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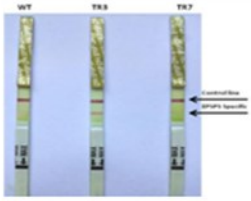
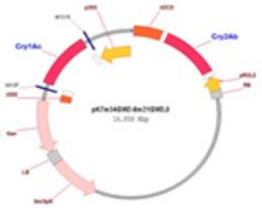
OVERVIEW

Any technological application that uses biological systems, living organisms, or their derivatives to make or modify products or processes for specific use called biotechnology. Plant breeder's objective is to improve the 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. So to improve quality and quantity of plants through innovative approaches mainly three areas are concerned including strategies to manipulate the genome. 1). Selection of desirable traits/diseases etc by using genomic tools especially molecular markers 2). Manipulation and subsequent growth of tissues, organs and plant cells in tissue culture 3). Genetic engineering/recombinant DNA technology which allows the direct transfer of single gene. Agricultural Biotechnology Research Institute is dealing with all these approaches of biotechnology for crop improvement of major cash crops including cereals, fiber crops, sugarcane, fodders, oilseeds, pulses and vegetables. This institute is helping the 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, disease free seed multiplication etc. ABRI also has Soil Bacteriology Section which deals with microbial biotechnologies for restoration of soil and plant health. During the year (2017-18), 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.



Qualitative ELISA for the verification of GT protein in transgenic tobacco

GT transgenic tobacco plants for acclimatization and seed formation



Final plant transformation vector having synthetic insect resistant genes

Drug resistant shoots in glass Jars for root and shoot development

A. GENETIC ENGINEERING

Codon optimization, characterization and expression analysis of genes for crop improvement

The objective of this experiment was to modify the nucleotide sequences of Glyphosate tolerant (*EPSPS)* and insect resistant (*Cry1Ac* & *Cry2Ab*) genes for higher

expression. Gene sequences were submitted to GenBank and accession no. was allotted. Characterization and expression studies of modified genes were carried out in model system (tobacco).

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

The objective of this research work was the development of herbicide tolerant transgenic cotton using pollen tube pathway transformation technology. For this experiment two Bt cotton genotypes (FH-142 & MNH-886) were targeted for the optimization of PTP genetic transformation protocol. Infected developed bolls (169) were raised and plantlets were screened using low dose of Roundup Ready i.e. 600 ml/acre. After 12 days of spray only two plantlets showed some resistant, hence genomic DNA was isolated. PCR confirmed the transgene integration while no protein was detected using strip test. After about 20 days of spray these plants also died. Experiment is being continued with modifications in the process of inoculations, flower selection etc.

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

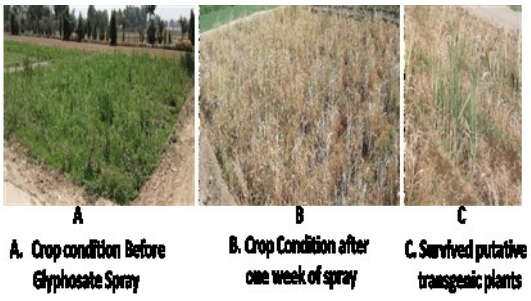
Integration of modified GT gene in brassica was planned for the development of glyphosate herbicide resistance for the effective weeds control.

8500, cotyledonary leaf petioles were inoculated with Agrobacterium strain LBA4404 having synthetic GT gene with selection marker. 1750 antibiotic resistant shoots were screened on selection media containing respective antibiotic (PPT @ 3mg/L & Kanamycine @ 50 mg/L. 280 putative antibiotic resistant plantlets were survived on regeneration and rooting media and 45 putative transgenic plants were developed and their confirmation was under process.

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

Three Weeks old calli were inoculated with Agrobacterium strain LBA4404 having modified GT gene with selection marker. 210 putative antibiotic resistant plants were screened on selection media containing Kanamycine @ 50 mg/L.

Previously developed transgenic sugarcane plants were subjected to herbicide (Glyphosate) spray trial. Two month old plants were sprayed @ 1900 ml/acre. Observations were recorded for 30 days on regular basis and 3 herbicide tolerant plants were observed and their confirmation via PCR was under process.



Integration of modified GT gene in wheat.

Experiment on integration of modified Glyphosate Tolerance (GT) gene in wheat varieties viz. Galaxy-2013, Ujala-2016 and Faisalabad-2008 was planned for the development of roundup herbicide resistance for the effective weeds control. Approximately 1500 immature embryos of wheat were excised and inoculated with agrobacterium strain LBA-4404 harboring synthetic EPSPS (GT) gene, from which 13 putative transgenic plants were screened on nutrient media containing PPT herbicide @ 3mgL^{-1} as selection. LBA4404 is a dicot specific agrobacterium strain. Hence, it did not perform well in monocot wheat and resultant putative plantlets were unable to survive on selection media. For the next year, wheat varieties will be transformed with a hyper-virulent monocot specific agrobacterium strain AGL1.

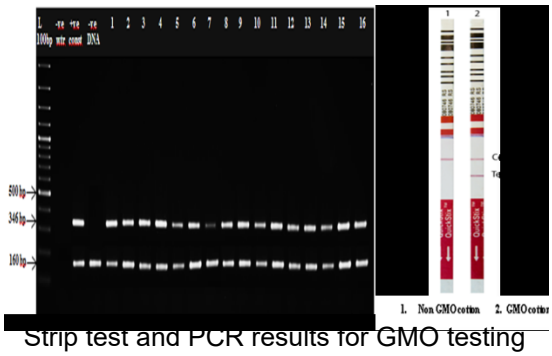
Different stages of wheat calli

Testing of biotech crops

The objective of experiment was to test the presence 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: 119 entries of NCVT, received from PCCC, Multan and 5 plants from each entry were tested for the presence of Cry1Ac, Cry2Ab and RR genes. 103 entries were found positive for Cry1Ac and MON531 event.

On demand testing: A total of 311 samples received from public and private sectors were tested for GM elements.



Strip test and PCR results for GMO testing

GMO lab accreditation under ISO-17025

The objective of this work was to develop and accredit the GMO testing activities from ISO-17025. Initially, 02 GM elements i.e. 35S promoter & NOS terminator were selected for accreditation work. For this purpose the GMO testing lab participated three time in international Proficiency Testing (PT) program with 100% consensus. The application for assessment and accreditation was send to Pakistan National Accreditation Council (PNAC), Islamabad. After through assessment GMO testing Lab has been Accredit for ISO-17025 from PNAC on 28-03-2018. Further scope enhancement and inclusion of new tests for accreditation is in progress.

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

Planned 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.

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 training of 200-300 personals about detection, identification & quantification of Bt cotton. Training manual was prepared and printed. The training was imparted to 301 trainees in 10 batches. There detail is given below.

