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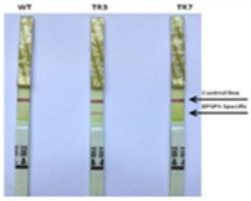
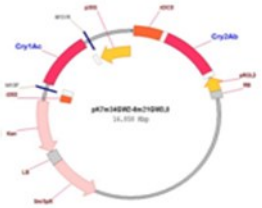
OVERVIEW

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 (2016-17), 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.

Agrobacterium inoculation With synthetic insect resistance genes

Inoculated developed bolls

Boll to row progenies grown in filed

Survived plants after spray with Roundup Ready

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.

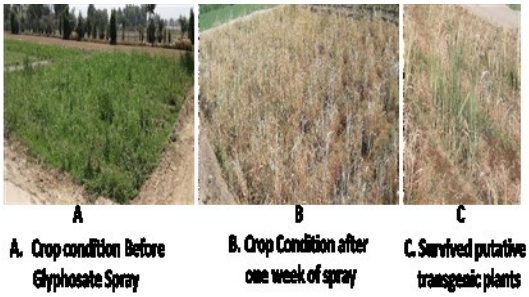
8000, four days old cotyledonary leaf petioles were inoculated with Agrobacterium strain LBA4404 having modified GT gene with selection marker. 2500 antibiotic resistant shoots were screened on selection media containing respective antibiotic (PPT @ 3mg/L & Kanamycine @ 50 mg/L. 500 putative antibiotic resistant plantlets were survived on regeneration and rooting media and 80 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. 230 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 40 days on regular basis and 2 herbicide tolerant plants were observed

and their confirmation via PCR was under process.



Integration of modified GT gene in wheat.

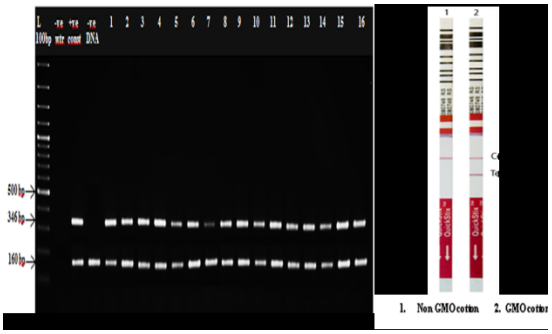
Experiment on integration of modified Glyphosate Tolerance (GT) gene in wheat varieties viz. Galaxy-2013, Ujala-2016 and Faisalabad-2008 was planned for the development of roundup herbicide resistance for the effective weeds control. Approximately 1500 immature embryos of wheat were excised and inoculated 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.

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: 72 entries of NCVT, received from PCCC, Multan and 5 plants from each entry were tested for the presence of Cry1Ac, Cry2Ab and RR genes. 56 entries were found positive for Cry1Ac and MON531 event.

On demand testing: A total of 236 samples received from public and private sectors were tested for Bt elements. Two samples received from private sector were positive for Cry1Ac. From public sector 234 samples were assayed and 202 samples were found positive for Cry1Ac.



Strip test and PCR results for GMO testing

Lab accreditation under ISO-17025

The objective of this work was to accreditation of the GMO testing activities. Lab successfully participated in international Proficiency Testing (PT) & Inter-Lab Comparison (ILC) programs with 100% success. Pre-assessment was done by an assessor from

PNAC. Documentation has been updated and revised in the light of observations and NCs raised during pre-assessment. Application for full assessment & certification has been sent to PNAC. Data recording and documentation as per instructions of ISO-17025 is continued.

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

Assessed 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)

a. Nitrogen nutrition: (Continues cotton)

	Cry1ac (ug/g of fresh sample weight)					
N applied Kg/ha	Leaf (Top)	Leaf (Middel)	Leaf (Bottom)	Square	Boll (10 days old)	Boll (15 days old)
0	2.6	2.1	0.00	2.5	0.8	0.25
100	3.9	3.3	0.3	3.8	1.75	0.60
200	4.5	3.8	0.35	4.2	1.90	0.56

b. Nitrogen nutrition: (Cereal rotation)

	Cry1ac (ug/g of fresh sample weight)					
N applied Kg/ha	Leaf (Top)	Leaf (Middel)	Leaf (Bottom)	Square	Boll (10 days old)	Boll (15 days old)
0	0.56	0.2	0.00	1.3	0.25	0.03
100	1.83	1.5	0.25	2.0	0.9	0.00
200	1.90	1.92	0.30	2.5	1.2	0.18

c. Potassium nutrition:

Potassium application did not significantly affected Cry1Ac protein expression.

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. Sixty wheat genotypes were used for identification of 2 optimized HMW-GS linked makers. Marker Dx2, Dx5 was present in 52 genotypes while By8 was found in 05 genotypes. New primers for Zinc and Iron have been identified and will be used in future work.

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. Forty five entries from CB (08), R_1 (10), R_2 (12), R_3 (11) & R_4 (04) were screened against Lr resistant markers. Seven cross combinations

were found positive for Lr-34/Yr-18, Lr-46/Yr-29 & Lr-19. 09 crosses were positive for Lr-46/Yr-29 & Lr-19. 05 cross was positive for Lr-34/Yr-18 & Lr-19.

