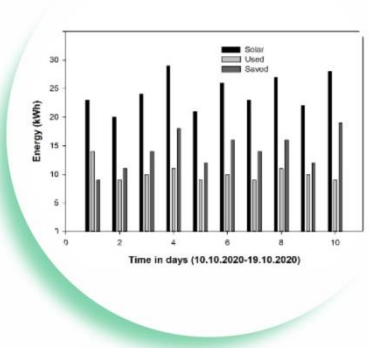
AGRICULTURAL LINKAGES PROGRAM

ANNUAL PROGRESS REPORT 2020-21



ALP SECRETARIAT
PLANNING & DEVELOPMENT DIVISION
PAKISTAN AGRICULTURAL RESEARCH COUNCIL
ISLAMABAD-PAKISTAN

Published by

Agricultural Linkages Program, PARC, Islamabad
ISBN 978-969-409-249-2

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Citation:

Asif, M., T.Z. Chohan and Ya.Sakina (2021). Annual Progress Report 2020-21. Agricultural Linkages Program, PARC, Islamabad, pp. 123

ANNUAL PROGRESS REPORT
OF
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2020-21

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Foreword

Agricultural sector is indispensable to the country's economic growth, food security, employment generation and poverty alleviation particularly, at the rural level. It contributes 19.2 percent to the GDP and provides employment to around 38.5 percent of the labour force. More than 65-70 percent of the population depends on agriculture for its livelihood. The agriculture sector's performance during 2020-21 broadly stands encouraging as it grows by 2.77 percent against the target of 2.8 percent. The growth of important crops (wheat, rice, sugarcane, maize and cotton) during the year is 4.65 percent.

Agriculture sector has a unique position in Pakistan's economy. It supports a growing food demand, stimulates growth and development in industries and services sectors and promotes overall development of the country. There are vast tracts of potential culturable wastelands, perennial rivers to provide water for irrigation, suitable climate for raising different crops and sturdy human resources to exploit these resources.

The prime objective of agriculture research is to feed the growing population across the world. In developing countries like Pakistan, agriculture is having key role in stability and growth of economy. Its contribution in terms of new varieties, machinery and technology consistent with efforts farmers have made Pakistan self-sufficient in food. Pakistan Agricultural Research Council (PARC), through coordination, capacity building, successful implementation of national level research and development programs, contributes tremendously to growth of agriculture.

Since 2000 dilemma of lack of financial recourses has been addressed amicably with creation of Agricultural Research Endowment Fund (AREF) under ALP. So far PARC has launched nine batches of ALP and 416 research projects have been completed successfully. In the 9th batch more than 1500 preliminary proposals were received. At present, 97 are in operation and 32 projects have been completed successfully so far.

There is no doubt that ALP is a gigantic task in which hard work of many professionals of PARC, GOP & USDA is involved. I appreciate the efforts of the group of expert consisting ALP Secretariat, the Technical Divisions and Finance Division for working beyond their capabilities to implement this program successfully. ALP has not only improved research culture, but also upgraded most of the research labs with latest scientific equipments and material at different institutes as well as universities.

The findings/ research achievements of the projects highlighted in this report are evidence of the diligent work of the scientists engaged in ALP. It also mirrors the excellent performance of ALP Secretariat and Technical Divisions as well as Finance Division of PARC. At end I heartedly appreciate efforts of Mr. Muhammad Asif and his crew for compiling this progress report and sailing this titanic carefully/efficiently.

(Dr. Ghulam Muhammad Ali)
Chairman, PARC

ACKNOWLEDGEMENT

Pakistan has tremendous potential for the development of its agriculture in view of land, water, suitable climate and human resource base. However, the lack of ability to exploit the potential needs an in-depth analytical review of constraints. The removal of these constraints through appropriate strategy, technology and financial resources would lead to productivity enhancement in agriculture sector.

Since the inception of ALP the deficiency of financial resources has been addressed adequately. Agricultural scientists from all over the country have the opportunity to win the ALP grants for operational funding through a highly competitive grant system. The overall allocation to the operational funding through this program is over Rs. 200 million per year. This program is fully functional since 2000. So far under ALP 416 projects have been completed successfully since its inception and more than 97 projects of the 9th batch are on-going. The success of this program is due to tireless efforts of ALP Secretariat. The contribution of USDA in establishing and running ALP is highly appreciated. The role of Technical Divisions of PARC for providing continuous guidance and support to ALP Secretariat in monitoring & evaluation of the projects is deeply acclaimed. The effective fund management by Finance Division of PARC is also commendable.

The prudence of Technical Advisory Committee (TAC) and Board of Directors (BOD) of ALP must be acknowledged for approving the projects of priority in the agriculture sector and providing policy guidance for the smooth implementation of the funds and project grants.

The findings/ research achievements of the projects highlighted in this report are evidence of the diligent work of the scientists engaged in ALP. It also mirrors the excellent performance of ALP Secretariat and Technical Divisions of PARC. The implementation of ALP would not have been possible without the firm commitment and substantial contribution of my colleagues namely Mr. Tahir Zahoor Chohan, Director (CG, ALP), Mrs. Naheed Zahra, Senior Scientific Officer (CG, ALP), Mrs. Ya.Sakina, Scientific Officer (CG, ALP), Mr. Mushahid Raza, Assistant Director (MIS), Mr. Nouman Badar, Research Fellow and Mr. Yaseen Khan, Research Assistant.

The working of ALP Secretariat squad consists of highly qualified and devoted scientists in processing, monitoring, review, evaluation and day to day management of ALP projects is deeply acknowledged. Similarly, contribution of Mr. Mudassar Hussain Satti, Mrs. Madeeha Shaikh, Ms. Aamash Haqqani and Ms Sumbul Gull are deeply appreciated.

(Muhammad Asif)
DG (P&DD)/Executive Director (ALP)

SUMMARY

Agricultural Linkages Program (ALP) focus on food security, poverty alleviation and promoting broad based equitable and sustainable agriculture. During 2020-21, three Technical Advisory Committee (TAC) meetings and one Board of Directors (BOD) meeting was conducted, in which 49 projects were recommended by TAC meetings and 22 Projects were approved by BOD for implementation by different Institutes/Universities.

During the year, twenty (33) projects were completed, out of them 08 projects were in Animal Sciences, 17 in Plant Sciences and 04 in Natural Resource, one project in Social Sciences Sector and 03 in Agricultural Engineering Sector.

Altogether nineteen (19) new projects were started. Out of them 09 projects were in Animal Sciences, 17 in Plant Sciences, 09 in Agricultural Engineering Sector and one (01) each in Natural Resource and Social Sciences Sector were implemented during the year.

A total number of 97 projects were ongoing under ALP during 2020-21 with total approved cost of Rs. 571.7539 million. Out of 97 ongoing projects, 34 projects were implemented at Federal level (PARC/NARC) with total approved cost of Rs. 233.6969 million. Nine (09) projects were executed by Pakistan Agricultural Research Council (PARC) Outstations (located in Provinces) with total cost of Rs. 72.426 million. Ten (10) projects were implemented at Federal level (other than PARC/NARC) with the total cost of Rs. 54.522 million, whereas thirty one (31) projects were executed in Punjab with total cost of Rs. 145.066 million. Six (06) projects were funded in KPK with total cost of Rs. 34.133 million, Four (04) projects in Azad Jammu and Kashmir (AJK) with total approved cost of Rs. 17.797 million and Three (03) projects were funded in Gilgit Baltistan (GB) Province with total approved cost of Rs. 14.113 million.

SECTION-1

ALP MANAGEMENT SYSTEM

ALP MANAGEMENT SYSTEM

1.1 Introduction

The Agricultural Linkages Program (ALP) was established through an agreement between the Govt. of Pakistan and United States of America signed in February, 1999. Under this agreement USA supplied wheat (200,000 tones) valued at US$23.222 million as a grant to Pakistan in March 1999. The agreement provided that the sale proceeds of this wheat amounting to Rs.1.3 billion will be invested in banks and income generated from this investment used by Pakistan Agricultural Research Council to establish the Agricultural Linkages Program (ALP) for promotion of agricultural research and development. Agricultural Research Endowment Funds (AREF) was approved to support/implement ALP. The Economic Coordination Committee (ECC) of the Govt. of Pakistan while approving the Agricultural Research Endowment Fund for ALP in its meeting held on 14.07.2000 also approved procedures for its implementation. The Govt. of Pakistan made PARC responsible to use this Endowment Fund, establish the Agricultural Linkages Program, manage its affairs and award the grants through an approved mechanism.

The income generated by investing principal amount of the endowment fund in the Government approved schemes and banks is used for ALP activities/ projects in line with the Pakistan's long term research/goals for the Agriculture Sector. The goals of ALP are to improve food security, alleviate poverty and to promote broad based equitable and sustainable agriculture.

