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OVERVIEW

Biotechnology (commonly abbreviated as biotech) is the broad area of biology involving living systems and organisms to develop or make products, or "any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use". Depending on the tools and applications, it often overlaps with fields of molecular biology, bio-engineering, biomedical engineering, bio-manufacturing, molecular engineering, etc. Ultimate objective of Plant Breeding programs is to efficiency of a crop variety which is different from the force operating in nature where the selection operates at the survival of the fittest rather than the efficiency. For improvement of any crop specie mainly three steps are followed i.e. 1). Selection of desirable traits by marker assisted breeding 2). Manipulation and subsequent growth of tissues, organs and plant cells in tissue culture 3). Genetic engineering/recombinant for direct transfer of single gene. 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 2018-19, 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.

A. GENETIC ENGINEERING

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

Integration of modified GT gene in brassica Juncea was planned to develop glyphosate herbicide resistance for the effective weeds control.

12376, cotyledonary leaf petioles were inoculated with Agrobacterium strain LBA4404 having synthetic GT gene with selection marker. 323 antibiotic resistant shoots were screened on selection media containing respective antibiotic (PPT @ 3mg/L & Kanamycine @ 50 mg/L. 56 putative antibiotic resistant plantlets were survived on regeneration and rooting media and 25 putative transgenic plants were developed.

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

4020, inner most leaf (spindle) of sugarcane were cultured for callus induction. 2210 three Weeks old calli were inoculated with Agrobacterium strain LBA4404 having modified GT gene with selection marker. 315 putative antibiotic resistant plants were screened on selection media containing Kanamycine @ 50 mg/L. 415 putative antibiotic resistant plantlets were survived on regeneration and rooting media and 181 putative transgenic plants were developed and their confirmation was under process.

Pollen tube pathway mediated genetic transformation for herbicide tolerant gene in cotton

The main goal of this plant transformation experiment was to utilize the pollen tube pathway (PTP) of cotton plant for the genetic transformation to develop roundup herbicide tolerance in cotton varieties without going through tedious process of tissue culturing. Three Bt cotton genotypes (MNH-886, FH-142, Lalazar and FH-490) were utilized in this work. In total 2400 flowers of three cotton varieties were inoculated with Agrobacterium LBA-4404 strain harboring EPSPE-Bar gene for glyphosate herbicide tolerance. To reduce the flower shedding after inoculation 80 ppm GA3 hormone was applied at the petiole of flowers. Overall 202 cotton seeds obtained from 11 surviving bolls were sown in plastic trays containing peat moss. Out of these 110 plantlets germinated. At 4 leaf stage these T1 generation seedlings were screened for transformants using 50% of recommended dose of Roundup chemical spray. Nine (09) seedlings survived the glyphosate spray. Leaf samples were taken and DNA was isolated. PCR with EPSPS gene specific primers was unable to amplify the gene from these samples. The seedlings of putative transgenic plants were shifted to earthen pots for further investigation. Further optimization of PCR profile and synthesis of primers from different region of expression cassette will be performed in the next season. Also apical meristem mediated transformation method will be performing next year to insure the success of experiment.

Fig 1. Procedure for transformation of Cotton Plants through In-Planta transformation by inserting the agrobacterium plasmid into petioles directly.

Integration of modified glyphosate tolerance (Gt) gene in wheat

An experiment on integration of synthetic Glyphosate Tolerance (GT) gene was planned and executed in wheat varieties viz. Faisalabad-2008, AARI-2010 and Anaj-2017 for the development of roundup herbicide tolerant wheat having effective weeds control capabilities.

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, five (05) Liter Agro inoculation Media (AIM) and 4 Litter Plant Selection Media (PSM) was prepared, sterilized and poured in Petri plates. About 1500 immature embryos of three wheat varieties 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 850 are on AIM media whereas 650 calli were shifted on PSM media. After subsequent sub-culturing 221 calli showing regeneration on PSM media having 3mgL-1 PPT (Phosphinothricin) herbicide were shifted on Rooting initiation media (RIM). From RIM media, 74 wheat putative transgenic plantlets were shifted to pots having sandy loam and peat moss mixture in the ratio of 1:1 for hardening and further testing. The pots were placed in the incubation room at ±25°C for 16 hour day and 8 hours night conditions.

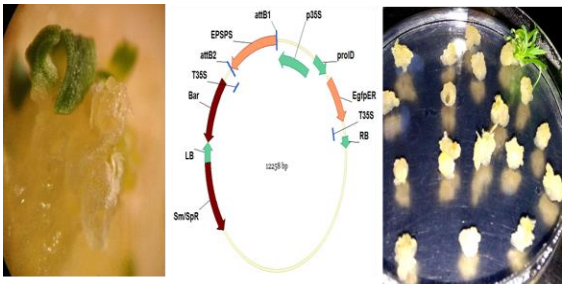

Fig 2. Tranformation procedure of Wheat Crop using GT gene culture for developing herbicide tolerant wheat plants.

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).

Regeneration percentage of calli was 15 percent at 120 minutes treatment of EMS (0.5%) and percentage of plantlets developed at PEG levels 10 % were 20 percent respectively.

Testing of biotech crops

The objective of experiment was to test the presence/absence of Biotech elements in newly developed crop varieties and to verify the claim regarding presence of the gene, type of protein expressed and level of expression of the gene product including National Coordinated Varietal Trials (NCVT) and on demand testing of samples from public sector, Research organizations/ private companies.

NCVT:

132 entries of NCVT, received from PCCC, Multan and 5 plants from each entry were tested for the presence of Cry1Ac, Cry2Ab and RR genes using strip test (Fig 1), ELISA and PCR.

On demand testing:

A total of 728 samples received from public and private sectors (Table 1).

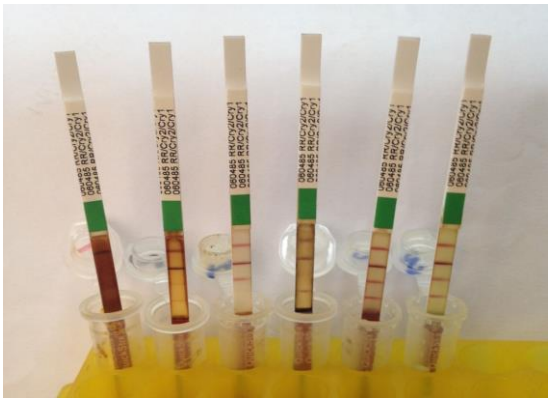

Fig 3. Imunostrip assay for detection of Bt genes (Cry1Ac, Cry2Ab) and herbicide tolerant gene (RR) in cotton. From left side Strip 1 is showing Non-Bt plants, Strip 2 have Cry1Ac nly, Strip 3 have Cry1Ac and Cry2Ab, Strip 4 have Cry1Ac and RR gene, Strip 5 and 6 contains Cry1Ac, Cry2Ab and RR genes.

Table 1. Detail of samples received from different Government and Private organizations for testing of GM elements, Cry1Ac, Cry2aAb and RR genes and their quantification through ELISA.

Samples Received	Total No. of samples	Remarks
NCVT - 18	132 Entries 132×5=660 samples	Entries Positive for Cry1Ac= 118 Entries Positive for RR= 14 All entries negative for Cry2Ab gene
On-demand	728 (Private: 490) (Public: 238)	Genetically Modified (GM) samples tested for crop events and GMO Elements and quantification of Cry proteins
Total samples analyzed	1388	

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).

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 2 and Table 3. Potassium application was not significantly affected Cry1Ac protein expression**.**

Table 2. Effect of Cotton-Cotton crop rotation on Quantification of Bt endotoxin. The results showed that Cotton-Cotton crop rotation has upregulated Cry1Ac protein.

	Cry1ac (ug/g of fresh sample weight)				
N applied Kg/ha	Leaf (Top)	Leaf (Middel)	Leaf (Bottom)	Square	Boll (10 days old)
0	2.24	1.75	0.00	1.80	0.6
100	2.65	2.24	0.00	1.65	1.20
200	3.84	2.54	0.30	2.76	1.82

Table 3. Effect of Cereal-Cotton crop rotation on Quantification of Bt endotoxin. The results showed that Cereal -Cotton crop rotation has downregulated Cry1Ac protein.

	Cry1ac (ug/g of fresh sample weight)				
N applied Kg/ha	Leaf (Top)	Leaf (Middel)	Leaf (Bottom)	Square	Boll (10 days old)
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

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 and RR genes in cotton genotypes received from Cotton Research Institute and Cotton Research Stations and sub-stations. 19 cotton genotypes were received from different cotton research stations and sub-stations. Purity test (strip test): Five consecutive plants in a row were tested for the presence of Bt (Cry1Ac & Cry2Ab) and RR proteins through lateral flow strip test. 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. Protein Quantification (ELISA): quantification of the gene product i.e. Bt toxin through quantitative ELISA (Enzyme Linked Immuno-sorbent Assay).

Training of Agri. Extension Wing, Federal Seed Certification & Registration Department and Private sector personnel in detection, identification & quantification of Bt cotton

The objective of this experiment was trained 200-300 personals about detection, identification & quantification of Bt cotton and Cotton crop survey for testing the purity of Bt cotton by testing 20-30 samples for presence/absence of Bt at about 200 locations of cotton zone in Punjab.

More than three hundred personnel's were trained in detection, identification & quantification of Bt cotton. In survey ten thousand leaf samples were tested from 400 farmers' field in 15 main cotton growing districts to check the status of approved and unapproved Bt cotton. The variety IUB-13 was grown in 14 out of 15 districts, while BS-15, FH-142, BS-18, SS-32, NIAB-878, IUB-15, MNH-886, MNH-1016 and Z-33 also dominating because 62% of samples were belonged to these varieties. Under Bt cotton category 9694 leaf samples were tested, from which 8340 positive for Bt Cry1Ac protein while 1354 samples were Non-Bt. The purity %age of Bt cotton was 86%. In non-Bt cotton category, 306 leaf samples were tested, 231 were Non-Bt and 75 samples were found positive for Bt protein in strip test showing 75% purity of non-Bt cotton. 49 leaf samples were found positive for three toxic proteins (Cry1Ac, Cry2Ab and RR), 52 samples for Cry2Ab and 236 leaf samples were carrying RR protein.