PCR based identification of sugarcane white leaf disease (SCWL)

The objective of this work was to standardize the protocol for the identification & confirmation of Phytoplasma responsible for SCWL disease through PCR. 06 sugarcane leaf samples were tested (04 from Habib Sugar Mills Limited, Nawab Shah & 02 from Mirpur Khas Sugar Mills, Sindh). Total genomic DNA was isolated using CTAB methods & DNA integrity was verified using universal primers for sugarcane. Phytoplasma specific primers were used in PCR but no amplification was detected. Isolated DNA was also got verified from Plant Pathology Division, NIAB, but the presence of disease was not confirmed. It was concluded that leaf samples were not infected with Phytoplasma.

PCR based identification of *Xanthomonas citri*

This research work was designed to standardize the PCR based protocol for the identification of *Xanthomonas citri.* 06 *Xanthomonas* infected leaves (02 from each Grapefruit, Lemon and Kinnow) and 01 healthy leaf were collected from nursery area of HRI, Faisalabad. Total genomic DNA was isolated using CTAB methods & used in PCR to standardize the *Xanthomonas* detection protocol using 04 different primer pairs. Primer Xac01/Xac02 was successfully optimize with expected fragment size with no amplification in healthy leaf sample. The amplified product was got sequenced which also confirmed the presence of *Xanthomonas citri* on citrus plants. The optimized primer was also tested on isolated DNA from purified culture of *Xanthomonas* and leaf samples collected from different locations.

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.

In the year 2016-17 screened a sum total of 260 maize line among which 13 were found to contain Opaque-2 gene.

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

The objective of the 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. For the identification of resistant source, 40 chickpea genotypes were collected from Pulses Research Institute, Faisalabad and DNA was isolated by using modified CTAB method, while DNA was quantified by using Nano Drop spectrophotometer. 3 RAPD markers (OPAC04, OPM02 and OPAI09) linked to resistance genes for Ascochyta blight disease were successfully surveyed on all genotypes. None of genotypes showed resistance against Ascochyta blight disease with all 3 RAPD primers.

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

The objective of the experiment was to find out the resistant genotypes against tomato mosaic virus in tomato with the help of DNA markers, for utilizing in the development of resistant varieties. For determining the resistant genotypes, 40 tomato genotypes were collected from Vegetable Research Institute, Faisalabad and DNA was isolated by using modified CTAB method, while DNA was quantified by using NanoDrop Spectrophotometer. One SSR marker ($Tm2RSf_2$-r_2) linked to resistance genes for TMV disease was validated during the previous year and it was successfully surveyed on all the genotypes. None of genotypes showed resistance against TMV disease for this primer.

Validation of Identified DNA Markers against Mungbean Yellow Mosaic Virus (MYMV) Disease in Mungbean [*Vignam Radiata (L.)*

The objective of the experiment was to verify the identified DNA markers for resistance against yellow mosaic virus (MYMV) disease for utilizing in the development of resistant varieties. For tagging the resistant genotypes, 45 mungbean genotypes were collected from Pulses Research Institute, Faisalabad and DNA was isolated by using modified CTAB method, while DNA was quantified by using NanoDrop Spectrophotometer. Two SSR marker linked to resistance genes for MYMV disease were assayed. None of genotypes showed resistance against TMV disease for this primer.

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

The objective of the experiment was to find drought related genes in spring wheat genotypes. For the identification of drought tolerant genotypes, leaves of 70 wheat genotypes were collected from Wheat Research Institute, Faisalabad and DNA was isolated by using modified CTAB method, while DNA was quantified by using Nano Drop spectrophotometer. Eight DNA markers linked to various traits of drought were surveyed on these wheat genotypes.

Five DNA markers (Pr8, Pr-9, WMC-156, XGWM-566 and KSUM-119) were found to be polymorphic, which differentiated Tolerant, susceptible and heterozygous genotypes.

Validation of identified DNA markers against sunflower head rot (Sclerotinia sclerotium LIB) disease in sunflower.

The objective of the experiment was to find out the resistant genotypes against sunflower head rot in Sunflower with the help of DNA markers, for utilizing in the development of resistant varieties. For this purpose, 22 sunflower genotypes were collected from Oilseed Research Institute, Faisalabad and DNA was isolated by using modified CTAB method, while DNA was quantified by using Gene Quant. One SSR marker (Ga5 & E10J20b) linked to resistance genes for SHR was validated and successfully surveyed on all 22 genotypes. None of these genotypes showed resistance against Sunflower Head Rot disease for this primer.

Validation of molecular markers for the screening of Hot Pepper lines against CLCuV disease

The objective of the experiment was detection of resistant source against Chilies leaf curl virus disease with the help of DNA markers for utilizing in the development of resistant varieties with the help of DNA markers. For determining the resistant genotypes against CLCuV disease, 45 hot pepper genotypes were collected from Vegetable Research Institute, Faisalabad and DNA was isolated by using modified CTAB method, while DNA was quantified by using NanoDrop Spectrophotometer. Five DNA markers were applied and two DNA Markers for resistance to CLCuV disease were validated.