The Fund provides for all the operational research and development expenditure including supplies and material and local travel for the research personnel to be deployed under the projects. Non-recurring expenditure on items of non-expendable equipment, capital goods, structures and transport facilities having anticipated usefulness beyond fixed duration of each project will be purchased sparingly.

1.2 Objective

The objective of the ALP shall be to promote and support agricultural research and development activities in accordance with the Pakistan's long term development goals and to promote long term scientific cooperation between Pakistan and the United States in Agricultural Sector.

The scope of agricultural research and development activities, which the ALP may promote and support shall cover all scientific activities related to agriculture including production, processing, marketing and agricultural services. The ALP gives emphasis, but not limiting its efforts to the support of strategic research, which improves farm and animal productivity and in the areas which directly or indirectly effect the achievements of these objectives. The ALP Fund is an independent entity and possesses all of the powers necessary to carry out its objectives including but not limited to the powers to:

- Promote and support by funding or otherwise agricultural research and development projects of mutual benefit;

- Encourage and support the exchange of agricultural scientists and other type of agricultural experts.

1.3 Management System

The Board of Director (BOD) is the governing body of ALP fund and is responsible for fund's program and its financial and managerial policies. The BOD comprises on Chairman, PARC as the Chairman of the Board, four Provincial Addl. Chief Secretaries, Senior Chief (Agri. and Food Section) P&D Division, Food Security Commissioner-I, M/o National Food Security & Research, all Members of PARC, two representatives of Stakeholders are its members and DG (P&D Division)/ Executive Director (ALP) is the Member/Secretary of the Board. The terms of reference (TOR) of Board of Directors are:

- Adopt bylaws, rules and procedures for the conduct of its activities;
- Establish the organizational frame-work of the fund;
- Appoint Technical Experts and Reviewers and Technical Advisors for review and evaluation of the proposals;
- Accept contribution of property, funds and services;
- Exercise and delegate any other powers of the fund not otherwise specifically assigned by the document.

1.3.1 List of BOD Members

Chairman, PARC/BOD of ALP

Members

1. Provincial Addl. Chief Secretaries/Focal Persons (4)
2. Senior Chief (Agri. And Food) Ministry of Planning Division
3. Agricultural Development Commissioner/Food Security Commissioner, MNFS&R
4. All Members, PARC
5. Representation of Stakeholders (2)
6. Director General (P&D Division)/ Executive Director (ALP)

1.3.2 Processing of Projects

The project proposals are processed in accordance with the procedures approved by the BOD of ALP Endowment Fund. All the proposals are judged and evaluated by the technical experts/referees in Pakistan. On the recommendations of TAC, the BOD finally approves the project funding.

Technical Advisory Committee includes eminent scientists representing all the provinces as well as major disciplines. The TAC comprises of; Chairman, PARC as Chairman of the TAC, all Members, PARC, four Provincial DGs of Research, four eminent scientists are its member and DG (P&D Division), PARC is Member/Secretary of TAC. The P&D Division is working as

ALP Secretariat. The ALP Secretariat team consists of a small team of four scientists, who are the back bone of this program.

Once the projects are approved and implemented, its monitoring, review and evaluation are conducted and organized by ALP Secretariat with the involvement of Directorate of Monitoring & Evaluation, P & D Division. The progress is reviewed through mid-year/ annual progress reports, in-house review and visit of field/ onsite activities through a team of experts. This help in improving the progress and successful implementation of project in relation to their specific objectives.

1.3.3 List of TAC Members

Chairman, PARC/ TAC of ALP

Members

1. All members, PARC
2. Provincial Director General Research (4)
3. Eminent Scientist (4)
4. Director General (P & D Division)/ Executive Director (ALP), PARC

SECTION-2

TAC AND BOD MEETINGS (2020-21)

2. TAC AND BOD MEETINGS

2.1 Technical Advisory Committee (TAC) Meetings

Three meetings of TAC were arranged during 2020-21, in which total 62 projects were presented of all five sectors. TAC members recommended only 49 projects for further approval from BOD, while 06 projects were deferred. Sector-wise details of recommended projects are below:

Projects presented in the TAC

Sectors	Total Projects Presented	Projects Recommended	Projects Not Recommended	Deferred Projects
Animal Sciences	*11*	*9*	*02*	*0*
Plant Sciences	*37*	*31*	*01*	*05*
Natural Resources	*6*	*2*	*04*	*0*
Agri. Engineering	*8*	*7*	*0*	*01*
Social Sciences	*0*	*0*	*0*	*0*
Total	*62*	*49*	*07*	*06*

The following projects have been approved by three TAC meetings during 2020-21:

2.1.1 Animal Sciences Sector:

- Commercialization of Fish Breeding and Culture Technologies in Coastal Region of Pakistan (Follow-Up of on Going Project)
- Prospects of Biofloc Technology for Tilapia Culture in Pakistan
- Investigations and Optimization of Sperm Sexing and Cryopreservation Techniques in Water Buffalo
- Establishment of Angora Rabbitry at Juglote & Sakardu, PARC–Mountainous Agricultural Research Centre (MARC)
- Validation and Establishment of Intensive Production Packages for High Value Fisheries
- Exploring Genomic Architecture and Improving Selection Prediction for Milk Production Traits in Potential Cattle Breeds
- Introduction of a High Value Fish Channa Striatus (Soul) in Aquaculture System of Pakistan
- Evaluation of Antigenic Variations among Infectious Bronchitis Virus in Commercial and Backyard Poultry
- Nanotechnology-Enabled Improvement of Spermatozoa Cryopreservation Technique for Water Buffalos

2.2.2 Agri. Engineering Sector:

- Develop an Innovative Technique for Processing the Olive Fruit Waste to Achieve Leftover Oil for Edible Use

- Development of Prismatic Solar Collector for Drying of Fruits Vegetables, Grains and Feed Heating
- Development and Adaptation of Mechanized Transplanting Technology for Vegetables
- Design and Development of Indigenized Compost Windrow Turner for the enrichment of Soil Nutrients
- Development of Hybrid Indirect Type Solar Dryer for Drying of Fruits and Vegetables
- Development and Adaptation of Modified Atmosphere Packaging (MAP) for Fruits & Vegetables
- Development Of Dust Control Mechanism for Wheat Thresher to Minimize Environmental Pol lution and Control Health Hazards of Farming Community

2.1.3 Plant Sciences Sector:

- Adaptation of Good Agricultural Practices (GAP) for Ispaghol (Plantago Ovata), Production & Processing Technologies at Water Scared Areas of Lower-Sindh Component-I
- Promotion and Introduction of Improved Varieties of Ispaghol (Plantago Ovata) Following WHO Guidelines of Good Agricultural Practices (GAP) in Balochistan, Component-II
- Evaluation of Oat Germplsam for Better Adaptation in Changing Climate Scenario
- Molecular Characterization of Predatory Odonata Species of Pakistan
- Production of Gelatin from Halal Source: Plants
- Crop Value Enhancement in Peaches through Fruit Load Management
- CRISPR-Cas-Based Promoter Editing of Sweet Genes in Basmati Rice for Bacterial Blight Resistance
- Collection, Characterization, Evaluation and Multiplication of Walnut Germplasm in Hazara Division, Khyber Pakhtunkhwa, Pakistan
- Introduction, adaptability and intercropping of white Lupine (Lupinus albus) as winter legume forage in arid areas of Cholistan
- Development of Fruits Value Addition Business Entities: A Way Forward Towards Women Entrepreneurship
- Hybrid Innovations in Processing Technology of Dhakki Dates with Quality Retention, Packaging and Value-Addition: A Multifarious Approach
- Improvement of Mechanized Tea Production and Promotion of Green Tea Profitability
- Evaluation of Oat Germplasm for Product Development