Genetic diversity assessment of maize (*Zea mays* l.) inbred lines using chromosome specific simple sequence repeat (SSR) markers

The objective of this experiment was to asses genetic relationships among 200 adapted

cultivars or elite breeding material of Maize and Milles Research Institute, Sahiwal and Maize Research Station, Faisalabad using 101 SSR markers belonging to BNLG, PHI, PNC and UMC series (Table 4). All the 101 SSRs were found polymorphic amplifying a total of 828 alleles with an average of 8.28 alleles per primer. The UPGMA tree was generated from genetic similarity coefficients. Two hundred maize genotypes were clustered into 08 groups which were further divided into subgroups (Fig 4)

Table 4. Series wise Statistics of markers used in the study of genetic diversity of 200 maize genotypes

Primer Series	BNLG	PHI	PNC	UMC
Detail of Series	SSR1-SSR7	SSR8-SSR48	SSR48-SSR55	SSR56-SSR101
No of Alleles	67.00	328.00	100.00	332.00
Polymorphic Alleles	66.00	325.00	100.00	320.00
Max. PIC	0.91	0.93	0.92	0.92
Min PIC	0.75	0.00	0.78	0.00
Max Alleles	15.00	19.00	18.00	16.00
Min Alleles	6.00	1.00	8.00	2.00
Alleles/ SSR	9.57	8.20	12.50	7.22
Polymorphic Alleles/SSR	9.43	8.13	12.50	6.96

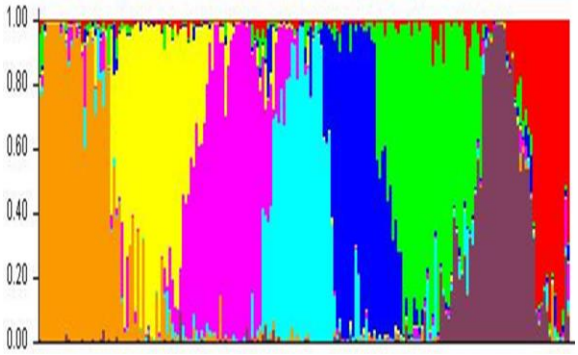

Fig 4. Structure Analysis of 200 maize inbred lines. Indicating the Grouping of 200 maize genotypes to eight groups as indicated by eight different colors.

SSR markers as a tool for preliminary screening of maize lines (*Zea mays* l) for drought tolerance

drought tolerance

The objective of this experiment was to assess the drought tolerance status of maize genotypes with the help of SSR markers. 200 Leaf samples were collected from Maize and Milles Research Institute and Maize Research Station Faisalabad. DNA extraction of 200 leaf samples was completed and PCR was assembled using 20 SSR markers.

DNA barcoding of maize genotypes with SSR markers for rapid varietal identification

The objective of this experiment was to develop DNA barcodes for rapid varietal identification of maize genotypes using chromosome specific simple sequence repeat (SSR) markers. Leaf samples of 08 maize genotypes were collected from Maize and Milles Research Institute and Maize Research Station Faisalabad and DNA was extracted and quantified. 215 SSR markers were searched and got synthesized. PCR was assembled on 08 genotypes using 215 SSR markers. Out of 215 primers, 201 were amplified. Out of 201 amplified SSR primers, 25 were monomorphic and 176 were polymorphic. All the eight maize genotypes were identified successfully by six SSR markers (M-45, M-51, M-64, M-75, M-78 and M-83). In addition to that there are number of combination that can differentiate all the eight maize genotypes (Fig 5).

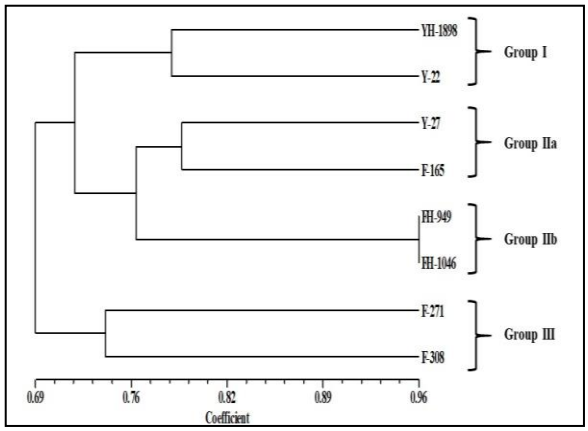

Fig 5. Dendrogram of 08 maize genotypes showing their interrelationship. Genotypes were clustered to three groups. Among which Group II was further subdivided to two subgroups.

Development of varietal identification key and genetic diversity analysis of Pakistani cotton varieties

The objective of this experiment was to develop variety specific DNA marker Key and study the genetic diversity for the present cultivated varieties of cotton using simple sequence repeats (SSR) markers. Leaf sample of 21 genotype were collected from different cotton research institutes in Punjab and DNA extraction was completed. 313 SSR markers were selected with wide genome coverage or evenly distributed on chromosomes from genomic databases and got synthesized. PCR was assembled using 313 SSR markers. Dendrogram was constructed using similarity matrix and UPGMA approach of 21 cotton genotypes as shown in Fig 6.

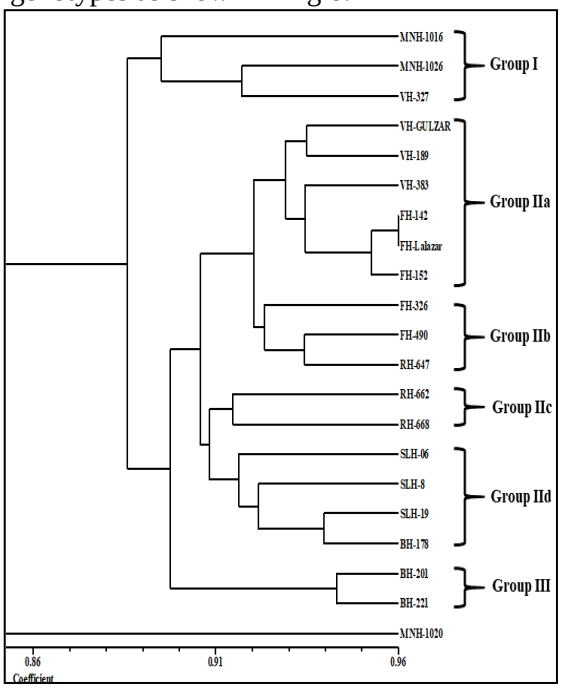

Fig 6. Dendrogram of 21 cotton genotypes showing their interrelationship. Genotypes were classified to three groups. Among which Group II was further classified to four subgroups.

DNA barcoding/fingerprinting for identification of date palm varieties

The objective of this experiment was the development of variety specific DNA marker key for genetic identification of date palm varieties at early developmental stages. Leaf sample of 13 date palm genotype were collected from Horticulture Research Institute Faisalabad and DNA extraction was completed. 210 SSR markers were searched and got synthesized. PCR was assembled on 13 genotypes using 210 SSR markers. Out of 210 primers, 194 were amplified. Out of 194 amplified SSR primers, 36 were monomorphic and 158 were polymorphic.13 date palm genotypes were identified successfully by using different combinations of thirty three SSR markers. The Dendrogram showing inter relationship of date palm genotypes is given below (Fig 7).

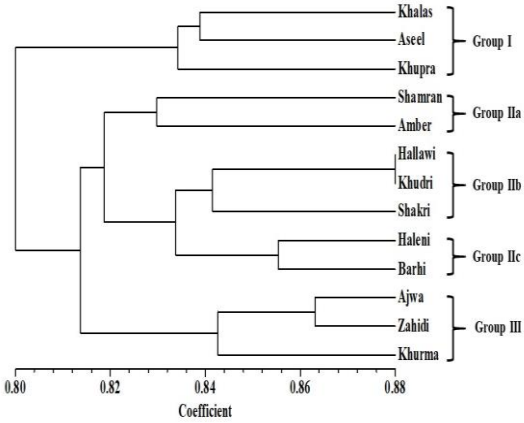

Fig. 7. Dendrogram of 13 date palm genotypes showing their interrelationship. Genotypes were classified to three groups. Group II was further subdivided to three clusters.

Utilization of DNA markers based on microsatellite polymorphism for identification of potato varieties

The objective of this experiment was to develop DNA fingerprinting key of 13 potato varieties using 200 linked DNA markers. Leaf of 13 potato genotypes were collected from Potato Research Institute, Sahiwal and DNA was extracted from all varieties. The detail of DNA extraction and Quantification is provided below in Table 4.

Table 5. DNA quantification, DNA quality and Dilution preparations of 13 potato genotypes to be used for DNA fingerprinting of Pakistani potato varieties.

Sr.	Name	260/ 280	DN Conc.	DNA Dil	
				DNA	Water
1	Faisalabad Red	1.97	577.5	13.8	386.2
2	Faisalabad White	1.97	462.0	17.3	383
3	SH-5	1.81	83.2	96	304
4	PRI-Red	1.94	651.8	12	388
5	Ruby	1.97	1301.1	6.2	394
6	Sadaf	1.88	562.3	14	386
7	Sialkot Red	1.98	870.2	9	391
8	FD 73-110	1.99	932.5	8.5	391.5
9	FD 78-51	1.98	1695.6	5	395
10	FD 76-67	1.99	944.8	8.5	391.5
11	FD 73-49	1.97	2112.9	4	396
12	FD 81-8	1.94	2638.6	3	397

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

The objective of this experiment was to develop varietal identification key of tomato genotypes using molecular markers. Leaf samples of 13 tomato genotypes comprising of hybrids, inbred and OPVs were collected from Vegetable Research Institute, AARI Faisalabad. 225 SSR markers were searched and got synthesized. DNA extraction and quantification of 13 samples were completed. PCR was assembled on 13 genotypes using 180 SSR markers a couple of representative gel images are given in Fig 8.

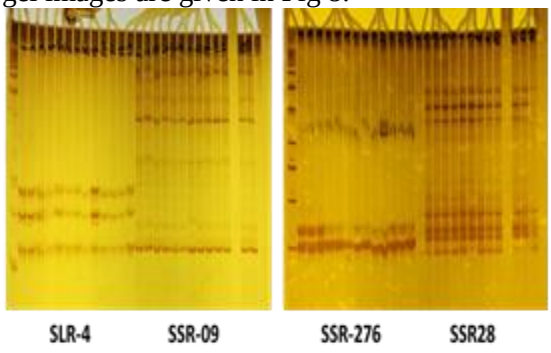

Fig 8. Representative gel images for tomato barcoding. The figure contains the Polyacrylamide gel electrophoresis detail of 04 primers of tomato used for DNA fingerprinting of 13 candidate tomato varieties/hybrids.

DNA barcoding of wheat genotypes with SSR markers for rapid varietal identification.

The objective of this experiment was to develop DNA barcodes for rapid varietal identification of wheat genotypes using chromosome specific simple sequence repeat (SSR) markers. Leaf samples of 13 wheat genotypes were collected from Wheat Research Institute, AARI Faisalabad. 220 SSR markers were searched and got synthesized. DNA extraction and quantification of 13 samples were completed. PCR was assembled on 13 genotypes using 190 SSR markers a couple of representative gel images are given in Fig 9.

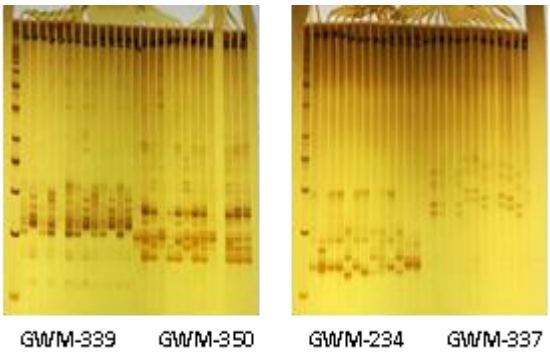

Fig 9. Representative gel images for wheat barcoding. The figure contains the Polyacrylamide gel electrophoresis detail of 04 primers of wheat used for DNA fingerprinting of 13 candidate wheat varieties.