Identification of QTLs for drought tolerance in wheat

The objective of this study was to determine the genes controlling the phenotype of interest by QTL analysis and to resolve the population structure and relatedness of wheat germplasm with genome-wide SSRs. Two contrasting parents (Chakwal 50 and Sehar-06) against drought were crossed and F_0 seed was collected. Data of morphological and drought related parameters (Flag Leaf senescence, leaf cuticular wax, Photosynthetic rate, Cell Membrane Stability (CMS) and relative water contents etc) was collected. Fifteen SSR primers were optimized through Gradient PCRs.

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 0, 30, 60 and 120 min. Screened EMS treated calli on selection media supplemented with different levels (0, 10 and 15 %) of PEG.

Regeneration percentage of calli was 62, 50 & 22 percent at 30, 60 and 120 minutes treatment of EMS (0.5%) respectively and percentage of plantlets developed at PEG levels 5, 10 & 15 % were 60, 50 & 20 percent respectively.

To develop drought tolerant genotypes of wheat through tissue culture

Immature embryos of four wheat varieties (Millet-11, Punjab-11, AARI-11 and Millet-11) were surface sterilized with 25% Sodium Hypochlorite followed by 70% ethanol and then rinsed three times with sterile distilled water. Immature embryos were aseptically excised from the caryopsis and placed with the scutellum in test tubes containing MS medium with different levels of 2, 4-D (2 and 3ml/L). The cultured tubes were placed in a growth chamber in the dark for 21 days for callus induction. Maximum callus (92%) was observed in Punjab-11and AARI-11 followed by Galaxy (90%) through immature embryo culture. The induced callus was transferred in PEG medium for four days and then shifted at shooting medium. Punjab-11 formed maximum and earlier shoots when callus was treated @ 2.5mg/L PEG. Best shooting was observed at medium MS having Kinatine (1mg/L) + BAP (1.50 mg/l). The rooting was found best at 1/2MS medium. 11 and 9 plants of Punjab-11 and AARI-11 respectively, were

selected on morphological and disease basis sown in field under water stress condition.

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

Dehusked seeds of five rice varieties (KS-282, PS-2, PK-386, Basmati-515 and Super Basmati) were surface sterilized in 20% clorex for 15 minutes followed by 70% ethanol and then rinsed four times in sterile distilled water in a laminar flow cabinet. Sterilized seeds were cultured in MS medium supplemented with different levels of 2, 4-D (2, 3 and 4 ml/L) and placed in a growth chamber under dark period for 21 days for callus induction. PS-2 induced maximum callus 97% followed by KS-282 (92%) at 2, 4-D (3mg/L) while super Basmati (90%) and PK-386 (86%) @ 4mg/L. Maximum shoots were observed in MS medium supplemented with Kinetin (3mg/L)+ BAP (1mg/L) **+** Sucrose (30g/L). Best rooting were observed at half MS medium. About 63 somaclones of PK-386 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 the study was development of high yielding and disease free somaclones of wheat through callus cultures derived from immature /mature embryo. For this purpose 10 F_2 crosses and two wheat varieties Fsd-08 and Seher-06 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, somaclones were transferred to pots. This year sixteen somaclones of different crosses and varieties were shifted to pots and spikes of developed somaclones have been sown in field as R_1.

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. Two hundred forty plants of S2006-SP-93 and 40 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 and 1 plant of S-2008-US- 375 were selected on the basis of red rot resistance.

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 augmented design. When crop was at tillering stage rust inoculums of leaf and yellow rusts were sprayed on the trial and rusted leaves were also rubbed on each entry. After 10th lines morocco was also planted as a spreader. Field observation was recorded for rust initiation and development. Amongst thirty eight entries under studies 9 and 34 entries showed immune response to leaf and yellow rusts whereas on 12 and 2 entries moderately resistant response to leaf and yellow rust was observed.

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

Genepool available at this institute was maintained and evaluated for different cross combinations. Two hundreds and eighteen (218) entries were sown in two different dates with a fortnight interval to synchronize early and late varieties to prolong the crossing program. Each entry was sown in two rows each of 2.5 meters length in non-replicated design. Data of different traits like germination percentage (80-95%), plant height (77-123cms), number of tillers per plant (3-12), days to heading (94-121) days to maturity (144-166), leaf rust (0-80S) and yellow rust (0-90S) were recorded.

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

Different filial generations (R1-R6) were studied. Each generation was promoted to next generation on selection basis against different rusts. 1267 entries of different generations were sown in the field and out of these, 744 entries were selected and harvested for further studies.

Preliminary wheat yield trials (A-Trials).

24 wheat lines were selected from advance generations and tested in A-1 and A-2 trials. On the basis of morphological characters and yield performance, three lines having high yield than checks were selected from the trials for further studies.

Regular wheat yield trial (B-Trial).

Twelve wheat promising lines including three lines selected from A trials along with two checks Galaxy-13 and Ujala-16 were tested in B trial for yield performance and other morphological characters. Out of these, three (3) lines 15BT001, 15BT019 and 14BT016 which gave higher yield than checks were selected and promoted to Micro trial.

E. SOIL SCIENCE

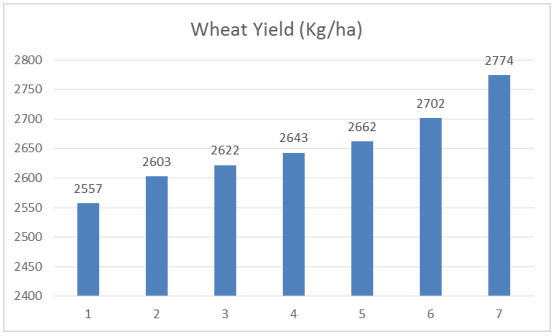
In-vitro studies in sugarcane for inducing salt tolerance.