Batch No. With Date	No. of Participants				Total Participants
	Agri. Ext.	FSC & RD	AARI	Seed Companies	
1st Batch (14th-16th February 2018)	38	-	-	-	38
2nd Batch (28-02-2018 to 02-03-2018)	38	-	-	-	38
3rd Batch (14-03-2018 to 16-03-2018)	38	3	-	1	42
4th Batch (28-03-2018 to 31-03-2018)	32	-	-	-	32
5th Batch (11-04-2018 to 13-04-2018)	34	1	-	1	36
6th Batch (18-04-2018 to 20-04-2018)	2	1	16	-	19
7th Batch (25-04-2018 to 27-04-2018)	15	1	-	4	20
8th Batch (02-05-2018 to 04-05-2018)	16	-	-	-	16
9th Batch (09-05-2018 to 11-05-2018)	13	-	9	-	22
10th Batch (14-05-2018 to 16-05-2018)	13	-	21	4	38
Total	239	06	46	10	301

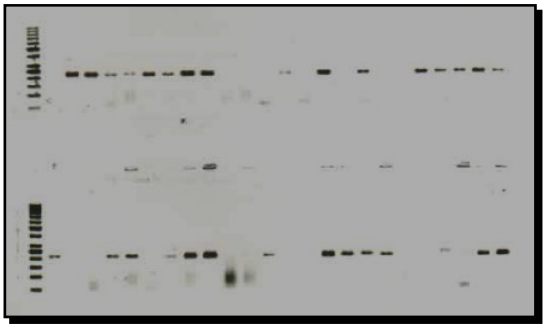
B. GENOMICCS

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 programs. Six DNA markers linked to resistance genes for wheat rusts were surveyed to identify stem, yellow and leaf rust resistance genes on 60 wheat advance lines. Out of these 06 DNA markers, 4 DNA markers for Lr resistant genes, 2 for Sr resistant genes. Marker genes were validated in advance lines. The genes Lr34/Yr18/Pm38 were present in 02 genotypes, Lr46/Yr29/Pm39 in 49 wheat, Lr-28 in 03 genotypes, Lr-19 in 55 genotypes, Sr2 in 28 genotypes and Sr-32 was present in 54 genotypes.

DNA markers based detection of quality related genes in spring wheat

This research work was planned to find quality related genes in spring wheat genotypes. 50 wheat genotypes were used for identification of 2 optimized HMW-GS linked makers. Marker Dx2, Dx5 was present in 44 genotypes while By8 was found in 26 genotypes. 05 new primers for Zinc and Iron were also optimized and above mention 50 wheat lines of micro trial 2017 were screened. Primer Xbarc-124 present in 45 lines, Xgwm-537 was present in 41 lines, Xgwm-577 was found 45 lines and Xcfa-2019 was amplified in 34 wheat genotypes. Optimization of more quality related genes in progress.

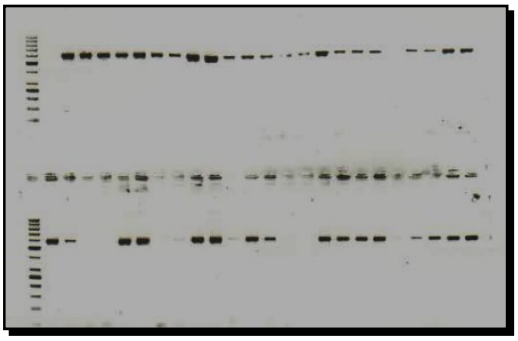


PCR amplification of Xgwm-537 quality primer

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.08 DNA markers linked to resistance genes for wheat rusts were surveyed to identify stem, yellow and leaf rust resistance genes on 50 wheat advance lines. Out of these 08 DNA markers, 05 DNA markers for Lr resistant genes, 03 for Sr resistant genes. Marker genes were validated on advance wheat lines. The genes Lr-19 was present

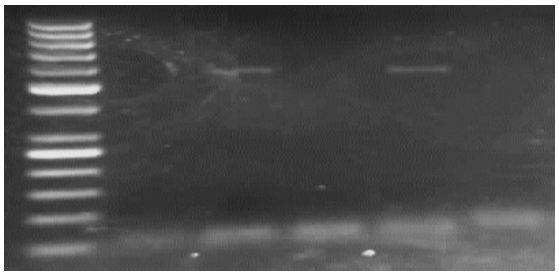
in 48 genotypes, Lr-28 in 47 genotypes, Lr-29 in 48 genotypes, Lr34/Yr18/Pm38 were present in 02 genotypes while 02 were heterozygous for this Lr-34, Lr46/Yr29/Pm39 in 47lines, Sr-2 in 47 genotypes, Sr-31 in 08 genotypes and Sr32 in 46 genotypes of wheat advanced lines of 2017.



PCR amplification of Lr-28 leaf rust primer

PCR based identification of Elsinoe

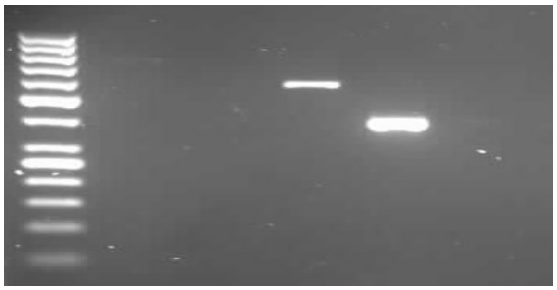
This research work was designed to standardize the PCR based protocol for the identification ofElsinoe, a casual fungus of citrus scab disease. For this purpose total genomic DNA was isolated from infected citrus fruits samples collected from Sargodha. The infected peel of fruit was frozen in liquid nitrogen and thoroughly grinded. DNA was isolated using CTAB protocol and used in PCR for the identification of Elsinoe. A Total 09primers pairs specific for Elsinoe were used in PCR to standardize the identification protocol. 02 primers i.e. EaNat1 & Eaut4 were successfully



PCR amplification of *Elsinoe*specific primers

PCR based identification of *Xanthomonas citri*

This research work was designed to standardize the PCR based protocol for the identification of *Xanthomonas citri*, a casual bacteria of citrus canker disease. Previously optimized primer i.e. Xac01/Xac02 was used to screen the about 50 samples collected from various citrus growing areas of Punjab. Total genomic DNA was isolated using CTAB method directly from infected leaves and used in PCR. Two new Xanthomonas genera specific primers i.e. XgumD & P16FR successfully optimized on DNA isolated from Xanthomonas infected citrus leaves.



PCR amplification of *Xanthomonas* genera specific primers

Characterization of maize genotypes for OPAQUE-2 gene

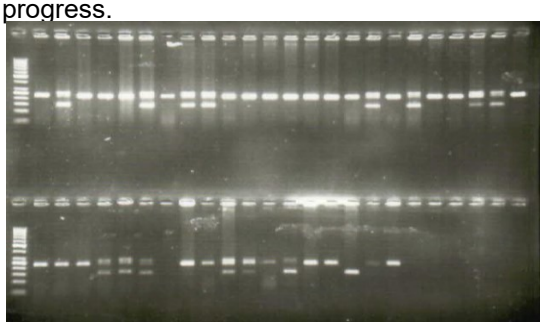
Screened maize lines for Quality Protein using molecular markers for higher tryptophan and lysine contents due to presence of opaque-2 gene.

Screened 260 maize genotypes for presence of Opaque-2 gene using linked DNA markers among which 13 were found positive and DNA was extracted second time from positive lines only for reconfirmation.

Rust resistance gene pyramiding in wheat using linked DNA markers

Rust resistance gene pyramiding in wheat using linked DNA markers

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. 38cross combination were selected from R-I (28), R-II (05), R-III (03) & R-IV (02) and screened against stem, yellow and leaf rust. 34 lines were positive for Lr-19 30 lines for Lr-28, 14 lines were heterozygous for Lr34/Yr18/Pm38, 37 lines for Lr46/Yr29/Pm39, 37 positive for Sr-2 and 30 lines were positive for Sr-32. Generation advancement for homozygous line sand development of more cross combination is in progress.