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

This research work was planned to find quality related genes in candidate cotton genotypes. Genomic DNA was isolated from 21 cotton genotypes. Primers related to fibre and yield related traits have been searched and got synthesized. PCR was assembled on 21 genotypes using 12 SSR markers.

Application of molecular markers for the identification of low erucic acid and glucosinolate contents in brassica genotypes

Breeding of oilseed has evoked a strong bottleneck selection towards double-low (00)

seed quality with zero euroic acid and low seed Glucosinolate content. DNA based molecular markers are important tools in breeding programmes for crop improvement. The main role of these makers is to detect the polymorphism. The objective of planning this work was molecular characterization of 50 brassica genotypes to identify candidate genes for low erucic acid and glucosinolate. Genomic DNA was isolated from 50 genotypes of *Brassica napus* and *Brassica Juncea* genotypes from Oilseed Research Institute. PCR and Gel electrophoresis of 50 brassica genotypes using primers related to erucic acid has been completed (Fig. 7). Screening for glucosinolate contents linked markers and data compilation is continued.

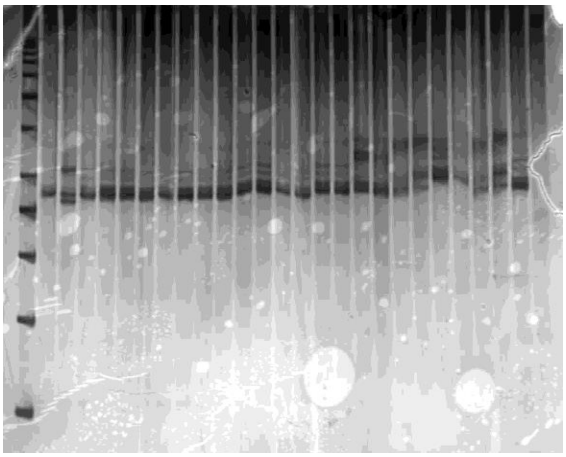

Fig 10. PCR Product of SSR marker Na10C01 showing the genetic relatedness of Cotton genotypes used in the study for testing of Fiber quality traits.

Characterization of maize genotypes for OPAQUE-2 gene

Screened maize lines for Quality Protein using molecular markers for higher tryptophan and lysine contents due to presence of opaque-2 gene. Screened 260 maize genotypes for presence of Opaque-2 gene using linked DNA markers among which 13 were found positive and positive lines were reconfirmed for Opaque-2 gene and among which 07 genotypes were confirmed again as shown in Fig 11.

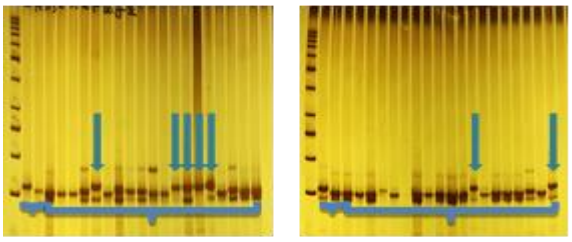

Fig 11. Screening of maize genotypes for QPM. The arrows indicating the genotypes that have Opaque-2 genotypes screened on the base of Phi-057 primer linked with Opaque-2 gene.

Marker assisted selection of pro-vitamin and carotenoids in maize inbred lines.

The objective of this experiment was to assess Pro Vitamin-A and carotenoids genes in 200 maize inbred lines using linked DNA markers. Leaf samples of 100 Maize genotypes were collected from Maize and Millets Research Institute, Sahiwal and 100 genotypes from Maize Research Station Faisalabad. DNA was extracted, quantification was done and PCR was assembly on 200 genotypes only 1 genotype was found positive for Pro Vitamin that too in heterozygote form as shown in Fig 12.

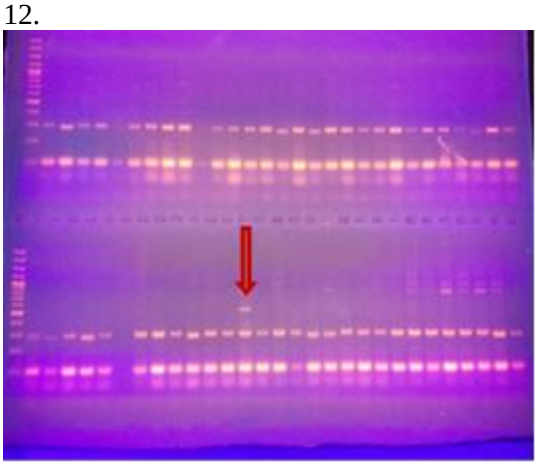

Fig 12. Screening of 200 maize genotypes for Pro Vitamin A. The arrow indicate the single genotype that possessed the desirable allele but in heterozygous form.

Protocol optimization to identify the limit of detection for GM contents.

The objective of this work was to optimize the limit of detection of GM elements in seed powder using PCR technology. For this

purpose GM and Non-GM maize and cotton were selected, seeds grounded into fine powder and verified at molecular level. GM seed powder was mixed at various levels with Non-GM seed powder and isolated DNA was used in PCR for the identification of limit of detection. It was observed that conventional PCR successfully detect GM element (TNOS) upto 1% mixing of GM seed with non-GM (Figure 13).

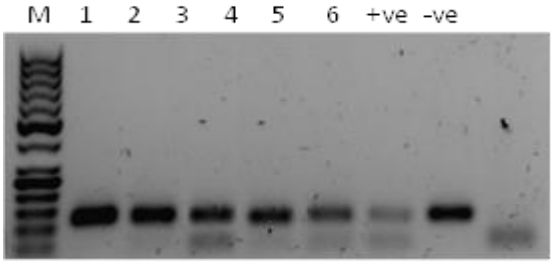

Fig 13. Optimization of limit of detection of GM elements. GMO Testing lab has established a protocol for identification of up1% of GM elements in a seed sample.

Rust resistance gene pyramiding in wheat using linked DNA markers.

The objective of this work was to combine the desirable rust resistance genes from different parents to get genotypes having different combination of various Leaf, Yellow and Stem rust resistance genes. 27 cross combination were selected from R1, R2, R3, R4 and R5 generations and screened against stem, yellow and leaf rust. Tracking of rust resistant gene for Lr-19, Lr-28, Lr-34/Yr-18 (Figure 14) using PCR technology has been completed. Screening for more rust resistant genes and compilation of data is in progress. Generation advancement for homozygous lines and development of more cross combination is in progress. 12 new cross combinations were also developed for disease resistant gene pyramiding.

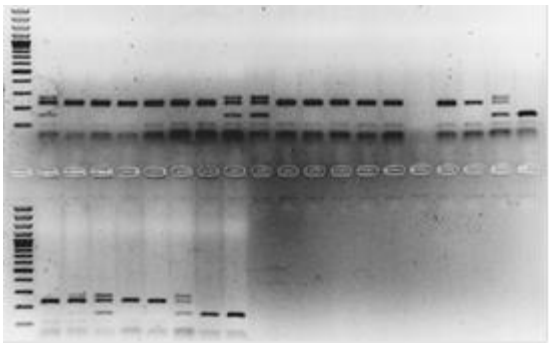

Fig 14. PCR amplification to track leaf rust resistance genes in wheat using linked DNA markers for marker assisted Breeding.

GMO lab accreditation under ISO-17025

The objective of this work was to develop and accredit the GMO testing activities from ISO-17025. 02 GM elements i.e. 35S promoter & NOS terminator were selected for accreditation work. GMO testing lab successfully participated two times in international Proficiency Testing (PT) program with 100% consensus (Figure 15). Application for surveillance visit of GMO testing lab has been sent to PNAC. 300 seed samples of various crops like rice, maize, sunflower, peas, sorghum, melons, vegetables etc. were tested for GM elements and reports have been sent to concerned authorities.

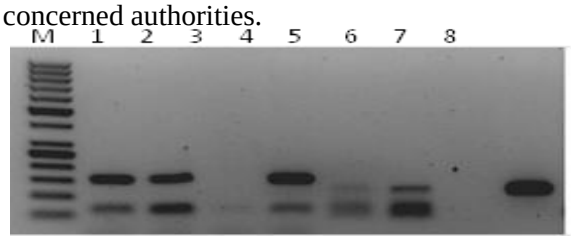

Fig 15. Detection of GM elements in PT samples. First samples from left indicate PCR amplification of Camv35S promotor whereas last four samples showed the PCR amplification of NOS terminator.

DNA markers based detection of quality related genes in spring wheat

This research work was planned to find quality related genes in candidate wheat genotypes in PUWYT-2018-19. Genomic DNA was isolated from 50 wheat genotypes and used to identify the linked DNA marker for quality parameters. Screening for 02 markers linked with HMW-GS, 01 for iron and 01 for zinc has been completed (Figure 16). Screening for more quality linked markers and data compilation is continued. DNA isolation from 33 wheat samples for the identification of quality genes from WRI also completed.

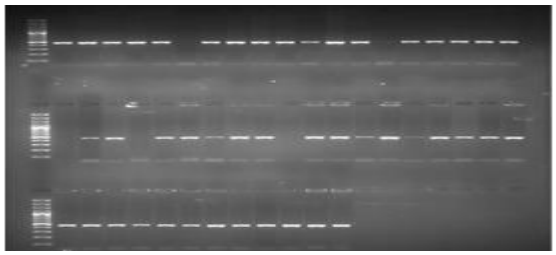

Fig 16. Screening of quality related genes in wheat. The figure holds the PCR amplification of gene linked to high Iron contents in wheat. The genotypes that possessed the band are positive while the genotype that does not possess the band are negative and have low Fe contents.

Identification of rust resistance genes in advance lines of wheat

The objective of this work was the molecular characterization of wheat genotypes to identify/tag rust resistance genes for use in the breeding programme. Genomic DNA was isolated from 50 wheat genotypes and used to identify the rust resistant linked DNA marker. PCR based screening against Lr/Yr and Sr genes (Lr-19, Lr-28, Lr-34/Yr-18, Lr-46/Yr-29 and Sr-2) has been completed (Figure 17) while tagging for more rust resistant genes and data compilation is continued. DNA isolation from 11 wheat samples for the identification of rusts resistant genes from WRI also completed.

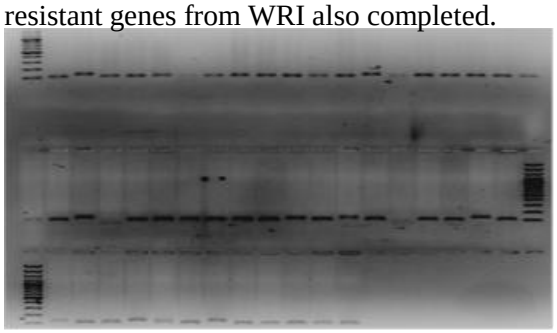

Fig 17. Identification of rust resistance genes in wheat advance lines through marker assisted selection using linked DNA marker.