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 varieties, FD-19, 248, 133, 134 and 658 were evaluated at different salt levels to see their inherited callogenesis potential.

Frequency of callus initiation, fair to excellent, was recorded in FD-19 followed by 134 at 50 m moles/L salt levels, 248, 658 and 133 at 100 m moles/L. Callogenesis was suppressed at higher salt concentrations; however variety/line 658 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.

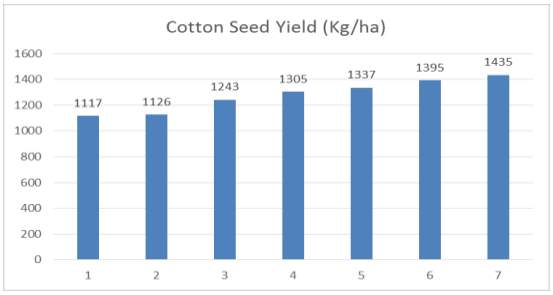
Diazotroph, L-tryptophan and phosphorous solublizing microbes (PSM) were applied alone and in combination to wheat crop for optimizing the yield. A field experiment was designed using RCBD with three repeats. Wheat variety Faisalabad-2008 was sown during Rabi season. Recommended dose of fertilizer was applied and agronomic practices were done. Maximum yield of 2894Kg/ha was obtained where diazotroph, L-tryptophan and PSM were applied in combination whereas minimum yield of 2557 Kg/ha was recorded in control.



Diazotroph + L-Tryptophan and phosphate solubilizing microbes (PSM)

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

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. Maximum seed cotton yield 1435 kg/ha was obtained where Diazotroph + L-tryptophan and Phosphorous solublizing microbes were applied in combination, compared with seed cotton yield 1117 kg/ha in control treatment.

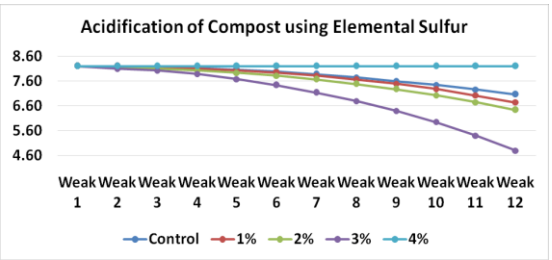


Isolation and screening of *Agrobacterium spp*

The experiment was planned to isolate *Agrobacterium ssp* for the use as vector in bio-transformation studies. For this purpose fresh crown galls/tumors were collected from different dicotyledonous plant i.e. Beri (Desi and Grafted) from different sites. These galls/ tumors were isolated and maintained on PDA media. Previously ten isolates are purified. DNA of these isolates was extracted and preserved for molecular studies.

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.



F. SOIL BACTERIOLOGY SECTION

Biofertilizer Testing Lab

Soil Bacteriology as "Biofertilizer Testing Lab" received 71 biofertilizer / biostimulant samples and analyzed for notified standards while 08 samples of bio-organo phosphate (BOP) were analyzed under FCO.

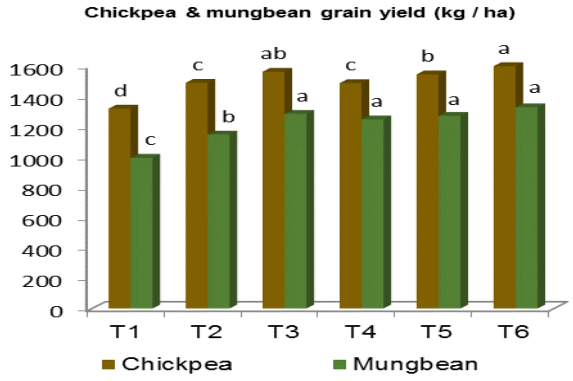
Biofert-plus: precursor enriched novel microbial formulations for legumes

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.

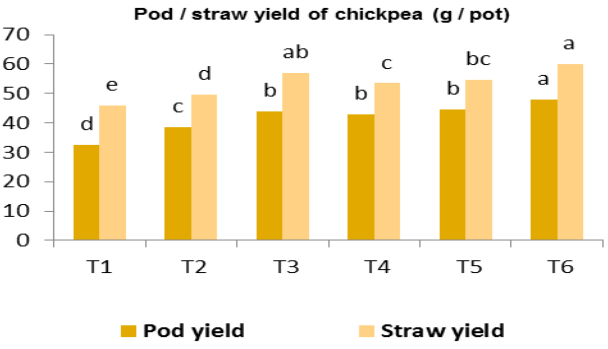
Results of chickpea and mungbean field trials at PRI, Faisalabad revealed that *Rhizobium* inoculum enriched with GA_3 produced the highest chickpea and mungbean grain yield i.e. 1598 and 1326 Kg/ha, as compared to control 1318, 993 Kg/ha, respectively. Results of nodulation in chickpea and mungbean field trials at PRI, Faisalabad revealed that *Rhizobium* inoculum enriched with GA_3 produced higher nodules per plant, nodular dry mass i.e. 19.8 and 15.0; 0.170, 0.105 g plant^{-1} as compared to control 11.8 and 5.5 nodules per plant, 0.088 and 0.055 g per plant, respectively (Fig 1).