- Molecular Identification of Indigenous Yeast Strains for Development of Yeast Extract Products
- Improving Sesame Productivity Through Better Management Practices in Balochistan
- Post-Harvest Fruits Management through Integrated Pre-harvest Management Practices in Balochistan
- Development of Bait Delivery Systems and Screening the Palatability of Baits Formulated for the Management of Wild Boar, Sus Scrofa
- Micropropagation of Elite Ginger Germplasm and Field Evaluation
- Prevalence and Management of Mycoflora Associated with Peach Orchards
- DNA Based Varietal Identification of Fruit Plants for True to Type and Healthy Fruit Plant Nurseries
- Strengthening of Intellectual Property Rights (IPRs) for PARC Technologies
- Distribution, Diagnostics and Containment of Panama Wilt of Banana Disease in Pakistan
- Development and Promotion of Improved Production Technology for Oil Palm Plantation and Production in Coastal Areas of Sindh Pakistan (CS 087)
- Development of Pure Lines for the Production of Hybrid Tomato Through Shuttle Breeding
- Ex-situ Conservation of Wild Edible and Medicinal Plants from Poorly Explored Bio-Diversity Hotspots of Pakistan
- Improving Indigenous Varieties of Vegetable (Onion, Tomato, Okra, Brinjal and Pea) Through Certified Seed Production Practices at Vegetable Seed Farm and Farmers Field
- Potato Varieties Development through Marker Assisted Selection (MAS) & Speed Breeding
- Implementation of GIS Models in the Movement Pattern of Whitefly Species and Biotypes and Associated Plant Viruses Along the CPEC Route
- Evaluation of Geophytes for Cut Flower Production in Different Ecological Zones of Pakistan (Component-I)
- Supply Chain Management of Cut Flowers through Optimizing Postharvest Handling Protocols (Component-II)
- Utilization of Nursery Sanitation Techniques in the Propagation of Economically Important Nursery Plants of Various Fruits Species for Peshawar Areas

2.2.4 Natural Resources Sector

- Development of Novel Bio fertilizer consisting of Consortium of Strains to Cope the Emerging Challenges of abiotic Stresses under the Climate Change
- Binding and Fate of Micronutrients Blends to Urea and DAP Fertilizers for Value Addition

2.2 Board of Directors (BOD) Meetings

One meeting of BOD were held during 2020-21, in which total 24 projects were presented of all five sectors. BOD members approved only 22, while 02 projects were deferred. Sector-wise details of approved projects are below:

Projects presented in the BOD

Sectors	Total Projects Presented	Projects Approved	Projects Not Approved	Deferred Projects
Animal Sciences	*06*	*05*	*0*	*01*
Plant Sciences	*12*	*11*	*0*	*01*
Natural Resources	*02*	*02*	*0*	*0*
Agri. Engineering	*04*	*04*	*0*	*0*
Social Sciences	*0*	*0*	*0*	*0*
Total	***24***	***22***	***0***	***02***

The following projects have been approved by two BOD meetings during 2020-21:

2.2.1 Animal Sciences Sector:

- Commercialization of Fish Culture Technology in Coastal Region of Pakistan (Follow-Up of a Completed Project
- Investigations and Optimization of Sperm Sexing and Cryopreservation Techniques in Water Buffalo
- Establishment of Angora Rabbitry at Juglote & Sakardu, PARC Mountainous Agricultural Research Centre (MARC)
- Validation and Establishment of Intensive Production Packages for High Value Fisheries
- Exploring Genomic Architecture and Improving Selection Prediction for Milk Production Traits in Potential Cattle Breeds

2.2.2 Agri. Engineering Sector:

- Develop an Innovative Technique For Processing the Olive Fruit Waste to Achieve Leftover Oil For Edible Use
- Development of Prismatic Solar Collector for Drying of Fruits, Vegetables, Grains and Feed Heating

- Development and Adaptation of Mechanized Transplanting Technology of Vegetables
- Design and Development of Indigenized Compost Windrow Turner for the enrichment of Soil Nutrients

2.2.3 Plant Sciences Sector:

- Evaluation of Oat, Vetch and Cowpea Germplasm for Better Adaptation in Changing Climate Scenario
- Production of Gelatin from Halal Source: Plants
- Crop Value Enhancement in Peaches through Fruit Load Management
- CRISPR-Cas Based Promoter Editing of Sweet Gene (s) in Locally Adapted Rice Cultivar (s) in Pakistan for Bacterial Blight Resistance
- Collection, Characterization, Evaluation and Multiplication of Walnut Germplasm in Hazara Division, Khyber Pakhtunkhwa, Pakistan
- Introduction, Adaptability and Intercropping of White Lupine (Lupinus Albus) as Winter Legume Forage in Arid Areas of Cholistan
- Development of Fruits Value Addition Business Entities: A Way Forward Towards Women Entrepreneurship
- Hybrid Innovations in Processing Technology of Dhakki Dates with Quality Retention, Packaging and Value-Addition: A Multifarious Approach
- Improvement of Mechanized Tea Production and Promotion of Green Tea Profitability
- Evaluation of Oat Germplasm for Product Development
- Molecular Identification of Indigenous Yeast Strains for Development of Yeast Extract Products

2.2.4 Natural Resources Sector:

- Determination of Fertilizer Requirements for Optimizing Tomato Productivity in Tirbal District Kurram of Erstwhile FATA
- Development of Novel Biofertilizer consisting of Consortium of Strains to Cope with the Emerging Challenges of abiotic Stresses under Climate Change

SECTION-3

PLANT SCIENCES SECTOR

3. Plant Sciences Sector

The completed projects of Plant Sciences Sector were Eighteen (18) under ALP during 2020-21, out of which eleven projects were completed in Punjab Province with the cost of Rs. 44.326 million, three in PARC/NARC with the cost of Rs. 21.299 million, two in Other federal with the cost of Rs. 12.536 million and one each in Khyber Pakhtunkhwa (KPK) and in GB Province with the cost of Rs. 5.735 and Rs. 4.979 million respectively. While seven (07) new projects were started during the reported period with the total cost of Rs. 35.232 million. Sixty (60) projects were on-going of this sector with total cost of Rs.354.969 million. Thirty-seven (37) projects Plant Sciences Sector were presented in three meetings of Technical Advisory Committee (TAC), out of these thirty-one (31) projects were recommended by TAC for further approval from Board of Directors (BOD). Only one meeting of BOD held in 2020-21, in which twelve (12) projects were presented for approval. BOD approved only 11 projects of Plant Sciences Sector for funding under ALP, while one project was deferred.

3.1 On-Going Projects

The progress of ongoing projects was monitored during the reporting period on the basis of technical and financial progress reports by ALP Secretariat as well as Technical and Finance Division of PARC. Region wise detail of on-going projects is given below in table.

Table: Region wise Plant Sciences Sector projects and approved cost of on-going projects (2020-21)

Region/Sector	No. of Projects	Total Cost (million Rs.)
Federal (PARC/NARC)	18	120.355
PARC (Outstation)	07	61.565
Other Federal	07	42.464
Punjab	19	86.954
Khyber Pakhtunkhwa	05	24.578
AJK	01	4.940
Gilgit Baltistan	03	14.113
Total	60	354.969

3.2 Achievements of Completed Projects

The total completed projects of Plant Sciences Sector during the year were Eighteen (18) under ALP during the year 2020-21 in various universities, agricultural research centers, institutes and stations. The completed projects have been contributed in screening/selection/identification/development of various cotton varieties, high yielding grain of Soyabean, hypolipidemic effect Citrus peel-derived pectin and its rootstock resistant against drought and salinity, optimized production technology of different fodder crops, wheat and different fruits etc. The salient achievements/progress of Eighteen (18) completed projects of Plant Sciences Sector is given below.

3.2.1: Development of heat tolerant cotton varieties having enhanced resilience against changing climatic scenario		
Name of PI/Institute and designation:	**Dr. Manzoor Hussain, T.I.,** **Deputy Chief Scientist/Group Leader Cotton** Nuclear Institute for Agriculture and Biology (NIAB)	
Postal Address:	P.O. Box 128, Jhang Road, Faisalabad, Pakistan Telephone: 041-9201784, Email: mhmanj@gmail.com	
Duration:	01.05.2016 to 31.12.2020	
Financial Status:	Total Cost: Funds Released: Funds Utilized:	Rs. 8.750 million Rs. 8.592 Million Rs. 7.985 Million

Objectives:

- Identification/screening for thermo-tolerant and sensitive cotton genotypes through growth indices, physio-biochemical and molecular characterization.
- Testing for the evaluation and promotion of potential performance heat tolerant cotton lines in F_7 and F_8 generations in different cotton growing zones for their adaptation by the end of the project.
- Identification of heat tolerant lines under IPCC RCPs scenarios 6.0 and 8.5 for mid and end century using crop growth modeling approach as a decision support system to quantify the benefits of genetic improvement and heat tolerance traits on the yield of cotton in current and future climates.

Achievements:

Project progressed well as per planned activities in terms of achievements i.e. with verifiable outputs/results- availability of 04 high yielding cotton varieties (i.e. NIAB-878B, NIAB-545, NIAB-1048 & NIAB-1011) with farming community. NIAB-878B approved in (2017 Punjab & 2021 Sindh), ranked top in its seed multiplication targets (60000 Mds), whilst NIAB-545 & NIAB-1048 approved in 2018 were also included in Seed multiplication targets of Punjab Seed Corporation (2020-21) as 2000 and 5000 Mds; respectively. NIAB-1011 was firstly recommended in 80th meeting of ESC of Punjab Seed Council for its approval held on 27th July, 2020. Latterly this was approved in 54th meeting of PSC (held on 28th January, 2021).