PCR amplification of Lr-34/Yr18/Pm38 rust primer

Identification of resistant genotypes for ascochyta blight in chickpea for marker-assisted selection.

The main objective of this experiment was to find out the resistant source against Ascochyta blight resistance in chickpea with the help of DNA markers for utilizing in the development of resistant varieties of chickpea. For achieving this objective, leaf samples of 50 chickpea genotypes were collected from Pulses Research Institute, Faisalabad and DNA was isolated by using modified CTAB method, After quantification of DNA with NanoDrop Spectrophotometer, 2 gene specific markers CaETR and SCY17 and 3 other Ascochyta blight disease resistant linked markers TA-34, TA-72 and TA-146 were surveyed on these chickpea genotypes and results are under process..

Application of molecular markers for the detection of resistant genotypes against tomato mosaic virus.

The objective of the experiment was to validate the DNA markers and detection of resistant source against Tomato mosaic virus with the help of linked DNA markers for utilizing in the development of resistant varieties. For the screening of tomato genotypes against mosaic virus, leaf samples of 50 tomato genotypes were collected from Vegetable Research Institute, Faisalabad and DNA was isolated by using modified CTAB method and quantification of DNA was carried out by using NanoDrop spectrophotometer. Two SSR markers, NCTm-019 and Tm2RSf2-r2 linked to resistance genes for TMV disease was successfully surveyed on all the

genotypes. Six genotypes showed resistance against TMV disease with these markers.

Validation of DNA Markers Linked to Traits that Confer Drought Tolerance in Wheat.

The objective of the experiment was to screen out the tolerant genotypes against wheat drought with the help of DNA markers, for utilizing in the development of drought resistant varieties. Leaf samples of 40 wheat genotypes were collected. DNA was extracted through CTAB method. 08 DNA markers linked to various traits of drought were surveyed on these 40 wheat genotypes. Three DNA markers (Pr-8, WMC-156 and KSUM-119) were found to be polymorphic, which differentiated tolerant, susceptible and heterozygous genotypes. Tolerant genes for Pr-8 (Flag Leaf senescence) were found to be present in 18 genotypes, for WMC-156 (leaf cuticular wax) in 21 genotypes and for KSUM-119 (Photosynthetic rate, Cell Membrane Stability (CMS) and relative water contents) were found in 22 wheat genotypes.

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 adapted cultivars or elite breeding material for crop improvement. Leaf samples of 200 maize inbred lines were collected from Maize and Milles Research Institute and Maize Research Station Faisalabad. 100 SSR markers were searched and got synthesized. DNA extraction of 200 leaf samples was completed and PCR was assembled using 02 SSR markers.

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

The objective of this experiment was to assess the drought tolerance status of maize genotypes with the help of SSR markers. Leaf samples of 200 maize inbred lines were collected from Maize and Milles Research Institute and Maize Research Station Faisalabad. 20 SSR markers were searched and got synthesized. DNA extraction of 200 leaf samples was completed and PCR was assembled using 05 SSR markers.

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. 200 SSR markers were selected with wide genome coverage or evenly distributed on chromosomes from genomic databases and got synthesized. PCR was assembled using 50 SSR markers.

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. 200 SSR markers were selected and got synthesized. PCR was assembled using 50 SSR markers.

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

The objective of this experiments covers the development of 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. 200 SSR markers were searched and got synthesized. DNA extraction of 08 leaf samples was completed and PCR was assembled using 50 SSR markers.

C. TISSUE CULTURE

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.

To develop drought tolerant genotypes of wheat through tissue culture

Immature embryos of four wheat varieties (AARI-11, Millet-11, Punjab-11, and Millet-11) were tested at two different levels of PEG containing MS media+ 2,4 D. Maximum callus (80%) was observed in Galaxy followed by Punjab-11 (78%), AARI-11(72) and (70%) recorded in Millet-11.

Effect of different media for callus induction and optimization of proficient regeneration system in rice

Dehusked seeds of six rice varieties (KS-282, PS-2, Basmati-385, Basmati 2000, Basmati-515 and Super Basmati) were cultured in two different levels of PEG (0.125 & 0.25mg/L) +2, 4-D (4 mg/L). At 0.125mg/L PEG level, maximum callus (88%) induced in coarse rice variety KSK-282 while minimum callus 60% observed in Super Basmati at 0.25% PEG. PS-2 formed maximum shoots in medium having MS +Kinetin (3mg/L BAP (1.5mg/L) + Sucrose (30g/L). Best rooting were observed at half MS medium. 131 soma clones were developed.

Use of Somaclonal variation for sugar cane improvement

The objective of this study was to develop the resistant somaclones 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 two sugarcane genotypes S-2006-SP-93 and S2008 AUS375 was develop on MS medium having 3mg 2, 4-D, sucrose 30g, pH 5.7 for mutation. Took the spindle portion of canes from sugarcane research institute, sterilize them with ethanol under aseptic conditions in air laminar flow. Inner most layer of leaves of spindle portion were used for Callogenesis. Culture was done and incubated for 14 days. Callus was shifted to regeneration medium. The plantlets shifted to rooting medium for root development. 240 plants of S2006-SP-93, 40 plants of S-2008-AUS- 375 were developed and planted in field for further studies.

Exploitation of somaclonal variation in wheat for yield and rust resistance.

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 two sugarcane genotypes S-2006-SP-93, S2008 AUS375 and SPF-234 were developed on MS medium having 3mg 2, 4-D, sucrose 30g, pH 5.7 for mutation. Took the spindle portion of canes from sugarcane research institute, sterilize them with ethanol under aseptic conditions in air laminar flow cabinet. Inner most layer of leaves of spindle portion were used for callogenesis. Culture was done and incubated for 14 days in dark after that check the callus and shifted to regeneration medium. The plantlets shifted to rooting medium for root development. 190 plants of SPF-234, 165 plants of S-2008-AUS- 375 were developed and planted in field for further studies.

Standardization of protocol for micropropogation in date palm

Suckers of Hilavi date palm variety removed from mother plant. Apical meristem were taken out from these suckers and shifted in citric acidascorbic acid or combination of both for different time intervals to control formation of phenolic compounds. Apical meristem were sterilized in different levels of clorex at different times intervals. Sterilized apical meri-stems were cultured in 21 different media and meristematic tissues of these apical meristem were also cultured in different levels of 2, 4 D for callogenesis. It was observed that date palm callus were only formed in medium having 5mg/L 2,4 D after four months sub culturing.

Maize mediated Doubled Haploid production in wheat

Four maize varieties were sown with one - week interval in tunnel to keep pollen available for pollination of wheat florets. Ninety F2 entries of wheat were sown in field. Three hundred ninety - five spikes of wheat were emasculated and pollinated with maize pollen. Two hundred thirty - seven immature embryos

were rescued under microscope. Immature embryos were cultured on half MS medium. Nine embryos regenerated and formed plantlets. Seven plants survived which were transferred on soil for normal growth.

D. FIELD TRIALS

Screening and selection of sugarcane somaclone 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 o SPF-234 and 125 plants of S-2008-US- 375 were developed and planted in field for evaluation for 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 (*Collitotrichum 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. 14 plants of S2006-SP-93 1plant ofS-2008-US- 375 were selected on the basis of red rot resistance. 17 plants of S-2006 SP-93 were selected out of 240 plants from R_1 generation and sown as R2 generation to asses red rot disease resistance in field. In R3 generation, 16 lines were planted, 6 lines of S-2006 SP-93, 2 lines of S-2007 AUS-375, one line of each S-2006 US-832, S-2006 US-665 and S-2006 AUS-628 showed resistant response were selected and planted.