Results of chickpea and mung bean pot studies at Soil Bacteriology Section, Faisalabad revealed that *Rhizobium* inoculum

enriched with GA_3 produced the chickpea and mung bean pod, straw yield i.e. 48.0, 41.0 and 48.8, 60.0 g/pot that was significantly higher than control, respectively. Results of nodulation in chickpea and mung bean pot studies revealed that GA_3 enriched *Rhizobium* inoculum showed higher nodules/pot, nodule dry mass g/pot i.e. 60, 42.5 nodules/pot and 0.25, 0.215 g/pot that was significantly higher than control, respectively (Fig 2 and 3).



Grain yield of chickpea and mungbean at PRI, Faisalabad



Pod and Straw yield of chickpea

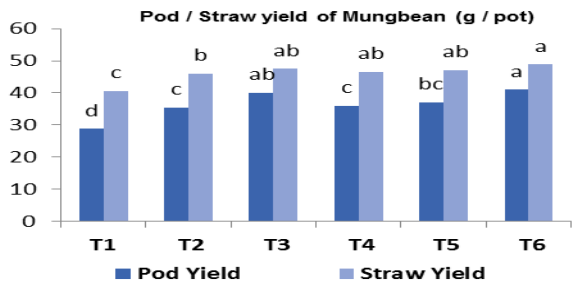


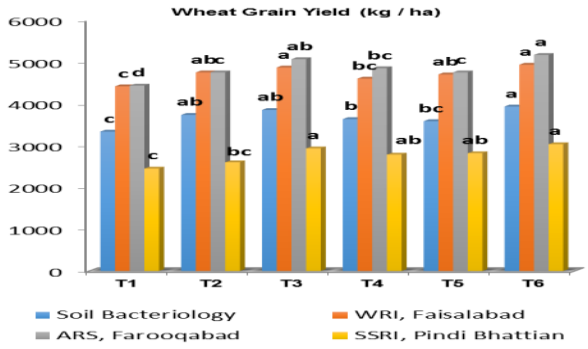
Fig. 3. Pod and Straw yield of mungbean

Biofert-plus: precursor enriched novel microbial formulations for cereals

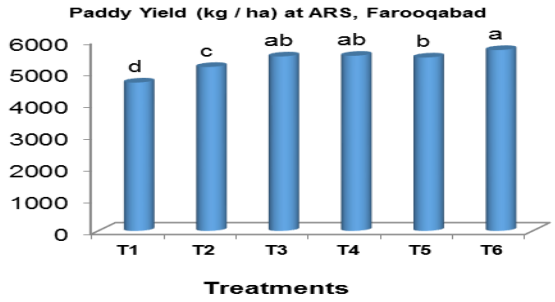
Plant hormones and their precursors exert beneficial effects on plant growth and development. Field studies were conducted to exploit the physiological precursors to improve the crop growth and yield in a cost effective and sustained way. Precursor enriched inoculum were applied along with 75% of recommended 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 at Soil Bacteriology Section revealed that PGPR enriched with GA_3 produced the highest grain yield i.e. 3933 as compared to control 3333 Kg/ha. Results at WRI Faisalabad revealed that PGPR enriched with GA_3 produced the highest grain yield i.e. 4933 as compared to control 4416.7 Kg/ha. Results at ARS, Farooqabad revealed that PGPR enriched with GA_3 produced the highest grain yield i.e. 5166.7 as compared to control 4433.3 Kg/ha. Results at SSRI, Pindi Bhattian revealed that PGPR enriched with GA_3 produced the highest wheat grain yield i.e. 3033 as compared to control 2450 Kg/ha (Fig 4). Results of rice trial at ARS, Farooqabad revealed that PGPR enriched with GA_3 produced the highest paddy yield i.e. 5696 as compared to control i.e. 4672 Kg/ha (Fig 5). Results of maize trial at Soil Bacteriology

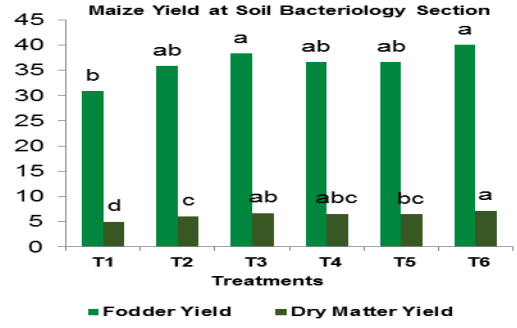
Section revealed that PGPR enriched with GA_3 produced the highest maize fodder yield, dry matter yield i.e. 40.0, 7.17 as compared to control i.e. 30.83 t ha^{-1} and 5.0 t ha^{-1}, respectively (Fig 6).



Wheat grain yield at multi-locations.



Paddy yield at ARS, Farooqabad.



Maize fodder and dry matter yield at Soil Bacteriology Section.