Application of molecular markers for the identification of red rot disease resistance in sugarcane (Saccharum spp.) genotypes

The genomic study was undertaken to optimize the screening of red rot disease resistant sugarcane genotypes by using genomics and molecular tools. 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 plant pathologist at Sugarcane Research Institute, AARI, Faisalabad. Twenty-Eight (28) SSR markers were optimized on the basis of differential amplification with respect to disease resistance and susceptibility. DNA from 50 somaclones of redrot susceptible variety SPF-234 was extracted and screened through PCR by employing 05 optimized polymorphic markers. SSR Markers SMC597CS and mSSCIR43 gave polymorphic amplification among somaclones and their susceptible parental line SPF-234. Further studies on linking the molecular based findings and there linkage with the field based morphological pattern of disease resistance in parental variety and its somaclones is needed to better understand and interpret the results of this study (Fig 18)

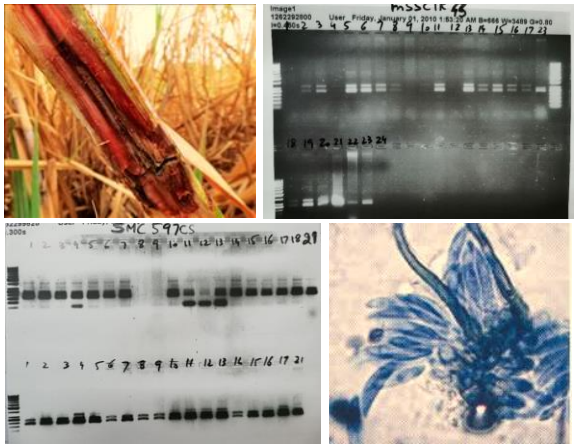

Fig 18. Screening of Sugarcane genotypes for Red rot resistance using linked DNA markers.

Evaluation of robust molecular markers linked to multiple disease resistances in tomato

The main objective of this experiment is to validate the DNA markers and detection of resistant source against tomato diseases with the help of linked DNA markers for utilizing in the development of resistant varieties. For this purpose, leaf samples of 50 tomato genotypes were collected from Vegetable Research Institute, Faisalabad. DNA of these genotypes

was extracted and DNA linked primers for tomato diseases were synthesized. PCR for Ty1-SspI, Ty1-BglII and Ty1-TaqI markers linked with tomato yellow leaf curl virus (TYLCV), Ph3-MspI,. Ph3-SCAR markers linked with Late blight (LB), Ve1-XbaI marker linked with tomato verticillium wilt, Sw5-f1, Sw5-r1 and Sw5-f2, Sw5-r2 markers linked with tomato spotted wilt virus was run to identify the resistance source for these four tomato diseases. No genotype was found resistant for these diseases with these markers.

Identification of resistant genotypes against powdery mildew in peas with allele specific markers.

This experiment is devised to find out the resistant source in pea (*Pisum sativum* L) against powdery mildew with the help of DNA markers for utilizing in the development of resistant varieties. Leaf samples of 50 peas genotypes were taken from Vegetable Research Institute, Faisalabad. After preparation of stock buffers for DNA isolation, DNA was extracted of these peas genotypes. DNA markers, ScOPD10, ScOPE16, ScOPO18, ScOPX04 and PsMlo1 linked to powdery mildew disease were applied on these peas genotypes. No genotype resistant for powdery mildew was found with these markers.

Validation of DNA markers against powdery mildew and karnal bunt diseases in wheat

This experiment was planned to verify the identified DNA markers for resistance against powdery mildew and karnal bunt diseases in wheat and detection of resistant genotypes for utilizing in the development of resistant varieties. Leaf samples of 50 genotypes were taken from micro yield trial sown at Agricultural Biotechnology Research Institute, Faisalabad. DNA was extracted from theses wheat genotypes and DNA markers R11, Me5/Em5-650, Me8/Em16 600 , Pm4b and STS-241 linked with powdery mildew and Karnal bunt markers, XGWM-337, XGWM-538, XGWM-539snp and XGWM- 637 were run on extracted DNA and no genotype exhibited resistant for these diseases with these markers.

Validation and identification of drought tolerant genes in spring wheat

The purpose of this trial is to find out drought related genes in spring wheat genotypes for utilizing in the development of drought tolerant genotypes. Leaf samples of 50 genotypes were collected from micro yield trial sown at Agricultural Biotechnology Research Institute, Faisalabad. DNA was extracted from theses wheat genotypes and 08 DNA markers linked to various traits of drought were surveyed on these 50 wheat genotypes. Pr-8 marker linked with Flag Leaf senescence was found in 06 genotypes, wmc-156 linked with leaf cuticular wax in 07 genotypes, KSUM-119 linked with Photosynthetic rate, Cell Membrane Stability (CMS) and relative water contents was found in 05 wheat genotypes.

Development of drought tolerant wheat plants through tissue culture

Seeds of three wheat varieties (Punjab-11, Galaxy and Pasban-90) were cultured in MS medium having two different PEG levels (0.25 and 0.125g/L) + 2,4 D (4mg/L). Developed callus were transferred on shooting and then rooting media. Regeneration was found best in Galaxy when MS medium had Kinetin (1.5mg/L) + BAP (0.5mg/L). Maximum shoots elongation observed when Gibberellic acid (1mg/L) added in MS medium. 13, 09 and 19 somaclones of Punjab-11, Chakwal-50 and Galaxy were developed, respectively.

Exploitation of somaclonal variation in rice for yield and other economic traits improvement.

Dehusked seeds of five rice varieties (KS-282, KSK-133, PS-2, Basmati-385 and Basmati - 515) were cultured in two different levels of PEG (0.125 & 0.25mg/L) +2, 4-D (4 mg/L). Developed callus were shifted on shooting and rooting media for regeneration. Maximum callus fresh weight was observed in PS-2 and minimum in Basmati-385 under control and PEG treatments. Maximum shoots initiation observed in MS +Kinetin (3mg/L) + BAP (1mg/L) while 1/2MS medium was noted best for rooting. 39, 61, 28 and 13 somaclones of KSK-282, PS-2, Basmati-2000 and Basmati-515 were developed and harvested, respectively.

Standardization of protocol for micropropogation in date palm.

Apical meristem were taken out from suckers of Hilavi date palm variety and dipped in ascorbic acid (150mg/L) for 3-4 hrs to control formation of phenolic compounds. Sterilized apical meristems were cultured in 10 different media and meristematic tissues of these apical meristem were also cultured in 2,4 D (5, mg/L) medium for callogenesis. Callus was formed in medium having 5mg/L 2, 4 D after four months sub-culturing. Little success of regeneration observed from callus of meristematic tissue. Another technique was adopted by selecting mature seeds of date palm for apicle meristem culture. In vitro roots initiation was observed after 27 days of date palm seeds cultured. Developed roots were cultured for callogenesis and shoot formation. Influence portion of female plants were also used for callogenesis at three 2, 4 D levels (5, 10 and 15mg/L) and experiment in progress.

Maize mediated doubled haploid production in wheat

Wheat spikes were detached from F2 segregating generation from field when spike present in flag leaf stage and emasculated the spikes. All anthers were removed with forceps carefully to avoid self-pollination. Each spike was covered with butter paper bag and placed in incubation room. After 2-3 days, emasculated spikes were pollinated with maize pollen and put into pollination medium for 3-4 days. Pollinated wheat spikes again kept in incubation room at temp 25C. Then spikes were shifted in growth medium and change it 2-3 regularly. After 12-14 days of pollination, haploid seeds were developed. Total 337 spikes were emasculated and 1202 haploid seeds were obtained. Haploid seeds were shifted into different medium for haploid plants development. 119 haploid embryos rescued under stereo microscope and only 06 haploid plants were developed. Moreover, response of various concentrations of 2, 4-D and sucrose observed for haploid seed development and noted that maximum haploid seed developed @ 100mg/L (2, 4-D) + 60mg/L (sucrose).

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 S2008 AUS375 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. The 840 plantlets were shifted to rooting medium for root development. 580 plants of SPF-234 and S-2008-AUS- 375 were developed and shifted in polythene bags for hardening. 300 plants of SPF-234 and AUS-375 were shifted in field for further studies.

Screening of wheat advanced lines against rust at ABRI

To screen the wheat genotypes for rust reaction 50 genotypes were sown 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 fifty entries under studies 15 entries showed immune response to leaf rust whereas 21 entries showed resistant response to leaf rust. 27 wheat entries showed immune response to yellow rust, 12 entries showed resistant response to yellow rust.

Screening of advanced wheat material against rusts at SARS, Kaghan

One hundred and sixty nine 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 60

entries escaped from rust diseases and the yellow rust incidence ranged 10 to 70 percent.

Somaclonal variation studies in wheat regeneration (R1-R6) on the basis of disease resistance

The objective of this study was screening and selection on the basis of disease resistance and other morphological parameters. 560 wheat entries were studied and 461 wheat entries were selected on the basis of disease resistance.

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 48 entries sown in A and B trial of wheat. 48 wheat lines in A-1, A-2 and B trials were tested against karnal bunt. Most of the wheat entries showed no disease symptoms. 0.5 to 1 percent bunted grains were recorded in 18BT007, 18BT013, 18BT026 and 17BT014.

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. 315 plants o SPF-234 and S-2008-US- 375 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. 50 plants were tagged for red rot disease incidence out of 300 previously sown somaclones of SPF-234 and AUS-375 plants. 43 plants were selected on the basis of red rot resistance for further studies in R1 generation.

Maintenance & utilization of wheat genepool for crossing and use as explant in tissue culture

Gene pool available at this institute was maintained and evaluated for different cross combinations. Two hundred and thirty four (234) entries were sown in two different dates with a fortnight interval to synchronize early and late varieties to prolong the crossing program and 1 crosses with desirable traits were attempted. Each entry was sown in two rows each of 2.5 meters length in non-replicated design. Data of different traits like germination percentage, plant height, number of tillers per plant, days to heading days to maturity were recorded.

Exploitation of Somaclonal variation in wheat for yield and rust resistance

The objective of the study was development of high yielding and disease free soma clones of wheat through callus cultures derived from immature /mature embryo. For this purpose ten R2 crosses and five wheat varieties Galaxy-13, Ujala-16, Fsd-08, Seher-06 and Ufaq-2002 were used. Callus was induced on MS media with different doses of 2,4D (0, 2, 4 and 6 mg/l). Data was noted for different parameters of callus. For regeneration, Kinetin and NAA was used. When roots and shoots were properly developed, fifty somaclones were transferred to pots. The seed of wheat somaclones of different crosses and varieties were sown in field as R1 generation for testing against disease resistance and other morphological parameters.

