with the objective of improving their ability to be consistent by ensuring valid results. ISO/IEC 17025:2017 accreditation minimizes risk by ensuring a technically competent lab that has a sound quality management system. The objective of study is to get accreditation of Biofertilizer Testing lab and related activities.

MATERIALS & METHODS

Biofertilizer Testing Lab, Faisalabad (Soil Bacteriology Section, Agri. Biotech. Research Institute, AARI, Faisalabad) has been seeking accreditation using

- Documentation
- Calibration of the equipments
- Visits of PNAC officials
- Participation in ILC / PT
- Uncertainty Measurement
- Internal Audit, MRM, Testing / Retesting / Intermediate Checks, Method Validation, Validity of Results etc.
- Implementation of the documented system.
- Preparation of documents as per requirements of ISO/IEC-17025:2017 standards.
- Preparation of 04 manuals, 13 policies and 28 procedures and their relevant record.
- Participation in Proficiency Testing /PT or Inter-Lab Comparison /ILC.
- Maintaining of quality indicators as per standard instructions.
- Application to PNAC for accreditation.
- Pre-Assessment followed by Final Assessment.

RESULTS

1. The complete documentation of Biofertilizer Testing Lab, Faisalabad, was prepared and PNAC officials evaluated them for Pre Assessment and Final Assessment.

2. Prepared 04 manuals, 13 policies and 28 procedures and with their relevant record / quality forms.
3. Biofertilizer Testing Lab, Faisalabad, successfully participated once in ILC organized by Provincial Reference Fertilizer Testing Lab, Raiwind, Lahore and got satisfactory Z score in each parameter.
4. The methods have been validated using techniques of method validation and desired performance characteristics.
5. The validity of results has been verified through retesting, uncertainty, method robustness and testing of blind sample etc.
6. Pre-Assessment was successfully conducted on 19-03-2020 and observations raised were rectified accordingly. Corrective actions for observations were taken and send to PNAC for evaluation.
7. CA's were thoroughly evaluated by PNAC and found satisfactory.
8. All the quality indicators like internal audit, MRMs, equipment calibrations, testing/re-testing, and data recording etc. were carried out as per plan to maintain the quality of work.
9. The Final Assessment was successfully conducted on 25, 26-06-2020 and raised NC's / observations were rectified after taking corrective actions.

4. ACHIEVEMENTS

1. *Rhizobium* species were isolated, purified and screened for their auxin biosynthesis potential that was ranged between 1.50-2.25 µg mL^{-1}. During 2019-20, 125 isolates of *Rhizobium* species were preserved after thorough screening.
2. PGPR of different kind viz. *Azotobacter* sp, *Azospirillum* sp, endophytes, *Acetobacter* sp, salt tolerant PGPR and Zn-solubilizers were isolated, purified and screened for their auxin biosynthesis potential that was ranged between 1.70-2.90 µg mL^{-1}. During 2019-20, 100 isolates of PGPR / free living diazotrophs were preserved after thorough screening.

3. Phosphate solubilizer microorganisms (PSM) i.e. *Bacillus* sp and *Pseudomonas* sp were isolated, purified and screened for their auxin biosynthesis potential that was ranged between 1.65-2.90 µg mL^{-1}. During 2019-20, 110 isolates of PSM/ free living diazotrophs were preserved after thorough screening.
4. Other 64 bacterial isolates pertaining to EPS, STB, SOB, DTB and siderophore were also preserved after detailed screening.
5. *Rhizobium* sp inoculation to chickpea and mung bean promoted the chickpea and mung bean crop growth and yield up to 13% and 16%, respectively and the effect was more pronounced with application of growth hormones and increase was 22-34% respectively.
6. PGPR inoculation to wheat, rice and maize promoted the wheat, rice and maize crop growth and yield up to 7-24%, respectively and the effect was more pronounced with application of growth hormones and increase was 12-24%, respectively.
7. 12,180 inoculum packets of *Rhizobium*, PGPR and P-Solubilizers were supplied to the farmers during 2019-20.
8. 73 biofertilizer / biostimulant samples were analyzed during the reported year, 2019-20.
9. 840 burnt vs. unburnt soil samples, after wheat and rice harvest, were analyzed during the reported year, 2019-20.
10. Ten students of BSc. (Hons.) Agriculture / BS Microbiology have completed their internship at the Section in 2020.