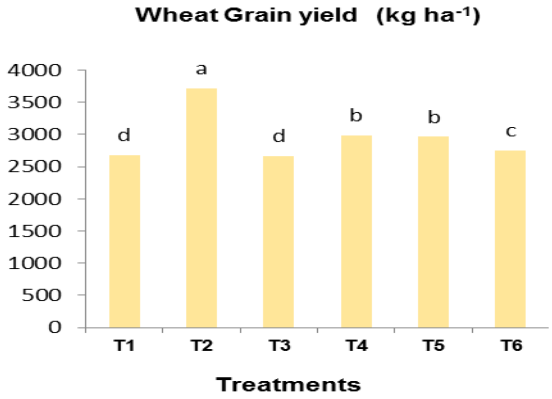
Effect of various crop residue management strategies on soil health in rice-wheat cropping system

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 eighty 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 35-40% 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

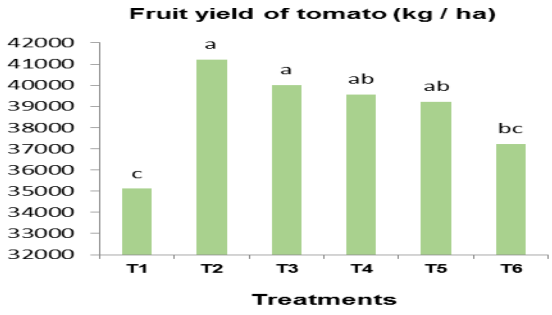
treatment i.e. 3713 as compared to control i.e. 2680 Kg/ha (Fig 7).



Wheat grain yield as affected by various weedicides.

Effect of different biostimulants on Rabi vegetables

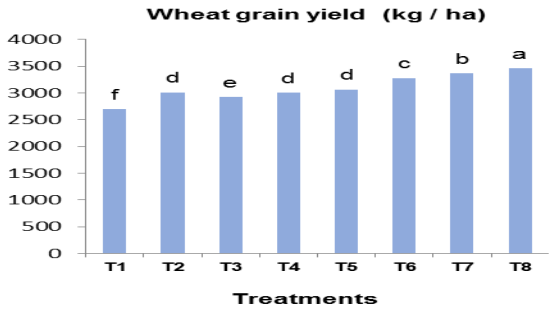
Field study was conducted to assess the effect of different biostimulants on growth and yield of tomato at Vegetable Research Institute, Faisalabad using normal soil having pH 7.9, ECe 1.2, organic matter 0.72% and available P 9.1 mg/kg. Treatments were control, Gibberon, Bio-hope, Fertigrain Start, Biomagic Super and BM Plus (T_6). Recommended dose of fertilizer @ 75-60-60 kg NPK per ha was applied. Parameters recorded were plant height, fruit yield, no of fruits plant^{-1} etc. Results showed that maximum yield was obtained from Gibberon i.e. 41222 as compared to control i.e. 35111Kg/ha (Fig 8).



Tomato fruit yield influenced by various biostimulants.

Effect of PGPR on biofortification of zinc in wheat

Field experiment was conducted at the Soil Bacteriology Section, Faisalabad to investigate the efficiency of PGPR (Zn-mobilizing bacteria) on biofortification of Zinc in wheat. Trial was carried out in sandy clay loam soil having pH 8.2, EC 1.6 dS m^{-1}, available P 7.7 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 for wheat viz. control, PGPR inoculation, $ZnSO_4.7H_2O$ @ 2.5, 5.0, 7.5 Kg/ha with and without PGPR Inoculation. Results clearly indicated that PGPR in combination with 5.0 Kg/ha $ZnSO_4.7H_2O$ give highest grain yield i.e. 3457 as compare to control i.e. 2700 Kg/ha(Fig 9).

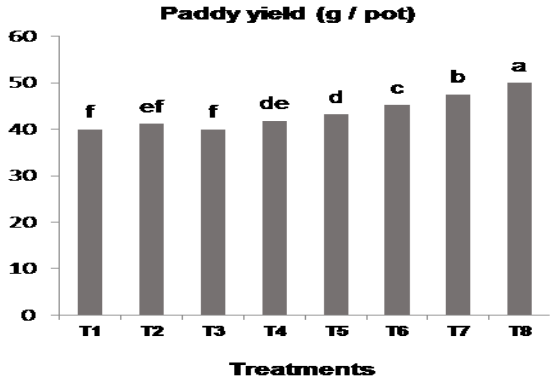


Grain yield of wheat affected by different zinc levels.

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 8.3, EC 1.3 dS m^{-1}, available P 7.3 mg/kg

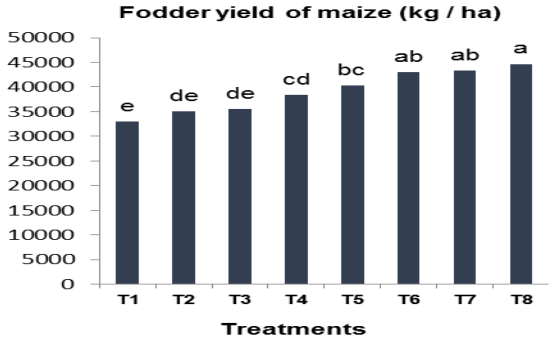
and organic matter 0.67%. Recommended dose of fertilizer @ 120-100-70 kg NPK/ha was applied to all treatments. Treatments include Control, PGPR inoculation and $ZnSO_4$ @ 5, 10, 15 Kg/ha with and without PGPR Inoculation. Results indicated that PGPR along with 15 Kg/ha $ZnSO_4.7H_2O$ significantly improved grain yield i.e. 50.0 as compared with its respective control i.e. 40.0 g/pot (Fig 10).