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

Different filial generations (R1-R6) were studied. Each generation was promoted to next generation on the basis of disease resistance and other morphological parameters. Selection on the basis of disease resistance and morphological traits is under progress.

Regular wheat yield trial (B-trials)

Sixteen wheat promising lines including two checks i.e Galaxy-13 and Ujala-16 from A trials were selected and tested in B trial for yield performance and other morphological characters. The experiment was sown in RCB design with three replications. . Three wheat advanced entries (17BT014, 17BT019 and 17BT024) surpassed the yields of check varieties (Galaxy-13 and Ujala-16).

Micro wheat yield trial

The experiment was sown in RCB design with two replications keeping row to row distance of 30cm. Fifty entries received from Wheat Research Institute, Faisalabad were studied for yield performance. Two advance lines (16BT015 and 16BT016)) of this institute which were found high yielder than checks were selected and sent to NUWYT trial.

Preliminary wheat yield trials (A-trials)

This experiment was designed to study the yield performance of promising lines selected from advanced generations of wheat. Thirty two regenerated promising lines including with two checks(Galaxy-13 and Ujala-16) were sown in RCB design with three replications. At harvesting and threshing data on grain yield was recorded. Six wheat entries(18BT011, 18BT004, 18BT008, 18BT026, 18BT027 and 18BT028 out yielded the check varieties (Galaxy-13 and Ujala-16).

Soil Science

In vitro studies in sugarcane for inducing salt tolerance

Sugarcane advanced lines / varieties viz. S2005-US658, S2006-AUS-133, S-2008-FD-19, CPF-248 and S2006-AUS-134 were evaluated at different salt levels to see their inherited Callogenesis potential. Response to callogenesis among sugar cane advance lines 133, 134, 246 and 658 were evaluated at different salt levels to see their inherited callogenesis potential.

Frequency of callus initiation, fair to excellent, was recorded in 133 followed by 134 at 50 m moles/L salt levels, while 246 and 658 at 100 m moles /L. Callogenesis was suppressed at higher salt concentrations; however variety/ line 658 and 133 fairly showed enough callogenesis potential even at 150 and 200 m moles / L salt level (Fig 19).

Fig 19. Procedure for development of salt tolerance sugarcane. From left to right this figure explains the procedural development of sugarcane plants under salt stress.

Combined application of Diazotroph + L-Tryptophan and phosphate solubilizing microbes (PSM) for improving the growth and yield of wheat.

The project was designed to face the changeling demand of surplus food for geometrically growing population of the country. Diazotroph, L-tryptophan and phosphorous solubilizing microbes (PSM) were applied alone and in combination to wheat crop for optimizing the yield. A field experiment was designed using RCBD with three repeats. Wheat variety Faisalabad-2008 was sown during Rabi-2015. Recommended dose of fertilizer was applied and agronomic practices were done. Maximum yield of 3213kg ha-1 was obtained where diazotroph, L-tryptophan and PSM were applied in combination whereas minimum yield of 2782 kg ha-1 was recorded in control.

Use of phytohormones and biofertilizers biotechnology for improving the growth and yield of cotton.

Pakistan is an Agricultural country and cotton plays the role of backbone of the economy. A field experiment was conducted at Agriculture Biotechnology Research Institute, AARI, Faisalabad. Six treatments were tested in the field along with control. FH-142 variety was

sown in the field and recommended dose of NPK @150-80-60 kg/ha was applied. The management practices were carried out throughout the season. Randomized Complete Block design was followed with 3 replications. Significant results were obtained when L-tryptone was applied in combination with phosphate solubilizing microbes over control (Table 6).

Table 6. Increase in yield by application of Phytoharmones on cotton crop. The results indicated that T6 possessed the highest yield as compared to rest treatments.

Sr. No	Treatment	Results
	Control	**1887^{c}**
T_1	**L-Trptophane**	**2398^{b}**
T_2	**Diazotrophs**	**2653^{ab}**
T_3	**PSB**	**2665^{ab}**
T_4	**T2+T3**	**2763^{ab}**
T_5	**T_2+T_4**	**2702^{ab}**
T_6	**T_2+T_3+T_4**	**2970^{a}**

51. Testing if Bio-fertilizer/Bio-stimulants as reference lab for confirmation

Agricultural Biotechnology Research institute is declared as Reference Lab by the Government of the Punjab to reanalyze the 30% of the tested samples of any other challenged sample of bio-fertilizer/bio-stimulant. Our lab re-analyzed 12 samples of bio-fertilizer/bio-stimulants till date.

Microbial-aided biosynthesis of 2-Acetyl-1-Pyrroline in Basmati and Non- Basmati rice

Isolation and screening of microbes producing secondary metabolite 2AP responsible for characteristics aroma in basmati and non-basmati rice, is in progress.

***In-vitro* production and quantification of microbial mediated bio-transformation of 2-Acetyl-1-Pyrroline**

Isolation and purification of microbes in question is in progress

Evaluation of biochar and phosphate solubilizing bacteria favors P availability and growth in maize

Soil samples of maize, rice and cotton rhizosphere were collected. Through dilution plate technique microbial isolates were purified. The phosphate solubilizing efficiency of purified isolates was measured on PIKOVASKAYA's media. Halo zone forming Isolates were considered to be positive for phosphate solubilizing microbes. Phosphate solubilizing index (PSI) of these isolates was calculated and it was in the range of 1.73-2.5. In addition to this, gram staining, catalase, oxidase and motility testes for these microbes were performed. Five best isolates with maximum PSI were selected for pot experiment**.**

Potential of endophytes for the growth promotion 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 was conducted to check the growth promotion of crops in lab. Five isolates were selected on the basis of their efficiency. Trail was conducted in collaboration with Soil Bacteriology section AARI, Faisalabad. In a field trail, seeds of Raya were inoculated with the selected microbes and sown. The routine cultural practices were done during the growth of crop. Harvesting has been done and threshing will be done at optimum level of moisture.

Soil Bacteriology Section:

Bio-fertilizer Testing Lab

Soil Bacteriology as "Biofertilizer Testing Lab" analyzed 79 biofertilizer / biostimulant / BOP samples for registration or under FCO.

Biofert-plus: precursor enriched novel microbial formulations for legumes

Study was conducted to test the precursor's enriched inocula to improve the chickpea and mungbean growth and yields for cost effective

crop production. Precursor enriched inoculum was applied at sowing. Field study was conducted at Pulses Research Institute (PRI) and Soil Bacteriology Section, Faisalabad. Treatments for mungbean include control, Rhizobium inoculation; Rhizobium enriched L-tryptophan, L-adenine, Tryptamine and GA3 each at 10-5M. Treatments for chickpea and lentil include control, Rhizobium inoculation; Rhizobium enriched L-tryptophan, Kinetin, L-Methionine and GA3 each at 10-5M. Results of mung bean field trial at PRI, Faisalabad revealed that Rhizobium inoculum enriched with GA3 produced the highest mungbean biomass / grain yield i.e. 3333 / 1517 kg ha-1, as compared to control 2600 / 1283 kg ha-1, respectively. Results of chickpea and lentil revealed that Rhizobium inoculum enriched with GA3 produced the highest chickpea and lentil biomass / grain yield i.e. 5333 / 1647 kg ha-1 and 3200 / 1427 kg ha-1 as compared to control i.e. 4383 / 1353 kg ha-1 and 2520 / 1040 kg ha-1, respectively. Results of nodulation in chickpea, mung bean and lentil field trials at PRI, Faisalabad revealed that Rhizobium inoculum enriched with GA3 produced higher nodules plant-1, nodular dry mass as compared to control (Fig. 20).

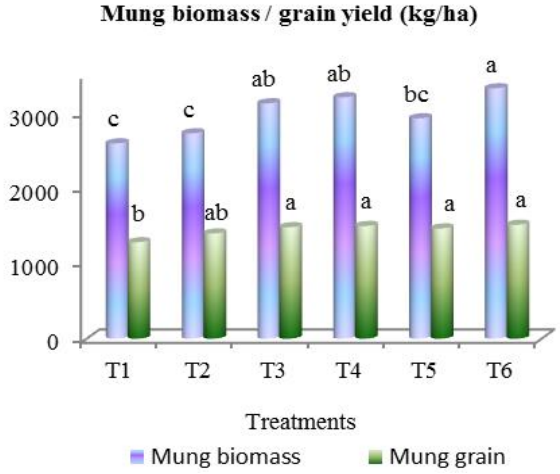

Fig 20. Grain yield of mungbean at PRI, Faisalabad. Results showed that Rhizobium inoculum enriched with GA3 produced higher nodules plant-1, nodular dry mass as compared to control.

Biofert-plus: precursor enriched novel microbial formulations for cereals

Plant hormones and their precursors exert beneficial effects on plant growth and development. Field studies were conducted to exploit the physiological precursors to improve the crop growth and yield in a cost effective and sustained way. Precursor enriched inoculum was applied along with 75% of recommended dose of NPK. Results revealed that physiological precursors improved the growth and yield at each location significantly. Treatment includes control, PGPR, PGPR enriched L-tryptophan, PGPR enriched L-adenine, PGPR enriched Tryptamine, and PGPR enriched GA3 each at 10-5M. Results of rice trials at ARS, Farooqabad and SSRI, Pindi Bhattian revealed that PGPR enriched with GA3 produced the highest paddy yield i.e. 4480, 3453 as compared to control 3613, 2787 kg ha-1, respectively (Fig. 21). Results of maize trial at Soil Bacteriology Section revealed that PGPR enriched with GA3 produced the highest maize fodder yield, dry matter yield i.e. 66.7, 14.44 as compared to control i.e. 48.3 and 9.01 t ha-1, respectively (Fig. 5). Treatments for wheat trials include control, Rhizobium inoculation; Rhizobium enriched L-tryptophan, Kinetin, L-Methionine and GA3 each at 10-5M (Fig 22).

Results of wheat trials at Soil Bacteriology Section, SSRI, Pindi Bhattian, WRI, Faisalabad and ARS, Farooqabad revealed that PGPR enriched with GA3 produced the highest wheat grain yields i.e. 3823, 1583, 3007 and 3460 kg ha-1 as compared to control i.e. 3153, 1120, 2180 and 2793 kg ha-1, respectively (Fig. 23).

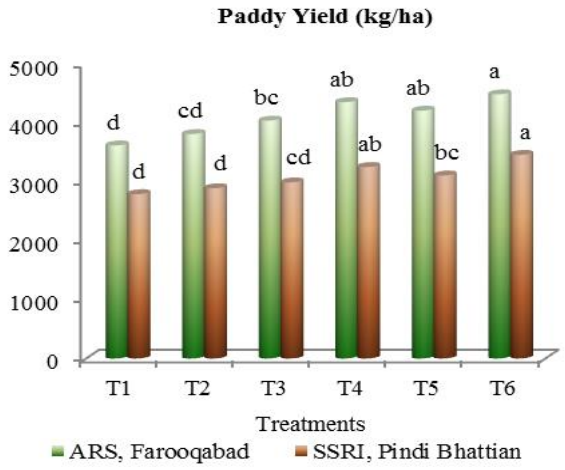

Fig. 21. Paddy yield at ARS, Farooqabad and SSRI, Pindi Bhattian. PGPR enriched with GA3 produced the highest paddy yield.