Screening of wheat advance lines against rusts (leaf and yellow rust) at ABRI

To screen the wheat genotypes for rust reaction 38 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 nine lines morocco was also planted as a spreader. Field observation was recorded for rust initiation and development. Amongst thirty eight entries under studies 4 entries showed immune response to leaf rust whereas 11 entries showed resistant response to leaf rust was observed. 35 wheat entries showed immune response and one entry showed resistant response to yellow rust.

Somaclonal variation studies in wheat Regenerations (R_1 – R_6).

856 entries of different generations were sown in the field and out of these, 587 entries were selected on the basis of rusts (leaf and yellow) disease incidence.

Rust resistance in wheat advanced lines of A and B trial under natural conditions

42 promising wheat lines including two checks were screened against rusts (leaf and yellow). 23 were found immune and 10 resistant to leaf rust, but all the tested entries showed immune reaction towards the yellow rust.

Screening and generation advancement of wheat material at SARS Kaghan

Out of 511 sown at Kaghan, 300 wheat entries were found resistant and selected for further studies at Faisalabad

Screening of wheat germplasm against Karnal bunt

42 wheat entries were inoculated with karnal bunt inoculums at booting stage. No symptoms on twenty six entries appeared. However 0.5 to 7 percent bunted grains were recorded.

Exploitation of somaclonal variation in wheat for yield and rust resistance

Ten R2 crosses and four wheat varieties Galaxy-13, Ujala-16, FSD-08 and Seher-06 were used for embryo culture. 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, 16 wheat somaclones developed and transferred in pots

Regular wheat yield trial (B-Trial).

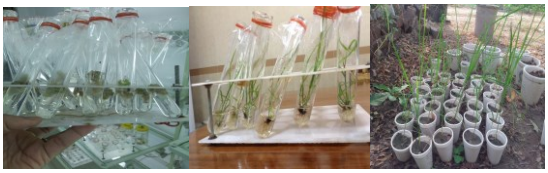
Fourteen entries including checks were sown in B trial for yield performance and results are waited.

E. 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 fairly showed enough callogenesis potential even at 150 and 200 m moles / L salt level.



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. Experiment is in progress.

Combined Efficiency of Acidified Compost and Microbial Inoculation in Pulses (Mung Beans)

This study was designed to manipulate pH of compost therefore to make it best use for high pH calcareous soil.

Compost was acidified using elemental sulfur @ 0, 1, 2, 3 and 4% w/w of compost. It was observed that on addition of sulfur, no significant change in pH was observed at all levels upto 4th week. At 4th week slight change in pH was observed at all levels i.e. control, 1, 2, 3 and 4%. Maximum change in pH was observed where sulfur was used @3% w/w. At the end of incubation study, pH of compost was decreased by 1.13, 1.45, 1.76, 3.3 and Zero units when elemental sulfur was used @ 0, 1, 2, 3, and 4% respectively. On the bases of this result elemental sulfur level 3% w/w was selected to acidified compost used for pot experiment.

In pot experiment, soil was mixed with sulfur affected compost @ 1% w/w. mung bean seeds were sown during autumn 2017. After seeding emergence, microbial inoculum was added to pots according to treatment plan. After 2 weeks of seedling establishment, thinning was done i.e. 3 plant per pot was maintained. On maturity, agronomic parameters i.e. shoot dry weight, root dry weight, 1000 grain weight and grain yield per pot was recorded.

Treatments	Root DW	Shoot DW	1000 GY	Grain Yield pot^{-1}
Control	1.23g	4.3f	127.67e	4.5f
S 0.4%	1.7e	5.47d	146.67cd	5.13de
S 0.45%	1.9d	5.8cd	151.33bcd	5.47cd
S 0.5%	2.2 c	6.2abc	162.33ab	6.1ab
Compost	1.46f	4.83e	139.00de	4.83ef
Comp+S 0.4%	2.1c	6.00bc	150.00bcd	5.65cd
Comp+S 0.45%	2.4b	6.3ab	159.67abc	5.9 bc
Comp+S 0.5%	2.6a	6.63a	167.67a	6.4a

Soil Bacteriology Section

Biofertilizer Testing Lab

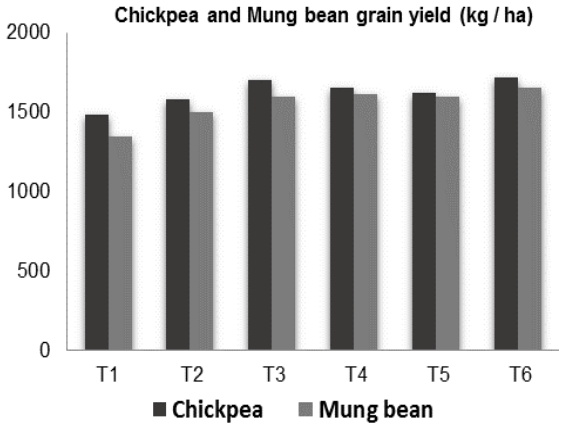
Soil Bacteriology as “Biofertilizer Testing Lab” analyzed 120 biofertilizer / biostimulant / BOP samples for registration or under FCO.

Biofert-plus: precursor enriched novel microbial formulations for legumes

Studies were conducted to test the precursor’s enriched inocula to improve the chickpea and mungbean growth and yields in a cost effective and sustained way. Precursor enriched inoculum was applied at sowing. Field and pot studies were conducted at Pulses Research Institute (PRI) and Soil Bacteriology Section, Faisalabad. Treatment includes control, *Rhizobium* inoculation; *Rhizobium* enriched L-tryptophan, L-adenine, Tryptamine and GA_3.

Results of chickpea and mungbean field trials at PRI, Faisalabad revealed that *Rhizobium* inoculum enriched with GA_3 produced the highest chickpea and mungbean grain yield i.e. 1720 and 1650 kg ha^{-1}, as compared to control 1480, 1350 kg ha^{-1}, respectively. Results of nodulation in chickpea and mungbean field trials at PRI, Faisalabad

revealed that *Rhizobium* inoculum enriched with GA_3 produced higher nodules plant^{-1}, nodular dry mass as compared to control.



Grain yield of chickpea and mungbean at PRI, Faisalabad.

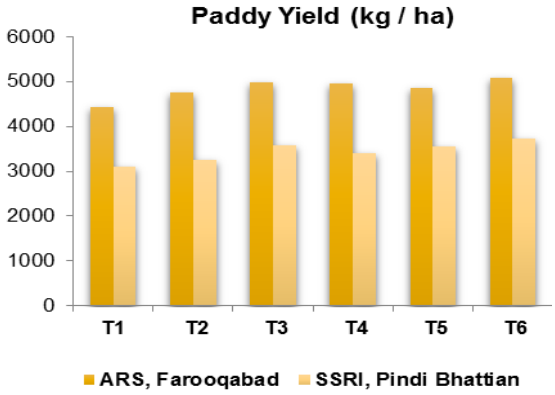
Biofert-plus: precursor enriched novel microbial formulations for cereals

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 GA_3.