Paddy yield of rice affected by different zinc levels.

Integrated use of mineral, organic and biofertilizer on maize fodder and soil health

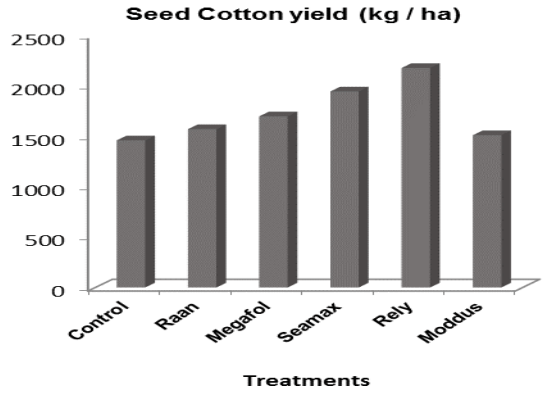
Field study was conducted at the Soil Bacteriology Section, Faisalabad to evaluate the effect of integrated application of inorganic/organic (biogas slurry) and PGPR on maize fodder yield in field conditions. Trial was carried out in sandy clay loam soil having pH 8.1, EC 1.49 dS/m, Olsen P 7.5 mg/kg and organic matter 0.90 %. Treatments include 100 and 75% N of recommended dose with and without PGPR inoculation and was applied with and without biogas slurry @ 600 Kg/ha. Recommended dose of fertilizer for maize fodder 100-60 NP Kg/ha were applied. Results showed that maximum fodder yield i.e. 44667 Kg/ha was observed when 75% N was applied along with biogas slurry as compared to alone 100% N i.e. 33000 kg/ha (Fig 11).



Fodder yield of maize affected by different zinc levels.

Cotton response to biostimulants under field conditions

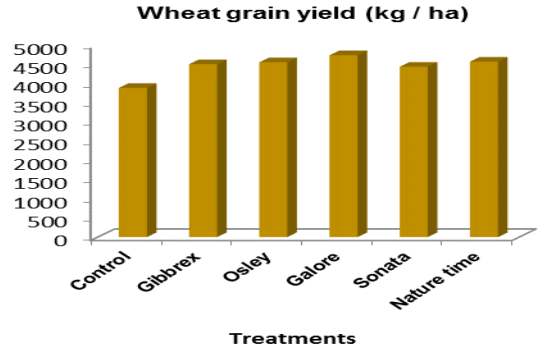
Biostimulants are the products that promote the plant growth in variety of means throughout the life cycle. Studies were conducted to assess the role of different biostimulants on growth and yield of cotton at Cotton Research Institute, Faisalabad. Different biostimulants were studied i.e. Raan, Megafol, Seamax, Rely and Moddus. These were applied according to their rate and time of application mentioned in their protocol. 80% dose of fertilizers was applied to all treatments. Results revealed that highest seed cotton yield was obtained i.e. 2177.9 Kg/ha with Rely followed by 1944.82 Kg/ha with Seamax as compared to 1459.02 Kg/ha of control (Fig 12).



Effect of different biostimulants on seed cotton yield.

Wheat and rice response to biostimulants under field conditions

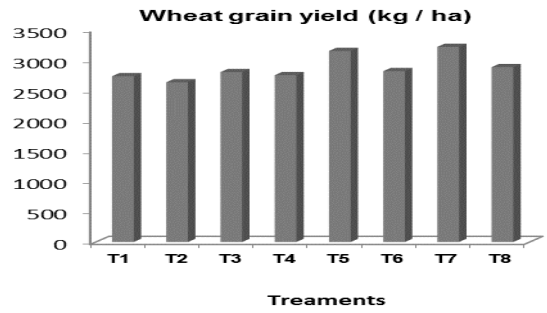
Studies were conducted to assess the role of different biostimulants on growth and yield of wheat at Wheat Research Institute, Faisalabad following RCBD. Different biostimulants were studied i.e. Gibbrex, Osley, Galore, Sonata and Nature time. These were applied according to their rate and time of application mentioned in their protocol. Uniform dose of fertilizers was applied to all treatments. First year results revealed that highest grain yield was obtained i.e. 4733 Kg/ha with Galore followed by 4567 Kg/ha with nature time as compared to 3883 Kg/ha of control (Fig 13).



Effect of different biostimulants wheat grain yield.

Integrated use of mineral, organic and biofertilizers on wheat and soil health

Field study was conducted to assess the effect of integrated application of inorganic, organic (biogas slurry) and PGPR on wheat and maize fodder yield. Recommended dose of P and K i.e. 114 and 60 Kg/ha and biogas slurry @ 600 Kg/ha was applied to wheat trial followed by RCBD layout. Two nitrogen levels i.e. 100% and 70% N were applied. Field trial was conducted at Soil Bacteriology research area. Biogas slurry was applied 15 days before sowing. Results revealed that 100% N coupled with biogas slurry @ 600 Kg/ha in combination with PGPR inoculation produced the highest grain yield of wheat i.e. 3217 followed by 3150 Kg/ha with 100% N and biogas slurry @ 600 Kg/ha (Fig 14).