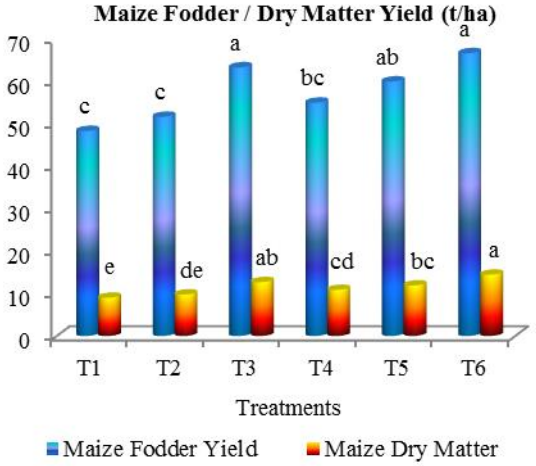

Fig. 22. Maize fodder and dry matter yield at Soil Bacteriology Section, Faisalabad. PGPR enriched with GA3 produced the highest maize grain and fodder yield.

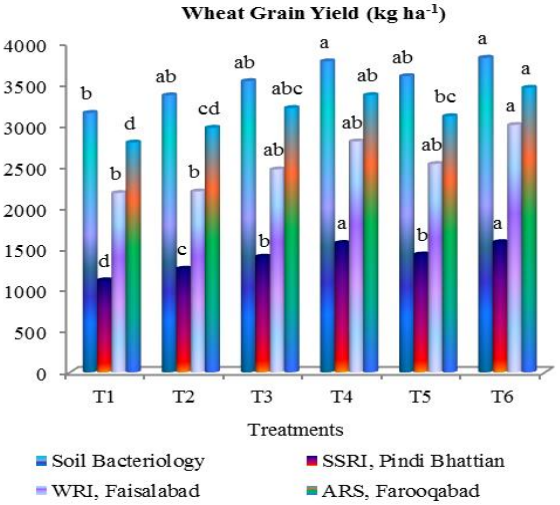

Fig. 23. Wheat yield at Soil Bacteriology Section, SSRI Pindi Bhattian, WRI, ARS Faisalabad. PGPR enriched with GA3 produced the highest wheat grain yields.

Effect of various crop residue management strategies on soil health in rice-wheat cropping system

In rice-wheat cropping system, farmer's burn crop residues for easy seed bed preparation. Study was conducted to note the ill effects of crop residue burning on soil health and environment. Crop residue burnt and unburnt soil samples were collected by Soil Fertility field staff from various districts of the Punjab and six hundred (960) samples were collected, processed and analyzed at Soil Bacteriology Lab for microbial count using dilution plate technique and soil organic carbon as per standard procedure. Results of burnt vs. unburnt soil samples after rice and wheat harvest showed significant reduction in microbial population expressed as CFU 107 per gram of soil. Measurable reduction in total organic carbon (TOC) because of residue.

Influence of L-methionine and rhizobium sp on growth and yield of mung bean

Field experiment was conducted at Pulses Research Institute, Faisalabad to test the precursor L-methionine (L-MET) and Rhizobium effect to improve the mungbean and chickpea growth and yield by using normal soil having pH 7.8, EC 2.4, Organic matter 0.68% and available P 8.3 mgkg-1. Treatments were control (T1), Rhizobium inoculation (T2), L-MET @ 5mg L-1 (T3), L-MET @ 10 mg L-1 (T4), L-MET @ 15 mg L-1 (T5), Rhizobium + L-MET @ 5 mg L-1 (T6) Rhizobium + L-MET @ 10 mg L-1 (T7), Rhizobium + L-MET @ 15 mg L-1 (T8). Recommended dose of fertilizer @ 30-60kg NP ha-1was applied. Results showed that in mung bean and chickpea maximum grain yield (1390, 1534 kg ha-1) was obtained from T6 treatment as compared to control (1067, 1096 kg ha-1), respectively (Fig. 24).

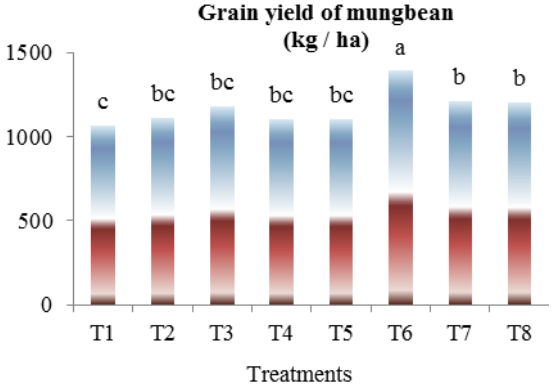

Fig. 24. Mung bean grain yield as affected by Rhizobium and L-Methionine. T6 (Rhizobium + L-MET @ 10 mg L-1) showed highest grain yield as compared to other treatments.

Potential of endophytic bacteria for wheat and maize growth

Endophytes enhance growth of plant by direct (nutrient acquisition, phytohormone production) or indirect mechanisms (biocontrol). Study was planned to check the potential of endophytic bacteria on growth and promotion of maize and wheat crop. Field study was conducted at the Soil Bacteriology Section, AARI Faisalabad using normal soil having pH 7.78, EC 2.3 dS m-1 and organic matter 0.54%. Treatments were control, and five endophytes as E-1, E-2, E-3, E-4 and E-5. Maize crop was sown during Kharif 2018 and wheat crop was sown during Rabi 2018-19. Recommended dose of fertilizer for maize (NP @ 100-60 kg ha-1) and wheat (NPK @ 120-100-70 kg ha-1) crop was applied. Result showed that inoculation with bacterial isolates showed significant increase in yield as compared to control. Endophytic bacteria E-3 produced maximum fodder yield (54.0 t ha-1) in maize (Fig. 25) and maximum grain yield (4030 kg ha-1) in wheat (Fig. 10) and it was significantly different from all other treatments including control followed by endophytic bacteria E-2 (Fig. 26).

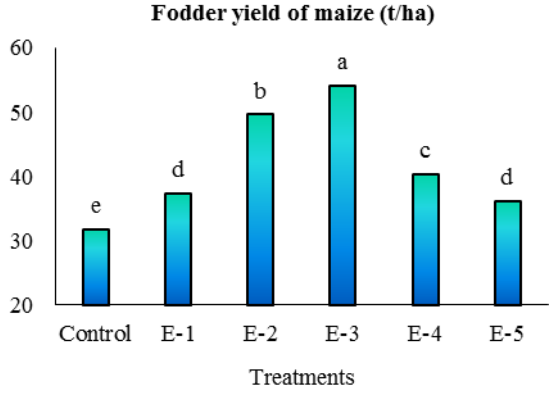

Fig. 25. Effect of Endophytic isolates on fodder yield of maize. E_2 showed maximum fodder yield as compared to other treatments.

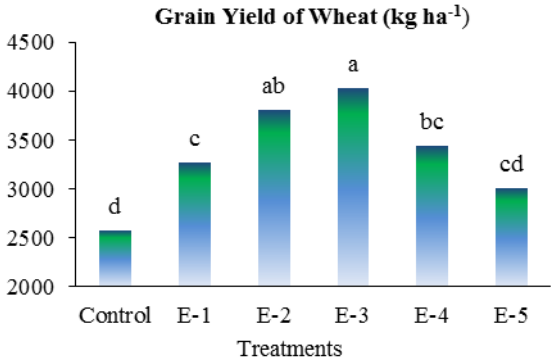

Fig. 26. Effect of Endophytic isolates on grain yield of wheat. Endophytic bacteria E-3 produced maximum fodder yield (54.0 t ha-1) in maize (Fig. 25) and maximum grain yield (4030 kg ha-1) in wheat.

Growth and yield response of oil seed crops to PGPR and microbially synthesized metabolites

Plant-growth-promoting rhizobacteria (PGPR) are soil bacteria which live in the rhizosphere of plants, where they stimulate plant growth and development of their hosts. Field study of sesame was conducted at the Oil Seed Research Institute, Faisalabad using normal soil having pH 8.01, EC 2.2 dS m-1 and organic matter 0.61% and of Raya at Agri. Biotech. Research Institute, Faisalabad using normal soil having pH 8.31, EC 2.1 dS m-1 and organic matter 0.64%. Treatments were control (T1), PGPR-Azotobacter (T2), PGPR-Pseudomonas (T3), Metabolite spray (T4), Metabolite spray + PGPR-Azotobacter (T5), Metabolite spray + PGPR-Pseudomonas (T6). Sesame was sown during Kharif 2018 and Raya was sown during Rabi 2018-19 and their recommended dose of fertilizer was applied. Result showed that inoculation with bacterial isolates along with metabolites spray showed significant increase in yield as compared to control. Maximum grain yield i.e. 2943 kg ha-1, 1396.3 kg ha-1) was produced with T6 in case of raya and sesame, respectively (Fig. 27, 28).

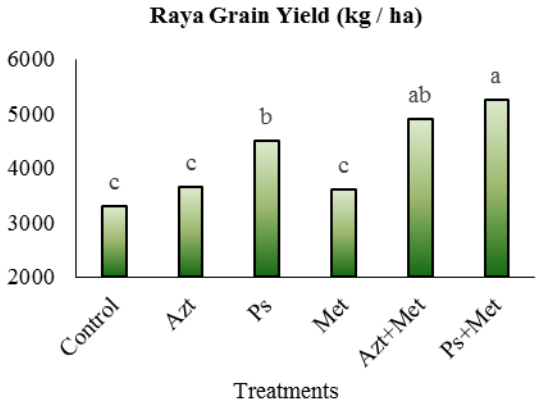

Fig. 27. Effect of PGPR and their metabolites on Raya grain yield. Result showed that inoculation with bacterial isolates along with metabolites spray showed significant increase in yield as compared to control.

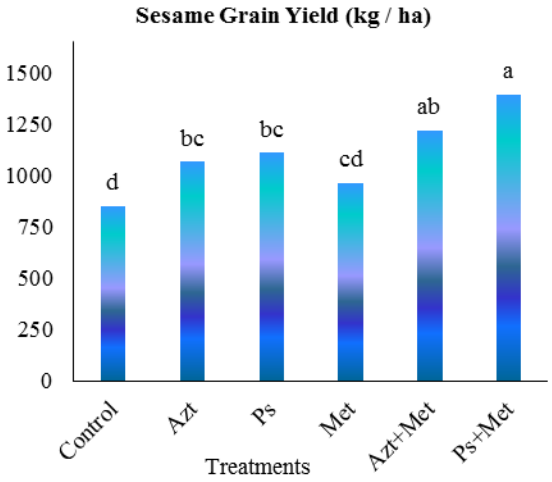

Fig. 28. Effect of PGPR and their metabolites on Sesame grain yield. Result showed that inoculation with bacterial isolates along with metabolites spray showed significant increase in yield as compared to control.