Publication: (Book Chapter)

Manzoor. Hussain; Ljupcho. Jankuloski; Massoud. Malek; Md. Kamrul Islam; M. Reza Raheemi; Jawdat. Dana; Khin Myat Lwin; Fiaz Ahmad; M. Habib-ur- Rehman; Muhammad Rizwan, Ghulam Mohyuddin Talha, M. Asif and S. Ali. "Improving Sustainable Cotton Production through Enhanced Resilience to Climate Change using Mutation Breeding." In Mutation Breeding, Genetic Diversity and Crop Adaptation to Climate Change, pp. 145-156. Joint FAO/IAEA Center, CABI, International 2021.

Sakhar

Bahawalnagar

3.2.2: Development of Sunflower Hybrids Modified for High Oleic Acid in Edible Oil		
Name of PI/Institute and designation:	**Dr. Saeed Ahmad Asad** Associate Professor, Department of Biosciences	
Postal Address:	Department of Biosciences COMSATS University Islamabad. Park Road, Tarlai Kalan, 45550 Islamabad *Telephone: +92 331 761 2373; Email: saeed.asad@comsats.edu.pk*	
Duration:	01.07.2018 to 30.06.2021	
Financial Status:	Total Cost: Funds Released: Funds Utilized:	Rs. 3.786 million Rs. 3.008 million Rs. 3.008 million

Objectives:

1. Investigate the yield (grain and roots) responses of Soybean under varying degrees of temperature, CO2 concentrations and moisture levels.

2. Identify the management interventions for mitigating impacts of climate change on soybean productivity using simulation model CropSyst/ DSSAT and suggest a complete production package to farmers for enhancing the productivity of Soybean under climate change scenarios.

Achievements:

Sowing dates and planting methods under irrigated and rainfed conditions were standardized. The simulations revealed that delayed sowing declined the soybean yield to a significant level. Crop model simulated that the cultivar C4 (NARC-16) produced higher grain yield when sown on 15 June, whereas seeds sown on 15 July were the least yielding both under rainfed and irrigated environments. It was observed that cultivar C4 (NARC-16) was less sensitive to temperature variations than others three cultivars and it also performed comparatively well during late planting (15 July). It was revealed that late planting on 15 July and afterwards had a heightened risk of low soybean grain yield. This impact was attributed to varying climatic conditions such as solar radiation, temperature and precipitation variations and low photo thermal units, when planting was too late or too early than 15th June under rainfed and irrigated conditions. The farmers in the Pothwar and Swat are suggested to plant NARC-16 between 15-30 June for a bumper yield. This short duration and high yielding cultivar can withstand the mild temperature stresses.

Publication:

1. Asad SA. (2020). Soybean, a miracle crop for national food security. Daily Dawn https://www.dawn.com/news/1527911 (public article).

2. Asad SA. Wahid MA. Shaheen F. Raza A. Farooq M. (2020). Soybean production in Pakistan: experiences, challenges, and prospects. International Journal of Agriculture and Biology. 24: 995–1005.

3. Asad SA. Shaheen F. Ahmad I. Farooq M. Mahmood I. Raza M. Ahmad F. (2021). Evaluating the thermo-temporal changes on Soybean productivity under rainfed and irrigated conditions in Pakistan (Submitted to journal of agronomy and crop science).

National Linkages Developed:

During project execution, collaboration with the following institutes were developed.

1. Institute of Environmental Sciences and the Department of Agronomy, PMAS Arid Agriculture University Rawalpindi, Punjab Pakistan.
2. Grains Quality Testing lab, National Agricultural research Centre (NARC), Islamabad.
3. Agricultural Research Institute (ARI), Mingora, Swat, Khyber Pakhtunkhwa.
4. Agricultural Research Station (ARS), Mansehra, Khyber Pakhtunkhwa.
5. Department of Botany, Hazara University, Mansehra, Khyber Pakhtunkhwa.

Degrees Awarded under the project:

One PHD student completed her degree under this project.

3.2.3: Exploring the potential of non-thermal techniques for quality analysis of citrus-based peel/pectin		
Name of PI/Institute and designation:	**Dr. Rana Muhammad Aadil** Assistant Professor, National Institute of Food Science and Technology,	
Postal Address:	National Institute of Food Science and Technology, University of Agriculture, Faisalabad, Pakistan	
Duration:	01-07-2018t to 30-06-2021	
Financial Status:	Total Cost: Funds Released: Funds Utilized:	Rs. 2.933 million Rs. 1.676 million Rs. 1.265 million

Objectives:

The objectives of this research include the following:

- Comparison and characterization of selected citrus varieties namely grapefruit, kinnow, and mandarin
- Optimization of pectin extraction efficiency from citrus peel by using non-thermal techniques
- Utilization of pectin extracted from citrus wastes for development of jelly and its product stability
- Authenticating the potential of citrus peel-based pectin against hyperlipidemia to ensure food security

Achievements:

Citrus (Citrus L. from Rutaceae) is one of the well-renowned world fruit crops, containing phytochemicals, flavonoids, phenolics, and antioxidants. Lemon and kinnow peels are the rich source of pectin as compared to the other plant sources. It is a significant polysaccharide having considerable applications in the food industry, pharmaceutics, and many others. It can be extracted by using many thermal as well as non-thermal techniques. In the present study, powdered peels were studied in respect of all the selected parameters. After the baselines testing of peel powder, it was subjected to pectin extraction in which grapefruit peel gave maximum yield through TS. The antioxidant contents of jelly were less prone to sonication and TS technique. From the results, it was clear that, if researchers only want a higher yield of pectin then TS is the finest technique but if the product is required with both quality and quantity then sonication is the best choice to obtain standard results. Results of the analysis also proved that citrus varieties by-products like peels contained a greater level of pectin than peels of other fruits. So, further investigations are required to evaluate the microbial status of jelly and similar products obtained by using non-thermal techniques.

Hyperlipidemia is a condition with increased blood lipid levels, which is a key factor for causing coronary heart disease. Side effects of medical treatments and increase in the incidence of cardiovascular diseases put strong interest in preventive medicine from natural

food as an alternative approach. Citrus peel waste accounts for almost fifty percent. For this purpose, the study is designed to explore the hypolipidemic effect of citrus peel-derived pectin. Two studies were conducted in this efficacy trial. Study I (normal rats) and study II (Hypercholesterolemic rats). Extracted pectin was given to the respective groups of both studies for 60 days. In both study I and study II, pectin showed improvement in all parameters (lipid profile, liver function markers, renal function tests, and glutathione and TBARS. Moreover, maximum feed and water intake were observed in rats given with citrus peel pectin as compared to control rats. In study II, as the concentration of citrus peel pectin increased, pectin showed more improvement in feed and water intake.

3.2.4: Value addition of guava through development of vitamin-A fortified fruit bar		
Name of PI/Institute and designation:	**Dr. Moazzam Rafiq Khan** Assistant Professor University of Agriculture, Faisalabad	
Postal Address:	National Institute of Food Science and Technology, University of Agriculture, Faisalabad, Pakistan. Telephone: 0321-8690691 Fax: N/A E-mail: mrkhan_ft@yahoo.com	
Duration:	15-10-2018 to 14-03-2021	
Financial Status:	Total Cost: Funds Released: Funds Utilized:	Rs. 2.124 million Rs. 1.820 million Rs. 1.799 million

Objectives:

- Antioxidant profiling of Guava collected from four different districts i.e., Sheikhupura, Faisalabad, Kasur and Pakpattan.
- Optimizing process conditions for development of Vitamin-A fortified Guava fruit bar.
- Assessing storage stability of developed fortified Guava fruit bar.
- Validate the efficacy of vitamin-A fortified value-added guava bar against Vit. A deficiency.