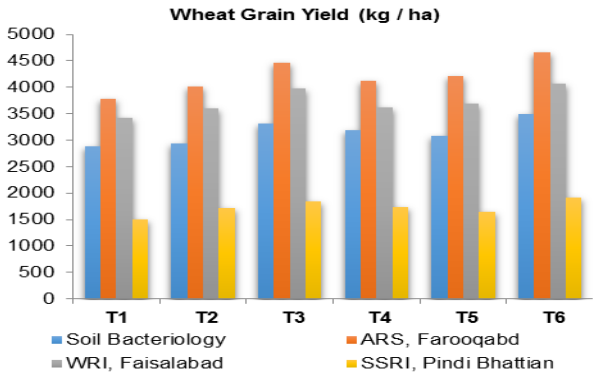
Results of rice trials at ARS, Farooqabad and SSRI, Pindi Bhattian revealed that PGPR enriched with GA_3 produced the highest paddy yield i.e. 5073, 3720 as compared to control 4433, 3107 kg ha^{-1}, respectively.

Results of wheat trials at Soil Bacteriology, WRI Faisalabad, ARS, Farooqabad and SSRI, Pindi Bhattian revealed that PGPR enriched with GA_3 produced the highest grain yields i.e. 3487, 4657, 4073 and 1920 kg ha^{-1} as compared to the rest of the treatments.

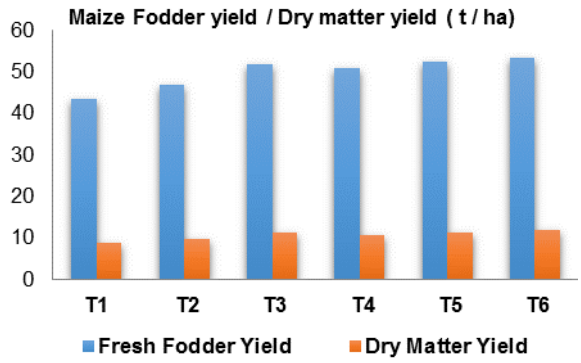
Results of maize trial at Soil Bacteriology Section revealed that PGPR enriched with GA_3 produced the highest maize fodder yield, dry matter yield i.e. 53.3, 11.82 as compared to control i.e. 43.3 and 8.71 t ha^{-1}, respectively.



Paddy yield at ARS, Farooqabad and SSRI, Pindi Bhattian.



Wheat grain yield at multi-locational trial.



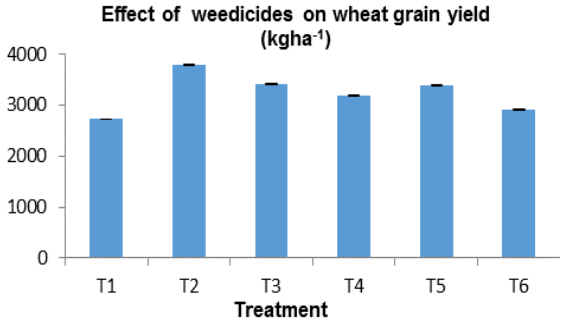
Maize fodder and dry matter yield at Soil Bacteriology Section.

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 eight hundred 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 10^7 per gram of soil. Measurable reduction in total organic carbon (TOC) because of residue burning was also observed. On an average at all locations 35-45% reduction in microbial count and TOC / organic matter content was observed.

Impact of various weedicides on soil microflora of wheat crop

Field study was conducted to evaluate the adverse effects of commonly used weedicides on useful soil microbes and their activities in wheat. Study was conducted at Soil Bacteriology Section, Faisalabad using normal soil having pH 7.8, ECe 1.2, Organic matter 0.69% and available P 8.3 mg kg^{-1}. Treatments were control (T_1), Atlantis (T_2), Affinity (T_3), Sulfan (T_4), Buctril Super (T_5) and Axial (T_6). Crop was sown during Rabi 2016-17. Recommended dose of fertilizer @ 160-114-60 kg NPK ha^{-1} was applied. Parameters recorded were plant height, grain yield, no. of tillers $plant^{-1}$, biomass, microbial count, total organic carbon etc. Results showed that maximum yield was obtained from Atlantis treatment i.e. 3787 as compared to control i.e. 2721 kg ha^{-1}.

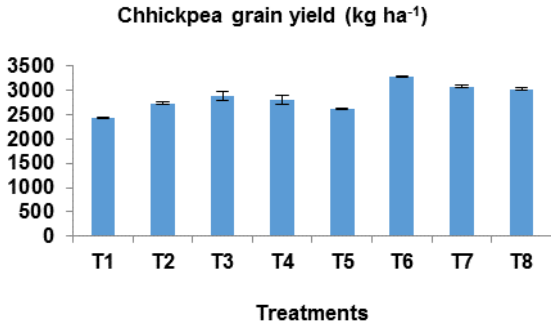


Wheat grain yield as affected by various weedicides.

Influence of L- methionine and rhizobium sp on chickpea

Field study was conducted to test the precursor L-methionine and *Rhizobium* effect to improve the chickpea growth and yield at Pulse Research Institute, AARI, Faisalabad by using normal soil having pH 7.7, ECe 2.2, Organic matter 0.68% and available P 8.1 mg kg^{-1}. Treatments were control (T_1), *Rhizobium* Inoculation (T_2), L-Methionine (L-MET) @

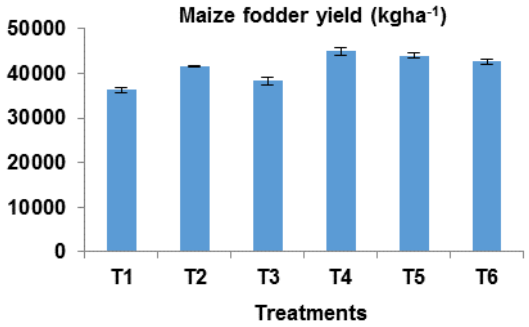
5mg L^{-1} (T_3), L-MET@ 10 mg L^{-1} (T_4): L-MET @ 15 mg L^{-1} (T_5) *Rhizobium* Inoculum+ L-MET @ 5 mg L^{-1} (T_6) *Rhizobium* Inoculum + L-MET @ 10mg L^{-1} (T_7) *Rhizobium* Inoculum + L-MET @ 15 mg L^{-1} (T_8). Recommended dose of fertilizer @ 30-60 kg NP ha^{-1} was applied. Parameters recorded were plant height, grain yield, no of nodules $plant^{-1}$ etc. Results showed that maximum grain yield (3283 kg ha^{-1}) was obtained from T_6 treatment as compared to control (2440 kg ha^{-1}).



Chickpea grain yield as affected by Rhizobium and L-Methionine.

Impact of various weedicides on soil microflora of maize crop

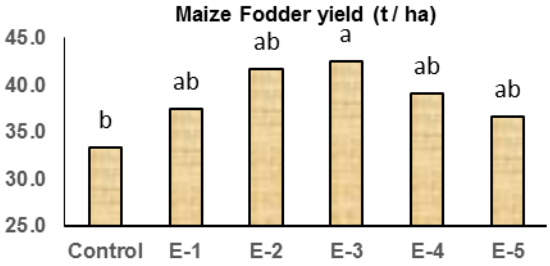
Field experiment was conducted at Soil Bacteriology Section, AARI, Faisalabad to evaluate the adverse effects of commonly used weedicides on useful soil microbes and their activities in maize by using normal soil having pH 7.6, EC 2.1, Organic matter 0.69% and available P 8.3 $mgkg^{-1}$. Treatments were control (T_1), Primextra gold 720 SC (T_2), Fallisto gold (T_3), Atrazine (T_4), Primextra gold (T_5), Dual gold 960 EC (T_6). Recommended dose of fertilizer @ 100-60 kg NP kg ha^{-1} was applied. Parameters recorded were plant height, fodder yield, microbial count, total organic carbon etc. Results showed that maximum fodder yield was obtained from T_4 treatment (45000 kg ha^{-1}) as compared to control (36333 kg ha^{-1}).