Isolation, characterization and screening of PGPR providing relief in abiotic stresses

Salt tolerant PGPR improves growth and yield of different crops in salt prone areas. Field study was conducted on rice and wheat crop at the SSRI, Pindi Bhattian using normal soil having pH 8.52, EC 6.3 dS m^{-1} and SAR 25. Treatments were control (T_1), Salt tolerant isolate KH-1 (T_2), KH-2 (T_3), KH-3 (T_4), and PGPR-I (T_5), PGPR-II (T_6). Recommended dose of fertilizer according to rice and wheat crop was applied. Parameters recorded were plant height, biomass, grain yield and no of tillers. Result showed that inoculation performed better as compared to control. Inoculation of salt tolerant isolate KH-2 produced maximum paddy yield in rice i.e. 4167 kg ha^{-1} followed by PGPR-I (*Azotobacter*) and it was statistically significant from control (Fig. 13). While maximum grain yield in wheat was also observed in KH-2 i.e. 1430 kg ha^{-1} which is at par with KH-3 (Fig. 29 & 30).

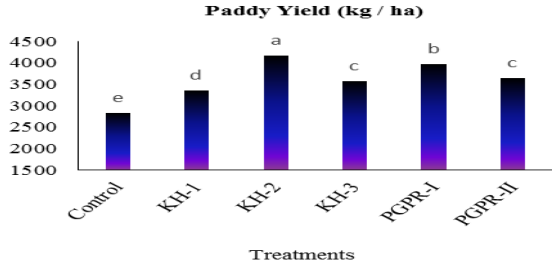

Fig 29. Effect of salt tolerant PGPR on paddy yield of rice crop. Result showed that inoculation performed better as compared to control. Inoculation of salt tolerant isolate KH-2 produced maximum paddy yield in rice.

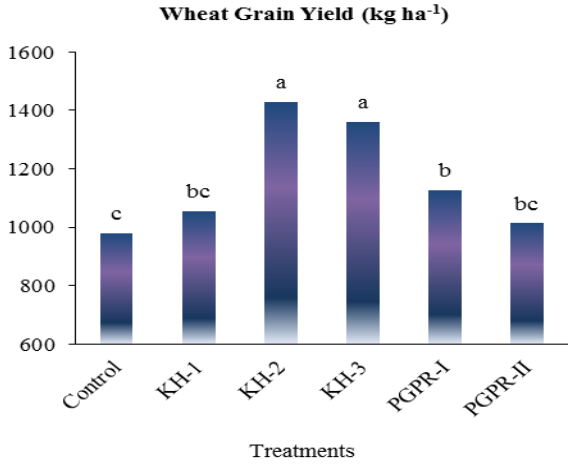

Fig 30. Effect of salt tolerant PGPR on grain yield of wheat crop. Result showed that inoculation performed better as compared to control. Inoculation of salt tolerant isolate KH-2 produced maximum wheat grain yield.

Co-inoculation of *Bradyrhizobium* and phosphate solubilizing microbes on growth promotion of groundnut under rain-fed conditions

Field study was conducted to assess the efficiency of *Bradyrhizobium* and phosphate solubilizing microbes (PSM) on growth and yield of groundnut crop under rain-fed

conditions. The *Bradyrhizobium* and phosphate solubilizing bacteria were characterized and 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 with three repeats in RCBD at Soil & Water Conservation Research Institute, Chakwal using normal soil and recommended dose of NPK (25:80:25) were applied. Treatments were control (T_1), *Bradyrhizobium* isolate-1, 2 and 3 (T_2, T_3, T_4), phosphate solubilizing microbe (PSM) (T_5), T_2 + PSM (T_6), T_3 + PSM (T_7), T_4 + PSM (T_8). Result showed that T_7 i.e. bacterial isolates-2 along with PSM produced 1211 kg ha^{-1} pod yield that is significantly higher than control (Fig. 31).

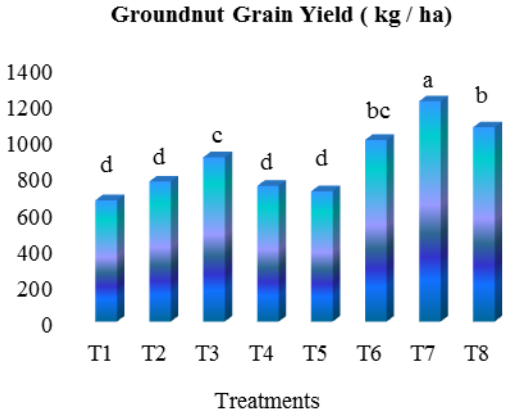

Fig 31. Effect of treatments on groundnut grain yield. Result showed that T_7 i.e. bacterial isolates-2 along with PSM produced 1211 kg ha^{-1} pod yield that is significantly higher than control.

Isolation, characterization of sulphur oxidizing bacteria for acidification of compost

The study was planned to isolate the efficient strains of sulphur oxidizing bacteria (SOB) to reduce pH of organic source. For this purpose, rhizosphere soil samples collected from different areas. Isolation was carried out on Thiobacillus agar media, Starkey broth and Beijerinck media by using dilution plate technique. Purified isolates were checked for reduction of pH in compost. Four purified isolates Thio-1, Thio-2, Thio-3 and Thio-4 were checked for microbial count, auxin biosynthesis and reduction in pH of compost. Isolate (Thio-4) showed maximum reduction in pH i.e. 3.92, highest microbial population 8.67 x 10^6 CFU g^{-1} and highest IAA i.e. 3.07 µg mL^{-1} (Fig. 32).

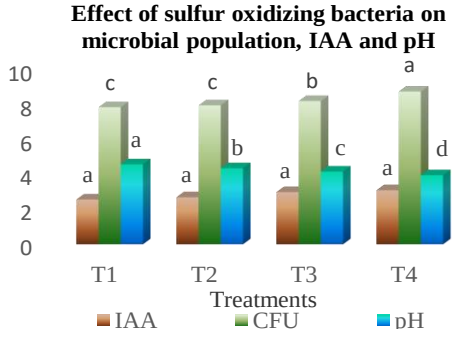

Fig 32. Effect of sulfur oxidizing bacteria on microbial population, IAA and pH. Isolate (Thio-4) showed maximum reduction in pH i.e. 3.92, highest microbial population 8.67 x 10^6 CFU g^{-1} and highest IAA i.e. 3.07 µg mL^{-1}.

Rice response to biostimulants under field conditions

Biostimulants are the products which foster the plant growth in a number of demonstrated ways throughout the life cycle that is from seed germination to crop maturity. These are the products which contain certain substances that when applied to the plants or crops, stimulate natural processes to improve nutrients availability, tolerance to abiotic stress and crop quality. Studies were conducted to assess the role of different biostimulants on growth and yield of rice at Agronomic Research Institute (ARS), Farooqabad, District Sheikhupur following RCBD design. Different biostimulants were studied i.e. Gibbrex, Osley, Galore, Sonata and Nature time. These were applied according to their rate and time of application mentioned in their protocol. Uniform dose of fertilizer was applied to all treatments. The results revealed that highest paddy yield was obtained with Galore i.e. 4333 kg ha-1 Followed by Nature Time i.e. 4247 kg ha-1 as compared to control i.e. 3227 kg ha-1 of control (Fig 33).

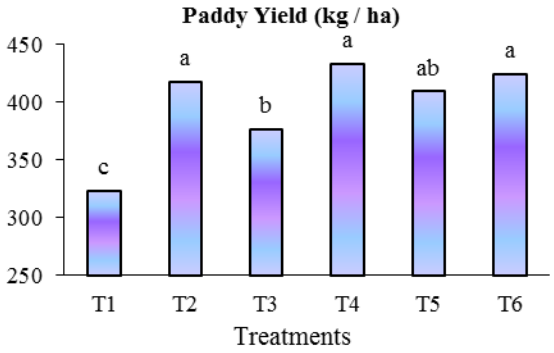

Fig 33. Effect of different biostimulants on paddy yield. The results revealed that highest paddy yield was obtained with Galore i.e. 4333 kg ha-1 Followed by Nature Time i.e. 4247 kg ha-1 as compared to control.

Precursor-inoculum interaction for Improving growth and yield of beans.

Field study were conducted at Oil Seed Research Institute and Pulses Research Institute, AARI, Faisalabad to assess the exploitation of *Rhizobium* species along with physiological precursor (L-tryptophan) to improve the growth and yield of soybean and mung bean. Treatments include control (T_1), three isolates of *Rhizobium* sp. (T_2, T_3, T_4), L-tryptophan @ 10^{-5} M (T_5), and interaction of Rhizobium species with L-Tryptophan (T_6, T_7, T_8). Recommended dose of fertilizer (NPK) was applied and layout was RCBD with three repeats. Results of mungbean revealed that Rhizobium species enriched with L-Tryptophan produced the highest mungbean yield i.e. 1457 kg ha^{-1} followed by 1380 and 1370 kg ha^{-1} as compared to control i.e. 1207 kg ha^{-1}. Results of soybean revealed that various Rhizobium species enriched with L-Tryptophan produced the highest soybean yield i.e. 2360 kg ha^{-1} followed by 2250 and 2186 kg ha^{-1} as compared to control i.e. 1850 kg ha^{-1} (Fig. 34).

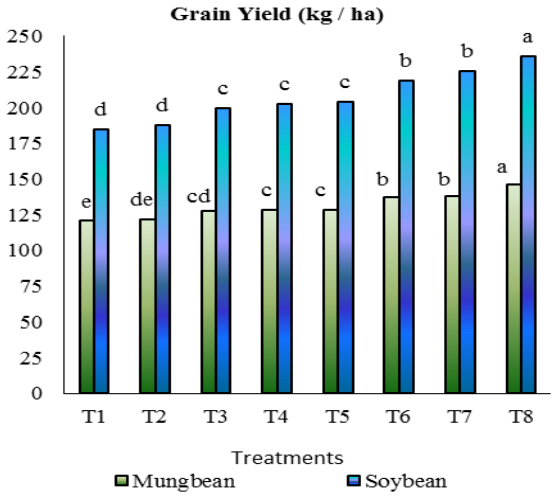

Fig 34. Effect of treatments on grain yield of mung bean and soybean. Results of soybean revealed that various Rhizobium species enriched with L-Tryptophan produced the highest soybean yield i.e. 2360 kg ha^{-1} followed by 2250 and 2186 kg ha^{-1} as compared to control i.e. 1850 kg ha^{-1}

Growth and yield response of rice to different levels of kinetin

Field experiment was conducted at the Soil Chemistry Section AARI, Faisalabad to check the best levels of kinetin along with PGPR to improve growth and yield of rice crop. Trial was carried out in clay loam soil having pH: 8.2, EC: 2.5 dS m^{-1}, available P: 7.5 mg kg^{-1} and organic matter 0.66%. Recommended dose of fertilizer @ 120-100-70 kg NPK ha^{-1} was applied to all treatments. Treatments were control (T_1), PGPR (T_2), Kinetin @ 10^{-3} M (T_3), Kinetin @ 10^{-4} M (T_4), Kinetin @ 10^{-5} M (T_5), PGPR + Kinetin @ 10^{-3} M (T_6), PGPR + Kinetin @ 10^{-4} M (T_7), PGPR + Kinetin @ 10^{-5} M (T_8). Results indicated that PGPR along with kinetin significantly improved paddy yield (4543.3 kg ha^{-1}) as compared to its control (4100.0 kg ha^{-1}) (Fig. 22). Similarly PGPR along with kinetin @ 10^{-5} M significantly improved wheat grain yield (4020.0 kg ha^{-1}), as compared to its respective control (3133.3 kg ha^{-1}). (Fig. 35 & 36).