Achievements:

Nutritional deficiencies, a severe worldwide health concern, are prevailing issue in developing countries. Vitamin-A deficiency (VAD) had been recognized as a major contributing factor perniciously influencing the health and nutritional security of Pakistan. Food fortification is an effective and low-cost approach to reduce the consequences of micronutrient deficiencies and increase the micronutrient supply. Guava (Paidium guajava L.) is characterized as fruit having pleasant flavor, high nutritive value, high palatability and antioxidant capacity but fresh fruits have limited shelf life (6-8 days). It requires attention to preserve it in the form of value-added product for the availability throughout the year. Present project was planned to alleviate the vit-A deficiency by providing vit-A fortified guava fruit bars. For the reason, fruit bars were made by adding two vit-A fortificants i.e., retinyl palmitate and retinyl acetate separately @ 40 and 50% RDA. Guava fruit of different verities (Gola, Surahi and pink flesh) and of different districts (Sheikhupura, Faisalabad, Kasur and Pakpattan) was analyzed for their antioxidants potential and organic acids and then processed in to bars with the fortification of vit-A (retinyl acetate, retinyl palmitate) and analyzed for vit-A and organic acids retention. The fortified bar was stored for 4 months at ambient temperature. Efficacy trials of fortified bars were conducted to validate the efficacy of vitamin-A fortified value-added bar against VAD. On the basis of antioxidants capacity and organic acids contents pink flesh guava was best verity, but due to insect pest attack its production is low and availability is less. Therefore, Gola verity from Sheikhupura district was selected to be fortified with vit-A. Physical analysis (TSS, pH, texture, titratable acidity, color, antioxidants study (total phenolic contents, flavonoids contents, DPPH and

FRAP), microbial study (total plate count) was conducted at the period of 30 days for the duration of 4 months of storage. Maximum vit-A was examined in T_2 (50% retinyl acetate) followed by T_4 (50% retinyl palmitate). Based on physico-chemical analysis, sensory attributes and vit-A retention T_2 (50% of RDA; retinyl palmitate) and T_4 (50% of RDA; retinyl acetate) were selected for efficacy purposes. The bio-efficacy trail was carried out on Sprague Dawley male rats. Depending on diet, the efficacy plan was categorized into two studies, study I, (normal rats), study II (vit-A deficient rats). Each study was further segmented into three groups; group fed on control bar (T_0), group fed on retinyl palmitate bar (T_2) and group fed on retinyl acetate bar (T_4) for 60 days to determine the effect of fortified bar against vit-A deficiency. Selected treatments (1 bar/day) along with control were provided to the respective groups of normal and vit-A deficient rats for 2 months. Retinyl palmitate and retinyl acetate fortified bars significantly enhanced the level of serum retinol by 20.68% and 20.97% in normal rats and by 22.80% and 23.15% in vit-A deficient rats, respectively. The serum retinol level was significantly decreased up to 5.14% in normal and 6.96% in vit-A deficient control groups. Collectively, the serum retinol level showed a significant increase of 43.68% and 44.12% in retinyl palmitate and retinyl acetate groups respectively, during two months whereas, a significant decrease 12.10% was found in control groups collectively. Due to retinyl palmitate and retinyl acetate antioxidants status of normal and vit-A deficient rats was also improved. The consumption of retinyl palmitate and retinyl acetate fortified bars exhibited non-significant ($p<0.05$) effects on renal and liver functioning tests showing safety and suitability of these fortificants. Retinyl acetate was found to be more effective than retinyl palmitate to uplift the serum retinol level in normal and vit-A deficient rats.

Graduate Students/Degrees:

One M.Sc.(Hons.) is awarded degree under this project.

3.2.5: Improving drought tolerance and quality of fodder maize genotypes for silage using growth hormone and selenium		
Name of PI/Institute and designation:	**Dr. Muhammad Rasheed** Associate Professor, Department of Agronomy	
Postal Address:	Department of Agronomy, Faculty of Crop & Food Sciences, PMAS-Arid Agriculture University, Rawalpindi Telephone: 0334-5204364, Fax: N/A E-mail: rasheed786@uaar.edu.pk, drrasheed786@gmail.com	
Duration:	01-07-2018 to 30-06-2021	
Financial Status:	Total Cost: Funds Released: Funds Utilized:	Rs. 5.737 million Rs. 4.934 million Rs. 4.913 million

Objectives:

- Screening drought tolerant fodder maize genotypes for higher quality fodder and silage production.
- Establishing the role of GA_3 and Se in fodder maize against water stress under rainfed conditions.
- Enhancing fodder maize quality and silage preservation to overcome the forage scarcity issue in rainfed areas.
- Capacity building of different stakeholders (livestock farmers, extension workers, students and researchers) through field demonstration of quality forage production and silage preservation technology in Pothwar.

Achievements:

Production of low-cost, high-quality fodder could be used to replace crop production in rainfed agriculture. In Pakistan, the demand for fodder crops for animal feed is steadily increasing due to geometrically growing mouths of the livestocks. Among the summer fodder crops, maize is capable of producing higher amounts of palatable and fibrous fodder without any poisoning substance (hydrocyanic acid etc.) and is extensively used as silage in advanced countries. Its digestibility is higher than sorghum, bajra and other non-leguminous forage crops being traditionally grown in the country. But yet no potential fodder maize drought tolerant variety have been screened and recommended for rainfed areas of Pothwar. Further, no investigations have been undertaken on the use of plant growth regulators (GA_3) and trace elements (Se) for improving the maize fodder yield and fodder/ silage quality. Hence, the current study was undertaken to screen the best performing maize genotypes (Out of 60 collected maize varieties/ hybrids) on the basis of their germination indices (In lab) and drought tolerance (in greenhouse) and then evaluated the effect of GA_3 and Se foliar application on maize fodder yield and fodder /silage quality under varied rainfall conditions of Pothwar.

During the first year (2018-19) among the collected genotypes, seven best performing maize genotypes (BPMG) viz. Sohni Dharti-623, Pahari, Afgoi, Sarhad yellow, C-905, Zaitoon Maize-

7786 and PSHW-0404 Hybrid, were screened in the lab (Petri dish experiments) on the basis of their germination indices. Then among the 7 BPMG, two best performing were selected against the drought stress developed by using varied levels of PEG hydroponically in the greenhouse.

During the second year (2019-20) two best performing variety/ hybrids (Afgoi, and Sohni Dharti-623) were tested at three different locations (with varied rainfall) of NARC, Islamabad, URF Koont, Gujjar Khan and Groundnut Research Station (GRS), Attock. The foliar application of graded combinations of GA_3 and Se at 4 leaf stage of fodder maize was done at all the three locations.

Analyzed data during the second year indicated that different treatments viz. varieties/hybrids (Afgoi, and Sohni Dharti-623), growth regulators levels (GA_0+Se_0, GA_{75}+$Se_{0.75}$ and GA_{50}+$Se_{0.50}$) and locations (NARC, Islamabad), URF Koont, Gujjar Khan and GRS, Attock) had significant effect on the overall agro-morphological parameters of maize plant at 3 locations, Of the two, the variety Afgoi performed better regarding the agro morpho parameters than Sohni Dharti-623.The climatic conditions at NARC, Islamabad were more conducive to fodder/grain maize production in rainfed regions of Pothwar followed by URF Koont, Gujjar Khan. Similarly, all agro-morphological parameters were improved significantly by GA_3 and Se @ GA_{75}+$Se_{0.75}$ followed by @ GA_{50}+$Se_{0.50\ at}$ three rainfall locations of Pothwar.

Similarly, for quality paramters (total dry matter, total digestible nutrients, Crude protein, neutral detergent fiber, Crude fiber acid detergent fiber, ash contents) the Afgoi was found superior to Sohni Dharti 623. Among locations, the above quality traits were recorded more at NARC followed by URF Koont and GRS, Attock which were comparable to each other. Among the graded levels of GA_3+Se, the combination/ level GA_{75}+$Se_{0.75}$ was found the most effective followed by GA_{50} +$Se_{0.50}$ at three locations.

During the third year (2020-21) the experiment (second year-2019-20) was repeated at aforesaid three locations at research stations (for data collection) as well as at farmer's field (for demonstration purpose). The results were demonstrated during the farmer's gathering. The data were recorded, analyzed which was statistically in coincidence with the second-year results (2019-20) regarding the agro-morpho and quality traits of the maize fodder / silage.

Publication / Patent Applications

- Rasheed, M., A. Hassan., M. B. Ali, M. Hussain and M. A. Hanif. 2021. Exogenously applied gibberellic acid improves growth of fodder maize (Accepted for publication in JAR-59 (4) (Acceptance letter attached).
- Muhammad Rasheed, Hussain Shah, Hafiz Muhammad Zia Ullah Ghazali, Saqlain Yaqoob, Hamid Shehzad, Salma Shujat and Masaood Khan.2021. Screening effect of fodder maize varieties under water stress conditions. Annals of plant sciences , 10(12) : 4350-4371
- Production technology of Fodder maize for Barani areas
- Preparation technology of Maize Fodder Silage.

Graduate Degrees:

One student completed his M. Sc. (Hons.) degree Agronomy thesis research in this project.

3.2.6 Demonstration of Technologies and Training of Growers for Handling and Value Addition of Fruits in Gilgit-Baltistan		
Name of PI/Institute and designation:	**Faizullah Khan** *Scientific Officer,* PCSIR Laboratories	
Postal Address:	PCSIR Laboratories Satpara Road Skardu, GB Telephone: 05815-920305 Fax: 05815-920259 E-mail: faizpcsir@gmail.com	
Duration:	02-11-2017 to 01-11-2020	
Financial Status:	Total Cost: Funds Released: Funds Utilized:	Rs. 4.979 million Rs. 4.736 million Rs. 4.735 million

Objectives:

- To control pre and post-harvest losses of fruits in Gilgit-Baltistan through demonstration of technologies and training of farmers/growers on fruit processing preservation, dehydration, value addition and use of fruit processing machinery.
- Income generation through sale of developed value-added products.
- To control food security issue of Gilgit-Baltistan.