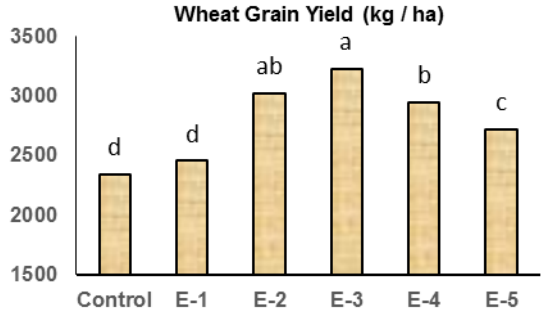
Maize fodder yield as affected by various weedicides.

Potential of endophytic bacteria for wheat and maize growth

Endophytic bacteria are generally colonized within plant tissues showing no external sign of infection or harmful effect on their host. 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 7.88, ECe 2.1 d Sm^{-1} and organic matter 0.69. Treatments were control, and five endophytes as E-1, E-2, E-3, E-4 and E-5. Recommended dose of fertilizer (NP @ 100:60 & NPK @ 120:114:90 kg ha^{-1}) for maize and wheat crop was applied. Parameters recorded were plant height, fodder yield, grain yield, fresh and dry mass, weight per plant and no. of tillers. Result showed that inoculation with bacterial isolates showed significant increase in yield as compared to control. Endophytic bacteria E-3 produced maximum maize fodder yield and wheat grain yield i.e. 42.5 t ha^{-1} , 3227 kg ha^{-1} and it was significantly different from control.



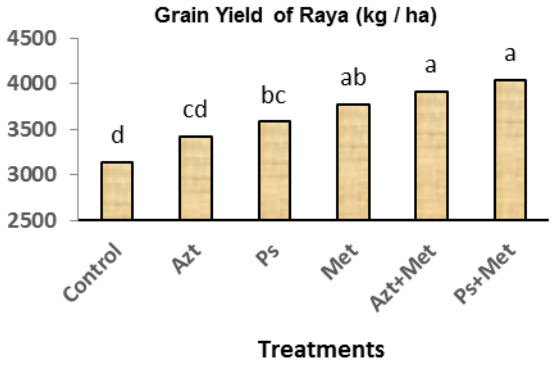
Effect of Endophytic isolates on fodder yield of maize



Effect of Endophytic isolates on wheat grain yield.

Growth and yield response of Raya to PGPR and 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. PGPR belong to the most important and agronomically useful soil micro-biota that involves free-living bacteria. Field study was conducted at the Agri. Biotechnology Research Institute, AARI Faisalabad using normal soil having pH 8.01, ECe 2.3 d Sm^{-1} and organic matter 0.72. Treatments were control (T_1), PGPR - Azotobacter (T_2), PGPR-Pseudomonas (T_3), Metabolite spray (T_4), Metabolite spray + PGPR -Azotobacter (T_5), Metabolite spray + PGPR -Pseudomonas (T_6). Crop was sown during Kharif 2017. Recommended dose of fertilizer was applied. Parameters recorded were plant height, yield, fresh and dry mass and no. of pods per plant. Result showed that inoculation with bacterial isolates along with metabolite spray showed significant increase in yield as compared to control. The treatment T_6 produced maximum yield (4036 kg / ha) and it was significantly different from control.

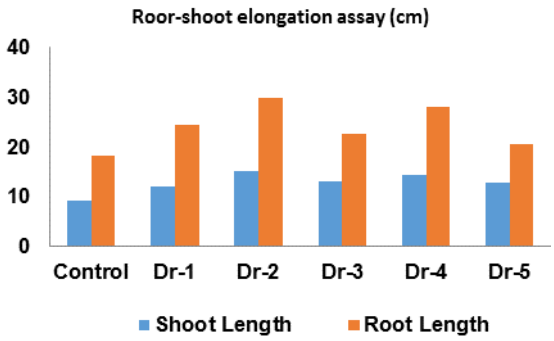


Effect of PGPR and their metabolites on Raya grain yield.

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

The main objective of the present study was to isolate and screen drought tolerant PGPR from drought prone areas and then check their effect for growth promotion of maize along with control condition. Root and rhizosphere soil samples were collected from drought prone areas of Punjab (Layyah, Multan, Khanewal etc.). Isolation was carried out on *Bacillus* media by using dilution plate technique after giving heat shock on 80°C for ten minutes. The isolates were further purified by repeated streaking. Five strains of drought resistance microbes such as Bacillus species were tested at the Soil bacteriology Section, ABRI, AARI, Faisalabad on the basis of growth promotion activities. The shoot length was increased upon inoculation over control

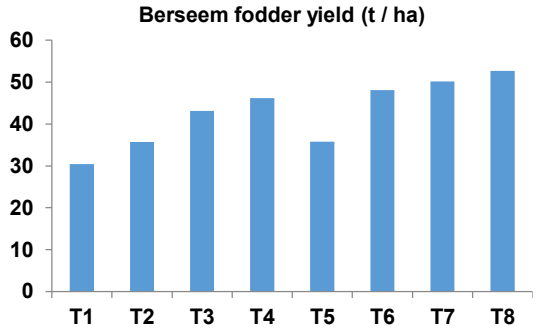
and maximum shoot length was observed in case of Dr-2 inoculation. The same trend was observed in case of root length and root, shoot mass where significant improvement was recorded over control. The maximum shoot mass was observed in Dr-2.



Root-shoot elongation assay of drought tolerant isolates.

Plant microbe interaction for the growth of berseem fodder

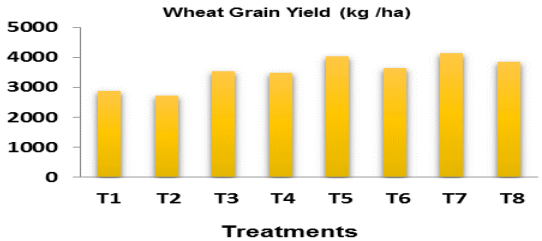
The mechanisms of PGPR include regulating hormonal and nutritional balance, inducing resistance against plant pathogens, and solubilizing nutrients for easy uptake by plants. Field trail was conducted on berseem fodder at the Fodder Sub Station, Faisalabad to check the combine effect of Rhizobium, PGPR and Tryptamine on yield of berseem. 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 and Co-Inoculation + Tryptamine @ 10^{-5} M (foliar spray) T_8.Crop was sown during Rabi 2017-18. Recommended dose of fertilizer was applied. Result showed that Co-inoculation of Rhizobium + PGPR with Tryptamine @ 10^{-5} M (foliar spray) showed significant increase in yield as compared to control and other treatments. This same treatment (T_8) produced maximum grain yield and it was significantly different from control.



Effect of treatments on berseem fodder yield.