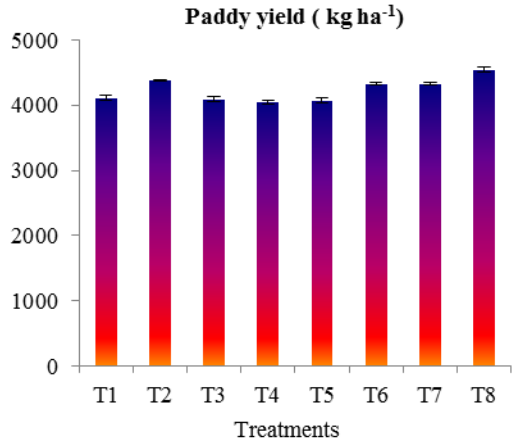

Fig 35. Effect of different levels of kinetin on paddy yield. Results indicated that PGPR along with kinetin significantly improved paddy yield (4543.3 kg ha^{-1}) as compared to its control (4100.0 kg ha^{-1})

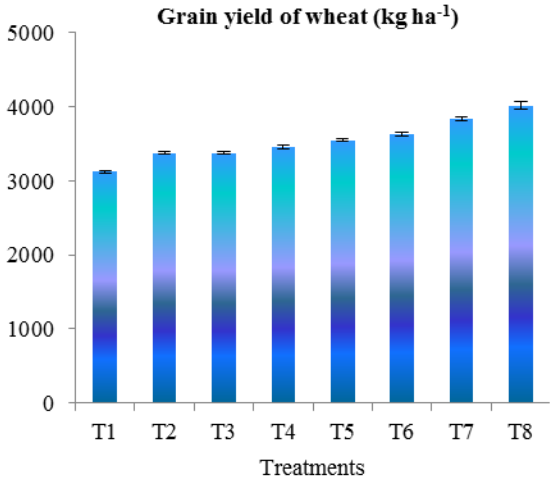

Fig 36. Effect of different levels of kinetin on wheat yield. PGPR along with kinetin @ 10^{-5} M significantly improved wheat grain yield (4020.0 kg ha^{-1}), as compared to its respective control (3133.3 kg ha^{-1}).

Effect of PGPR on biofortification of zinc in Rice

Pot experiment was conducted at Soil Bacteriology Section, AARI Faisalabad to study the effect of PGPR (zinc mobilizing bacteria) on biofortification of zinc in rice. Trial was carried out in sandy clay loam soil having pH: 8.0, EC: 2.8 dS m^{-1}, available P: 7.7 mg kg^{-1} and organic matter 0.79 %. Recommended dose of fertilizer @ 120-100-70 kg NPK ha^{-1} was applied to all treatments. Treatments include Control, PGPR inoculation with 5, 10, 15 kg ha^{-1} $ZnSO_4$, PGPR inoculation with 5, 10, 15 kg ha^{-1} $ZnSO_4$. Results indicated that PGPR along with 15 kg ha^{-1} $ZnSO_4.7H_2O$ significantly improved grain yield (52.17 g pot^{-1}), as compared with its respective control (41.67 g pot^{-1}) (Fig. 37).

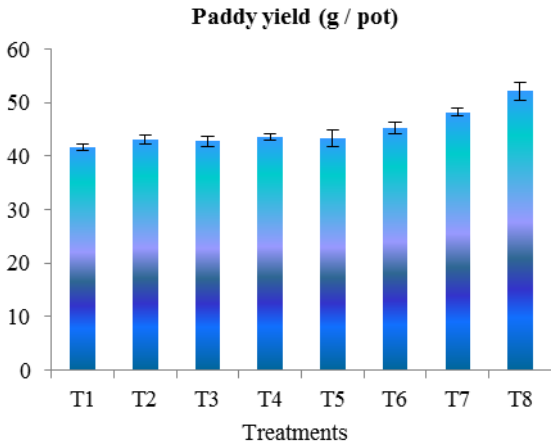

Fig 37. Effect of bio-fortification of zinc and PGPR on paddy yield of rice. Results indicated that PGPR along with 15 kg ha^{-1} $ZnSO_4.7H_2O$ significantly improved grain yield (52.17 g pot^{-1}), as compared with its respective control (41.67 g pot^{-1}).

Response of maize to slow releasing phosphatic fertilizers in comparison with other fertilizer sources

Pot study was carried out in the wire house of Soil Bacteriology Section, Faisalabad, to compare the response of maize to slow releasing P fertilizers in comparison with other fertilizer sources. Slow releasing P fertilizers with commercial names; Nutrafil, Marathon, Charger and Super Kissan were tested along traditional DAP and Rock phosphate + compost (70:30) + PSM. After four weeks of sowing, average shoot fresh weight (g pot-1) recorded was as follows; Nutrafil (242.5), Charger (220.0), DAP (198.8), Rock phosphate + compost (70:30) +PSM (195.0), Marathon (173.8), Super Kissan (168.8) and control, without any phosphatic input yielded 112.5 g pot-1 (Fig. 27). (Fig. 38).

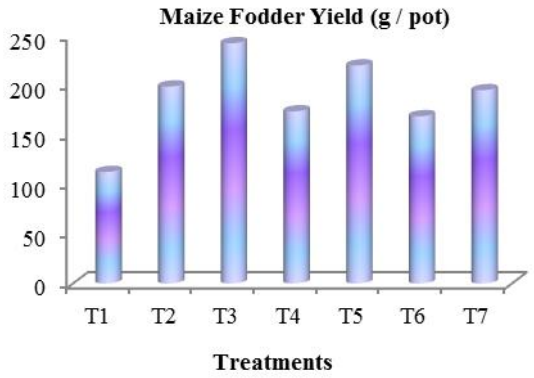

Fig 38. Effect of treatments on maize fodder yield. Slow releasing Phosphatic fertilizer significantly impacted grain yield as compared to control.

PARB Projects

Sr. No.	Title	Year
1.	Training of Agri. Extension Wing, Federal Seed Certification & Registration Department and Private Sector Personnel in Detection, Identification & Quantification of Bt Cotton	2018
2.	Acceleration of Maize Breeding Through Inducer Line Mediated Double Haploid Inbred Lines for Development of Climate Smart, High Yielding Maize Hybrids	2018-2019
3.	DNA barcoding/fingerprinting for identification of Cotton, Wheat, Maize, Potato, Tomato and Date Palm varieties	2018-2019

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3. Muhammad Zaffar Iqbal, Shakra Jamil, Abid Mehmood and Rahil Shahzad 2019. Identification of Seven Olive Varieties using RAPD Molecular Markers. Journal of Agricultural Research. 57(1): 07-14.
4. Munazza Rafique, Aneela Riaz, Ashfaq Anjum, M. Amjad Qureshi, Fakhar Mujeeb. 2018. Role of Bioinoculants for Improving Growth and Yield of Okra (*Abelmoshuses culentum*). Universal Journal of Agricultural Research 6(3): 105-112, 2018.
5. Muhammad Jamshed Anwar, Muhammad Aslam Javed, Muhammad Waqas Jamil, Imran Habib, Shahid Nazir, Sajid ur Rehman, Muhammad Zafar Iqbal, Muhammad Kamran, Muhammad Ehetisham ul Haq. 2019. Response of wheat genotypes for resistance against leaf rust (*Puccinia triticina* eriks.) Under field conditions. Plant Protection Journal.
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10. Muhammad Zaffar Iqbal, Shahid Nazir, Sajid-ur-Rahman, Muhammad Younas. 2018. Stability analysis of candidate bollgard bt cotton (Gossypium hirsutum L.) genotypes for yield traits. International Journal of Biosciences. 13(5) :55-63.
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Zaffar Iqbal. Variation in responses of genotypes of wheat to different tissue culture media. 2019 in 1st International Sino-Pak Wheat conference. Muhammad Nawaz Sharif University of Agriculture, Multan.

3. Shakra Jamil, Rahil Shahzad, Sajid Ur Rahman, Shamsa Kanwal, Erum Yasmeen, Raiza Sultana and Muhammad Zaffar Iqbal. Trangenes Purity and Cry1Ac expression in GM cotton grown in Punjab Pakistan. In 6th International Conference on Sustaiable Agriculture in Changing Climate; Strategies and Management. 2019.
4. Rahil Shahzad, Shakra Jamil, Sajid Ur Rahman, Erum Yasmeen, Shamsa Kanwal and Zaffar Iqbal. DNA Fingerprinting of Pakistani maize hybrids and Parental lines using SSR markers. In 6th International Conference on Sustaiable Agriculture in Changing Climate; Strategies and Management. 2019.
5. Shamsa Kanwal, Shakra Jamil, Rahil Shahzad, Sajid Ur Rahman and Muhammad Zaffar Iqbal. Comparison of different methods for DNA mini Prepratiobns from Fibrous leafs of Date Palm with and without use of Liquid Nitrogen. In 6th International Conference on Sustaiable Agriculture in Changing Climate; Strategies and Management. 2019.
6. Erum Yasmeen, Shakra Jamil, Rahil Shahzad, Muhammad Riaz and Muhammad Zaffar Iqbal. Genome Wide In Silico Expression Analysis of WRKY Transcriptional Factor Gene Family in Sorghum bicolor. 6th International Conference on Sustaiable Agriculture in Changing Climate; Strategies and Management 2019.
7. Razia Sultana, Shakra Jamil, Muhammad Aslam, Rahil Shahzad and Muhammad Zaffar Iqbal. Molecular and Morphological Characterization of Maize genotypes for Opaque-2 gene and yield contributing traits. In 6th International Conference on Sustaiable Agriculture in Changing Climate; Strategies and Management. 2019.

Miscellaneous:

Radio Talks Delivered	03
TV Talk	01
Visits of Delegations	20
Training Seminars Delivered	11
Class visits	10
Conference Abstracts	09
MPhil/PhD Students	03
Training Imparted to Scientist	301
PARB Projects	03
Internship Students	100
GMO Sample analyzed	1588
Income Generations	3646643

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