Achievements:

The basic aim of project is to control pre and post-harvest losses of fruits in Gilgit-Baltistan through demonstration of technologies and training of farmers/growers on fruit processing, preservation, dehydration and value addition. Processing booklet

سکر دو کے وضع کردہ طریقوں سے پھلوں کو محفوظ کرنا اور ان سے گھریلو پیمانے پر مصنوعات تیار کرنا

PCSIR and fruit survey forms printed during first year was given to each trainee as training material.

Conducted 40 group training courses each of two days titled “Two Days Fruit Handling, Processing, Preservation, Dehydration, Value-addition and use of Fruit Processing Machinery Training” and trained total twelve hundred and eleven fruit growers/ farmers in Fruit Handling, Processing, Preservation, Dehydration, Value-addition and use of Fruit Processing Machinery at ten districts during the three years project period. In each district two days training was given in four different locations for four different groups in each group there was 30 potential farmers/growers and total 120 farmers trained from one district and grand total 1211 from over all the (ten districts of Gilgit-Baltistan. The training was conducted at house hold level in development of fruit pulp, fruit jam, tomato paste, vegetable pickle and organic and inorganic dehydration of fruits. The farmers practically performed all the value-added product development activities to get hand on its development. They also trained in use of fruit processing machinery that is fitted in MTU (Mobile Training Unit). During the COVID-19 Pandemic (from March 2020 to November 2020) twelve group trainings conducted with main focused on COVID-19 awareness. The

title of training changed i.e. “Preventive and Precautionary Measures for COVID-19 and Fruit Processing, Preservation, Dehydration, Value Addition, use of Fruit Processing Machinery”. The training started with hand washing and hand sterilization, PPEs (personal protective equipments) Facemask, gloves and head cap given to each trainee. First lecture delivered on Preventive and Precautionary Measures for COVID-19 and practically demonstrated seven steps of hand washing and properly followed SOPs of social distancing.

The training was given in collaboration with Agriculture Department Gilgit-Baltistan, MARC Juglot, and Welfare and Development Organizations. Before Training MoUs were signed between Agriculture Department Deputy Directors and Principal Investigator Project and presidents of welfare and Development Organizations.

1128 Fruit survey questionnaires filled for total fruit production, fruit losses and income generated through sale of fresh fruit and value-added products before fruit processing training in Urdu (فروٹ پروسیسینگ ٹرینگ سے پہلے) was collected during 2nd day of training during the reporting period. The survey filled questionnaires data analyzed and average losses calculated. According to this survey total average fruit losses/wastages of fruits in Gilgit-Baltistan is 34.87%, income received through sale of fresh fruit is only 7% of fresh fruit sold out and 5% of fresh apricot processed for dehydration. other product development i.e. preservation of pulp, jam, tomato ketchup and tomato paste manufacturing is 1%.

District Kharmang four group trainings post training survey conducted and 108 post training questionnaires filled data analyzed and average wastages/losses of fruits and income generation through sale of fruits and developed products compiled. According to this post training survey total average fruit losses/wastages of fruits in district kharmang is 17.2%, income received through sale of fresh fruit is 11%, income received through sale of value added product i.e. fruit Jam and Dehydrated apricot is 22%. The data shows that the fruit processing trainings has great impact in increase in income generation of farmers and to control fruit losses/wastages.

P.I Project Practically Demonstrating Seven Steps of Hand

P.I project demonstrating how to fill fruit survey forms for total production, total losses, wastages and total income of fruits

Publications:

Demonstration of Technologies and Training of Growers for Handling and Value Addition of Fruits in Gilgit-Baltistan**.** Faizullah Khan, MSc1*; Tariq Umar Khan, Msc1; Muhammad Ayub, Msc2,3; Tajudin, MSc2,3; Rehmat Kabeer, MSc. Adv Food Technol Nutr Sci Open J, January 2019.

Graduate Students/Degrees:

- Two student of M.Sc. (Hons) completed their degrees under this project.

3.2.7 Collection, Conservation and Documentation of Traditional Heirloom Crop Germplasm of Pakistan		
Name of PI/Institute and designation:	**Dr. Tariq Rafique** Senior Scientific Officer, BCI, PGRP	
Postal Address:	Plant Genetic Resources Program (PGRP), BCI NARC, Park Road, Islamabad Telephone: 9073-3717 Fax: 9255201 E-mail: tariqrafique@parc.gov.pk	
Duration:	01-02-2018 to 30-06-2021	
Financial Status:	Total Cost: Funds Released: Funds Utilized:	Rs. 6.487 million Rs. 5.921 million Rs. 5.903 million

Objectives:

1. To collect and expand germplasm of existing heirloom traditional crops of economic significance from diverse ecologies of Pakistan.
2. To characterize the collected germplasm for various agromorphological traits and develop pre-breeding lines for traits of interest in the context of climate change adaptation.
3. To conserve selected heirloom fruit crops of economic significance using in vitro techniques.
4. To compile and establish a database of heirloom germplasm and associated traditional knowledge for intellectual property rights (IPR) issues.

Achievements:

Diverse genetic resources are important for maintaining an efficient and sustainable farming industry, as they allow the development of varieties to cope with new demands. It is pertinent to mention that today many traditional plants face extinction or severe genetic loss, but detailed information about their genetic diversity is lacking. Current project was aimed at devising strategies for conservation and sustainable use of traditional heirloom crop varieties of Pakistan so that local genetic resources can be efficiently utilized. To achieve this as per work plan, more than 1941 accessions of indigenous germplasm of field crops (including barley, buckwheat, common bean, faba bean, guar, maize, millets, rice, sesame, sorghum), vegetable crops (i.e., carrot, chili, cucurbits, fenugreek, onion, Sonchal, tomatoes) and 293 accessions of indigenous fruit crops (including apples, apricot, grapes, peach, pear) were collected from diverse ecologies having marginal lands including AJK, some parts of Balochistan, Chitral, Gilgit-Baltistan, Khyber Pakhtunkhwa, Punjab (Cholistan, Potohar, Thall), Thar desert of Sindh during the project period. Diverse gene-pool of said species has enhanced the existing limited genetic resources of traditional heirloom germplasm in Pakistan. Identification of different genotypes of crop plants is important when new crop cultivars are to be released, newly developed germplasm is to be registered, different accessions of wild relatives are to be characterized and purity of the cultivars is to

be determined. A total of 96 accessions of common-bean, 70 of guar, 90 of maize and 430 accessions of rice, 139 accessions of barley, 167 accessions of fenugreek, 100 accessions of sorghum, 47 accessions of chilies and 33 accessions of carrot were characterized under field conditions to assess variation for agro-morphological and traits of economic significance. A considerable level of intra-specific variability was detected among the accessions used based on various quantitative and qualitative agro-morphological traits. Correlation coefficient analysis suggested that many of the related traits had significant positive correlation with one another. On the basis of early flowering &maturity, leaf, pod & seed size/weight, branches per plant, improved plant stature and greater yield potential promising germplasm has been identified for future variety development programs. Clonal respository and field gene-bank of traditional heirloom vareties of targetted fruit crops varieties of apple, apricot, grapes, peach and pear has been established and. In vitro conservation of selected varieties of fruit species for medium-term storage and back-up is being carried out. Studies are being performed for standardization of protocols to establish in vitro cultures, multiplication and mid-term storage of established cultures. Explants from selected fruit germplasm from northern areas have been collected; standardization of surface sterilization and establishment of in vitro cultures on medium with different formulations of hormones was carried out. In vitro multiplication, culture maintenance and placement on slow growth medium for mid–term storage were carried out. A comprehensive national database including local name, local varieties name, cropping pattern, uses, specific traits, etc. of indigenous heirloom varieties of field and horticultural crops is being prepared with a view to assess their use and status within key ecosystems. Information has been assembled of the persistence of traditional heirloom field and horticultural crop species in AJK, Balochistan, Chitral, FATA, Gilgit-Baltistan, KPK, etc, and document heirloom varieties that are still being grown. A comprehensive inventory of 3 traditional heirloom varieties of field and horticultural crops currently used by the inhabitants of the target regions is being established for the protection of famous agriculture products as geographical indications and documentation of associated traditional agricultural knowledge.

e-1: Diversity in heirloom maize germplasm collected from Chitral, KPK.

Fig 8: a) In vivo propagation in sand, b) Showing roots and shoots, c) Ex situ conserved of peach in pots.