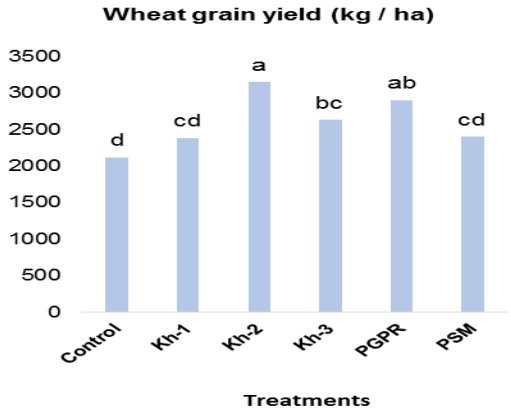


Effect of different treatments on wheat grain yield.

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

Isolation and exploitation were made of novel PGPR isolates to combat saline and drought stresses for different crops under climate change scenario. Roots and rhizosphere soil samples were collected from salt range (Kheura mines and its surroundings). Isolation were carried out on LB media by using dilution

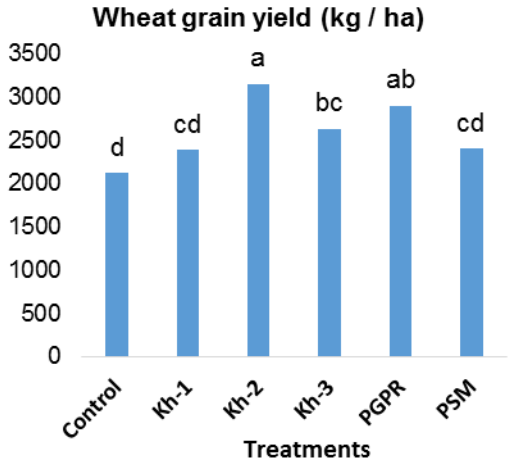
plate technique and screened out in lab. The purified isolates were assayed for growth promotion by root / shoot elongation. Twenty isolates were characterized / screened for their growth promotion activities and preserved during this year. The promising isolates were tested in field experiment at SSRI, Pindi Bhattian in saline sodic soils with following treatments, control, Kh-1 Inoculation, Kh-2 Inoculation, Kh-3 Inoculation, PGPR Inoculation and PSM Inoculation. Result showed that inoculation performed better as compared to control. Inoculation of salt tolerant strain KH-2 produced maximum grain yield i.e. 3150 followed by PGPR inoculation i.e. 2900 Kg/ha as compared to control i.e. 2117 Kg/ha (Fig 15).



Effect of bacterial isolates on wheat grain yield at SSRI, Pindi Bhattian.

Growth and yield response of wheat to different levels of kinetin

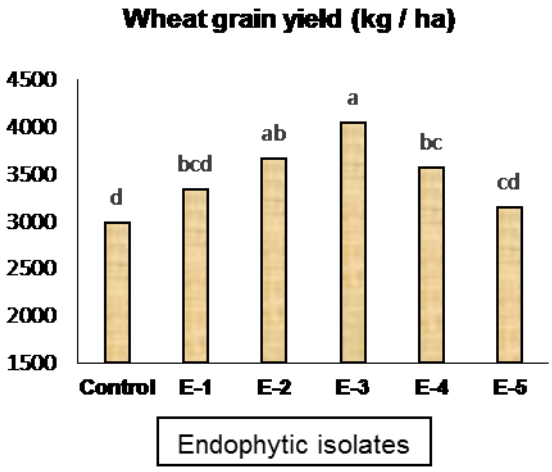
Field experiment was conducted at the Soil Bacteriology Section, Faisalabad to check the best levels of kinetin along with PGPR to improve growth and yield of wheat crop. Trial was carried out in sandy clay loam soil having pH 8.4, EC 2.8 dS m^{-1}, available P 7.9 mg kg^{-1} and organic matter 0.75 %. Recommended dose of fertilizer @ 160-114-60 kg NPK ha^{-1} was applied to all treatments. Treatments were control, PGPR Inoculation and three levels of kinetin i.e. 10^{-3}, 10^{-4}, 10^{-5} M with and without PGPR inoculation. Results clearly indicated that PGPR in combination with Kinetin @ 10^{-5} M produced the highest grain yield i.e. 3233 as compared to control i.e. 2643 Kg/ha(Fig 11).



Potential of endophytic bacteria for growth promotion of wheat and maize

Endophytes are microbial symbionts that colonize the interior of plant tissues without displaying any disease symptoms. They establish an association with a host plant that benefits the health of the plant in several ways including enhancing nutrient availability, degrading toxic substances, and producing plant hormones. Study was planned to check the potential of endophytic bacteria on growth promotion of wheat crop. Field study was conducted at the Soil Bacteriology Section Faisalabad using normal soil having pH 7.98, EC 2.2 dS m^{-1} and organic matter 0.76%. Treatments include five endophytic isolates with control named as E-1, E-2, E-3, E-4, E-5.

Recommended dose of fertilizer (NPK @ 120-90-60 Kg/ha was applied. Different physical parameters were recorded. Result showed that inoculation with bacterial isolates showed significant increase in yield as compared to control. Endophytic bacteria (E-3) produced maximum grain yield i.e. 4050 Kg/ha and it was significantly higher than control.



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