Integrated use of mineral, organic and biofertilizer on wheat yield

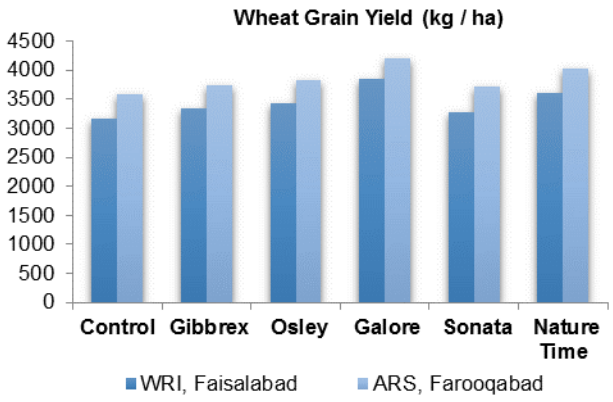
Field study was conducted at the Soil Bacteriology Section, Faisalabad to evaluate the effect of integrated application of inorganic/organic (biogas slurry) and PGPR on maize fodder yield in field conditions. Trial was carried out in sandy clay loam soil having pH 8.1, EC 1.49 dS m^{-1}, Olsen P 7.5 mg kg^{-1} and organic matter 0.90 %. Treatments include 100 and 75% N of recommended dose with and without PGPR inoculation and N was applied with and without biogas slurry @ 600 kg ha^{-1}. Results showed that maximum wheat grain yield i.e. 4135 kg ha^{-1} was observed when 100% N was applied along with biogas slurry coupled with microbial inoculation.



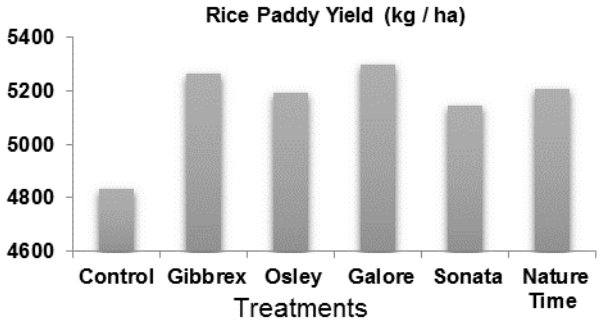
Effect of treatments on wheat grain yield.

Wheat response to biostimulants under field conditions

Studies were conducted to assess the role of different biostimulants on growth and yield of wheat at Wheat Research Institute (WRI), Faisalabad and ARS, Farooqabad following RCBD and rice yield at ARS, Farooqabad. 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. Results revealed that highest grain yield at WRI, Faisalabad and ARS, Farooqabad was obtained i.e. 3846, 4203 kg ha^{-1} with Galore followed by 3616, 4023 kg ha^{-1} with Nature Time as compared to 3173, 3573 kg ha^{-1} of control, respectively (Fig. 14). The highest paddy yield at ARS, Farooqabad was obtained with Galore 5300 kg ha^{-1} followed by Gibbrex i.e. 5266 as compared to control 4833 kg ha^{-1}.



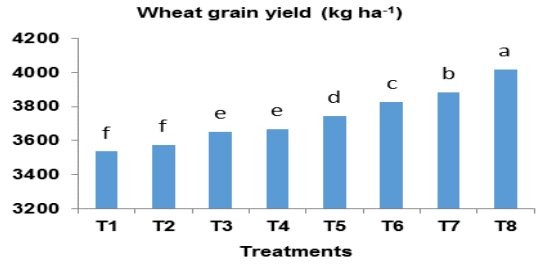
Effect of different biostimulants on wheat grain yield.



Effect of different biostimulants on wheat grain yield.

Effect of PGPR on biofortification of zinc in wheat

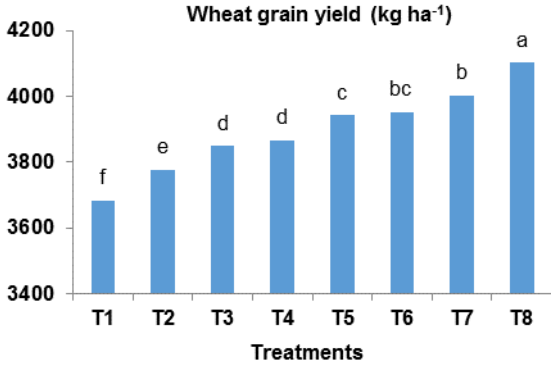
Field experiment was conducted at the Soil Bacteriology Section AARI, Faisalabad to investigate the efficiency of PGPR (Zn-mobilizing) on biofortification of Zinc in wheat. Trial was carried out in sandy clay loam soil having pH 8.5, EC 2.4 dS m^{-1}, available P 6.7 mg kg^{-1} and organic matter 0.86 %. Recommended dose of fertilizer @ 160-114-60 kg NPK ha^{-1} was applied to all treatments. Treatments were control (T_1), PGPR inoculation (T_2), 2.5 kg ha^{-1} $ZnSO_4.7H_2O$ (T_3), 5.0 kg ha^{-1} $ZnSO_4.7H_2O$ (T_4), 7.5 kg ha^{-1} $ZnSO_4.7H_2O$ (T_5), T_3 + PGPR Inoculation (T_6), T_4 + PGPR Inoculation (T_7), T_5 + PGPR Inoculation (T_8). Results clearly indicated that PGPR in combination with 5.0 kg ha^{-1} $ZnSO_4.7H_2O$ gave highest grain yield (4016.7) as compare to control (3536.7 kg ha^{-1}).



Effect of biofortification of zinc and PGPR on wheat grain yield.

Growth and yield response of wheat to different levels of kinetin

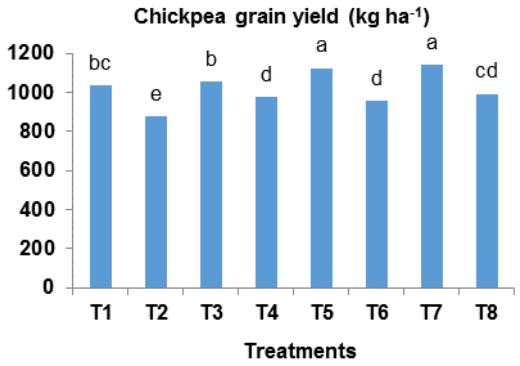
Field experiment was conducted at the Soil Bacteriology Section AARI, Faisalabad to check the best levels of kinetin along with PGPR to improve growth and yield of wheat crop. Trial was carried out in sandy clay loam soil having pH: 8.0, EC: 2.5dS m^{-1}, available P: 7.0 mg kg^{-1} and organic matter 0.76 %. Recommended dose of fertilizer @ 160-114-60 kg NPK ha^{-1} was applied to all treatments. Treatments were control (T_1), PGPR Inoculation (T_2), Kinetin @ 10^{-3} M (T_3), T_4: Kinetin @ 10^{-4}M (T_4), Kinetin @ 10^{-5}M (T_5), T_6: PGPR + Kinetin @ 10^{-3} M (T_6), T_7: PGPR + Kinetin @ 10^{-4} M (T_7), PGPR + Kinetin @ 10^{-5} M (T_8). Results clearly indicated that PGPR in combination with Kinetin @ 10^{-5} M give highest grain yield (4103.3 kg ha^{-1}) as compare to control (3683.3 kg ha^{-1}).



Effect of different levels of kinetin on wheat grain yield.