Fig 8: a) In vivo propagation in sand, b) Showing roots and shoots, c) Ex situ conserved of peach in pots.

3.2.8: "Genetic Transformation of Chickpea for Herbicide Resistance"		
Name of PI/Institute and designation:	**Dr. Shaukat Ali** PSO NIGAB,	
Postal Address:	National Institute for Genomics and Advanced Biotechnology (NIGAB), NARC, Park Road, Islamabad Telephone: 05190733805, Email: shaukat_parc@yahoo.co.in	
Duration:	31.07.2018 to 30.06.2021	
Financial Status:	Total Cost: Funds Released: Funds Utilized:	Rs.5.93 million Rs.5.93 million Rs.5.803 million

Objectives:

The main goal of the proposed project was the development of herbicide resistant transgenic lines of chickpea mediated by Agrobacterium tumefaciens methodology.

Specific Project Objectives:

- Synthetic modification of EPSPS gene and vector construction
- Incorporation of herbicide resistance gene into one desi and one kabuli variety.
- Evaluation of transgenic plants for target trait

Achievements:

- Optimized the in vitro regeneration protocols for Bittle 2016 and Noor 2013.
- Successfully optimized transformation protocols for Bittle 2016 and Noor 2013.
- Invitro transformation efficiency achieved for Bittle 2016 and Noor 2013 was 0.9 and 0.3 %, respectively.
- One novel inplanta transformation method was developed through which 2 % transformation efficiency was recorded for Bittle 2016.
- Patent application for novel method of transformation through meristem tip infection has been submitted to IPO, Pakistan.
- 3 transformation events for herbicide resistance in chickpea have achieved (2 of Bittle 2016 and 1 of Noor 2013).
- T1 and T2 generation of 3 transgenic events are successfully evaluated at molecular level (PCR and ELSIA) for the integration of herbicide resistance gene.

Publication:

- Title: Establishment of in vitro regeneration system in local Pakistani chickpea cultivars. Submitted to JAPS for publication.
- Title: Functional characterization of synthetically modified EPSPS gene in transgenic model plant. Submitted to SainsMalaysiana(In review)
- Title: Genetic transformation of herbicide resistant gene in local chickpea. Submitted to BMC Plant Biology
- One patent application for novel process of transformation through meristem tip infection has been submitted to IPO, Pakistan.

National / International Linkages Developed:

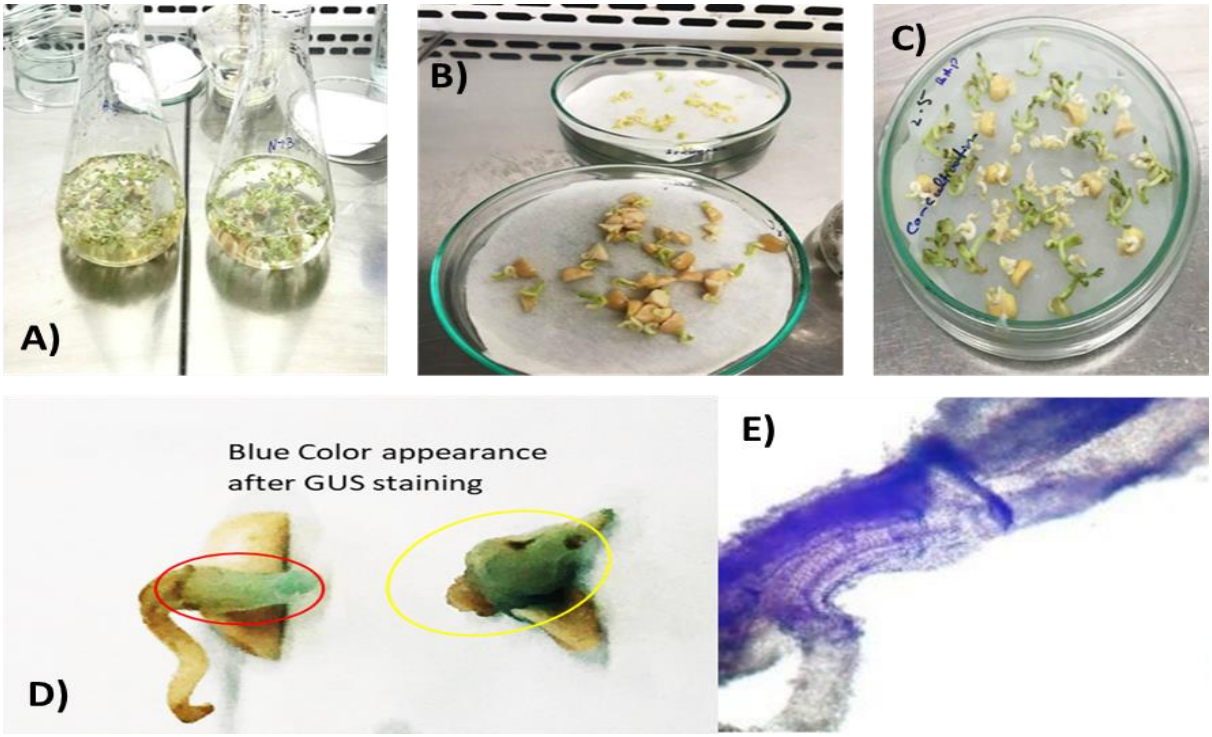
Department of plant sciences, QAU, Islamabad Pakistan.

Meanwhile, we have also in touch with Australian and Chinese scientists to develop strong link in future and for this purpose, PhD student, Ms Sarwat visited to CSIRO, Australia for carrying partial research work in chickpea transformation with Dr. T.J. Higgins.

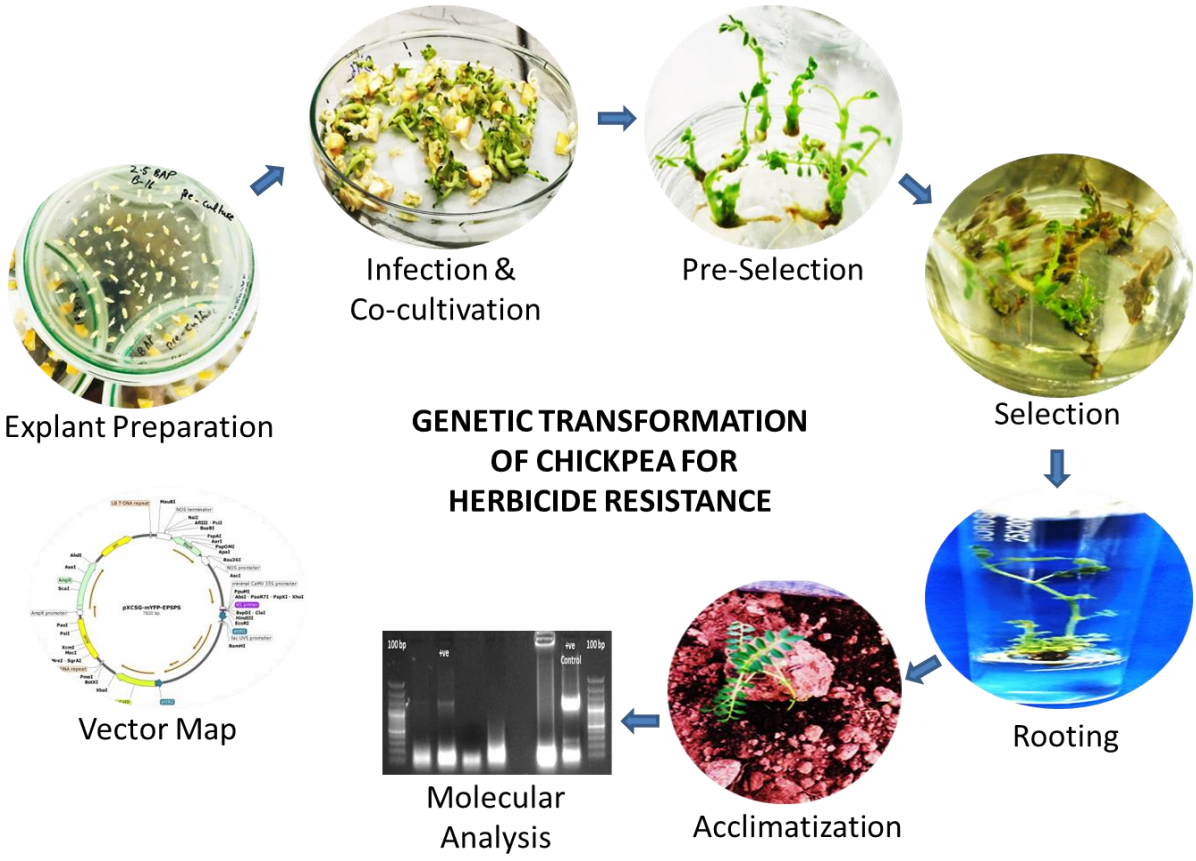
Degrees Awarded under the project:

One Ph.D. student, Ms. Sarwat Khan, enrolled in PIASA, NARC, worked on chickpea genetic transformation against herbicide. Her final thesis have been submitted, processed for foreign evaluation and will be awarded Ph.D. degree in near future.