Biocontrol of fusarium wilt in chickpea

Field experiment was conducted at the Pulses Research Institute, AARI, Faisalabad to check the antifungal aptitude of microbes against *fusarium* wilt and enhancing the yield of chickpea in diseased soil. Trial was carried out in normal and *fusarium* sick soil having texture sandy clay loam, pH 8.5, EC 2.5 dS m^{-1}, available P 7.6 mg kg^{-1} and organic matter 0.76 %. Recommended dose of fertilizer @ 30-60 kg NP ha^{-1} was applied to all treatments. Treatments were control in normal soil (T_1), control in *fusarium* infested soil (T_2), T_1+ *Rhizobium* inoculation (T_3), T_2 + *Rhizobium* inoculation (T_4), T_1+ PGPR (T_5), T_2+ PGPR (T_6), T_1 + co-inoculation (T_7), T_2 + co-inoculation (T_8). Results clearly indicate that co-inoculation give highest grain yield in normal soil (1140 kg ha^{-1}) as well as in sick soil (990 kg ha^{-1}) as compare to their respective control (1033.33 kg $ha^{-1}$and 876.67 kg ha^{-1}).

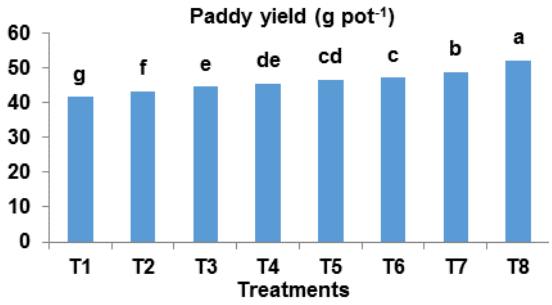


Impact of Biocontrol of fusarium wilt on chickpea.

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 8.1, EC 2.4 dS m^{-1}, available P 7.8 mg kg^{-1} and organic matter 0.77 %. Recommended dose of fertilizer @ 120-100-70 kg NPK ha^{-1} was applied to all treatments. Treatments include Control, PGPR inoculation, 5, 10, 15

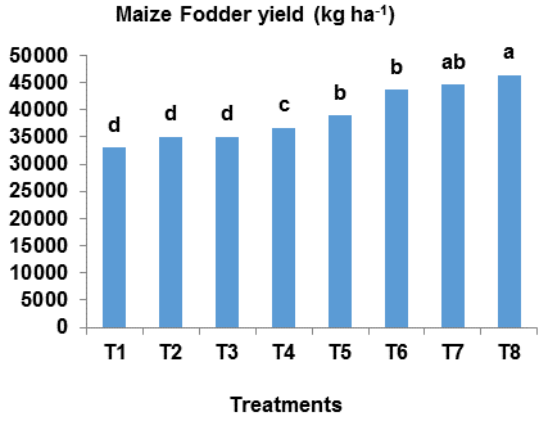
kg ha^{-1} $ZnSO_4$, PGPR Inoculation + 5, 10, 15 kg ha^{-1} $ZnSO_4$. Results indicated that PGPR along with 15 kg ha^{-1} $ZnSO_4.7H_2O$ significantly improved grain yield (52.17 g pot^{-1}) as compared with its respective control (41.67 g pot^{-1}).



Effect of biofortification of zinc and PGPR on paddy yield of rice.

Integrated use of mineral, organic and biofertilizer on maize fodder

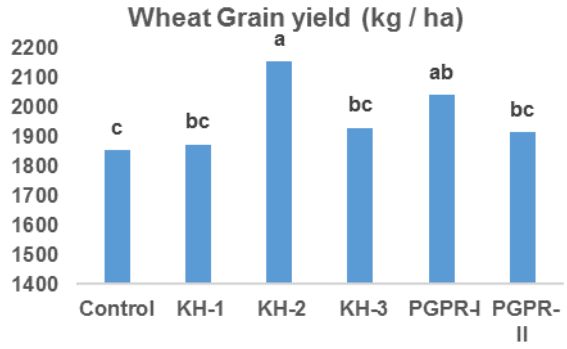
Field study was conducted at the Soil Bacteriology Section AARI, Faisalabad to evaluate the effect of integrated application of inorganic/organic (bio slurry) and PGPR on maize fodder yield in field conditions. Trial was carried out in sandy clay loam soil having pH: 8.1, EC: 2.89 dS m^{-1}, Olsen P: 7.5 mg kg^{-1} and organic matter 0.90 %. Treatments were 100% N (T_1), 75% N of Recommended dose (T_2), T_1 + PGPR Inoculation (T_3), T_2+ PGPR Inoculation (T_4), T_1+ biogas slurry (600 kg ha^{-1}) (T_5), T_2+ biogas slurry (T_6), T_3+ biogas slurry (T_7), (T_8) T_4+ biogas slurry (T_8). Recommended dose of fertilizer for maize fodder 100-60 NP kg ha^{-1} were applied. Results showed that maximum fodder yield (46333 kg ha^{-1}) was observed when 75% N was applied along with biogas slurry as compared to alone 100% N (36000 kgha^{-1}).



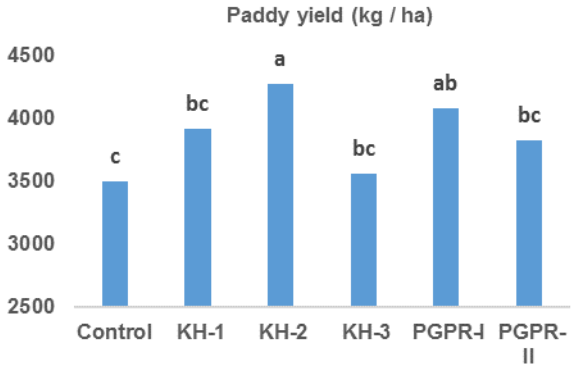
Effect of organic, inorganic fertilizer and PGPR on Fodder yield kg ha^{-1} of maize

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

Salt-tolerant plant growth-promoting rhizobacteria significantly influence the growth and yield of wheat crops in saline soil. Soil salinity causes plant stress in two ways: (1) making water uptake by the roots more difficult, and (2) causing plant toxicity via accumulation of high salt concentrations in the plant. Field study was conducted at the SSRI Pindi Bhattian using normal soil having pH 7.84, EC 6.4 dS m-1 and SAR 30. Treatments were control (T1), Salt tolerant isolate KH-1 (T2), Salt tolerant isolate KH-2 (T3), Salt tolerant isolate KH-3 (T4), PGPR-I (T5), PGPR-II (T6). Recommended dose of fertilizer according to rice and wheat 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 strain KH-2 produced maximum grain yield in wheat (2153 kg ha-1) and rice (4267 kg ha-1) followed by PGPR-Azotobacter and it was statistically significant from control.



Effect of salt tolerant bacteria and PGPR on wheat grain yield.



Effect of salt tolerant bacteria and PGPR on paddy yield.

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- Ahmed M, Shah AD, Rauf M, Habib I, Shehzad K. (2017) Ectopic Expression of the *Leptochloa fusca* and *Allium cepa* Lectin Genes in Tobacco Plant for Resistance against Mealybug (*Phenococcus solenopsis*). J Genetics Genomes 1(2): 108.
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- Nazir, S. and Khan, M.S., 2018. Microscopic detection of chloroplast transgenic plastids using fluorescent probe. *Advancements in Life Sciences*, *5*(3), pp.121-129.
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Miscellaneous:

1.	Radio Talks Delivered	04
2.	TV Talk	01
3.	Visits of Delegations	30
4.	English Articles in Magazines	20
5.	Training Seminars Delivered	08
6.	Class visits	20
7.	Conference Abstracts	10
8.	MPhil/PhD Students Supervised	05
9.	Training Imparted to Scientist	330
10.	Foreign Tours of Scientists	02
11.	PARB Projects	03
12.	Internship Students	100
13.	Sample analyzed	500
14.	Income Generation	1.8 M

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