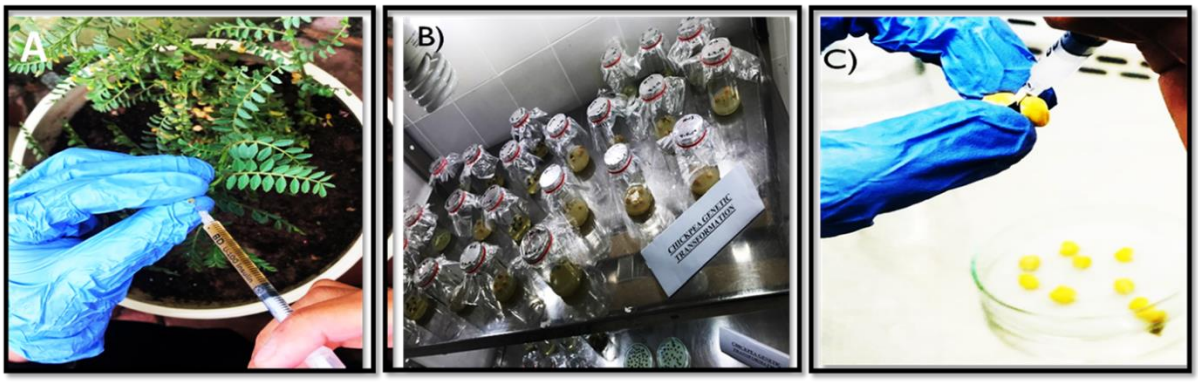
Invitro regeneration steps from multiple shoot regeneration to rooted plantlets, acclimatization and hardening stage



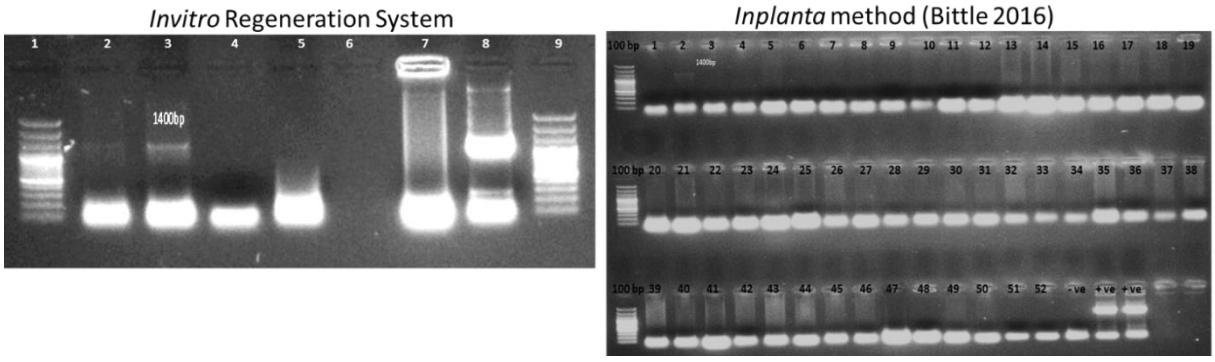
In-vitro Genetic Transformation step optimization via transient gene expression



In-vitro Gene transformation of herbicide gene in Chickpea



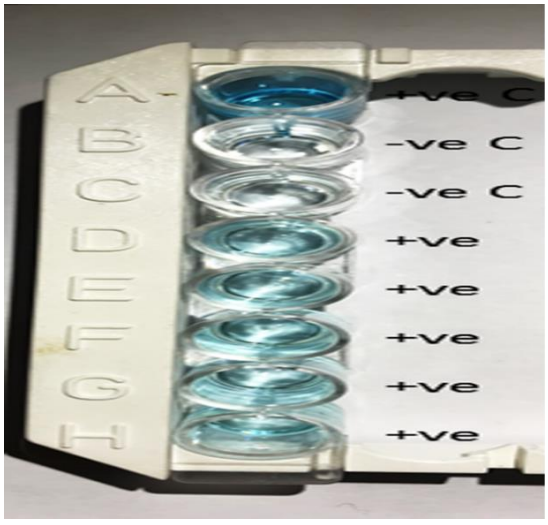
In planta Genetic transformation efficiency in chickpea *A) Flower injection method, B) Infection via seed imbibition, C) Infection on meristem tip*



T_o Generation Molecular Analysis (DNA/PCR based)

Invitro Regeneration System: PCR Screening Results for EPSPS gene in Chickpea, Lane 1 and 9: 100bp ladder Lane, Lane 2 and 3: Positive samples of Bittle 2016 and Noor 2013, Lane 5, 6 and 7: Negative samples, Lane 4: Negative Control, Lane 8: Positive Control

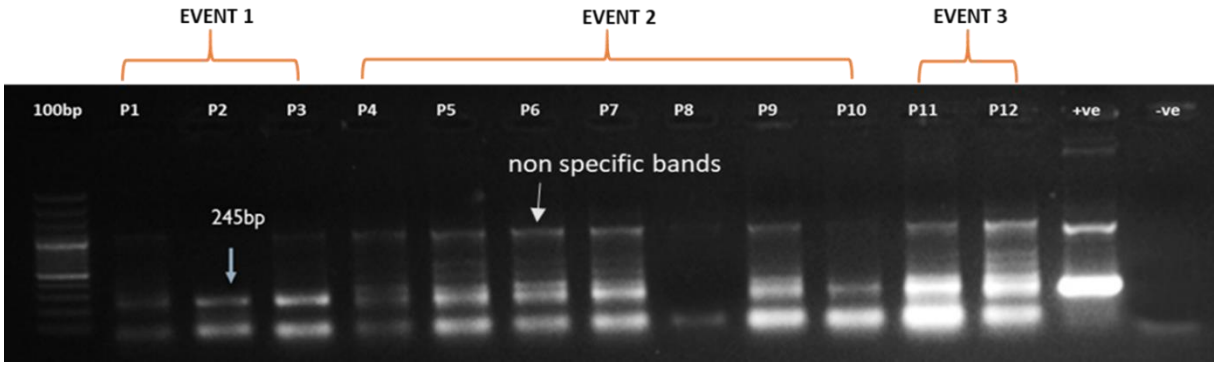
Inplanta method (Bittle 2016): PCR Screening Results for EPSPS gene in Chickpea plants obtained via inplanta method (meristem tip infection method)



Event 1 (Bittle 2016-*invitro*)
Event 2 (Bittle 2016-*inplanta*)
Event 3 (Noor *2013-invitro*)

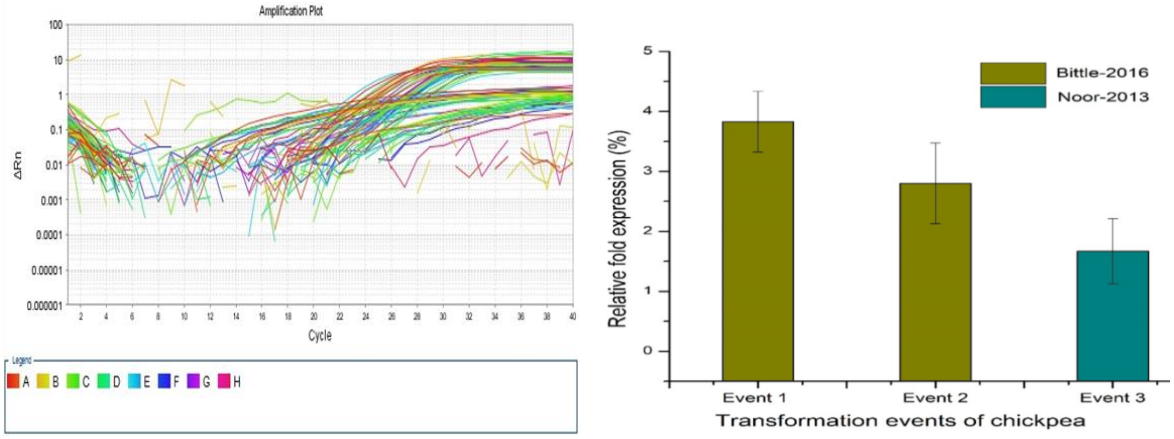
Samples	O.D
+ve	0.671
-ve	0.020
-ve	0.023
Event 1	0.21
Event 1	0.23
Event 2	0.20
Event 2	0.21
Event 3	0.17

T_o Generation Molecular Analysis (Protein based): EPSPS Expression Analysis via ELISA

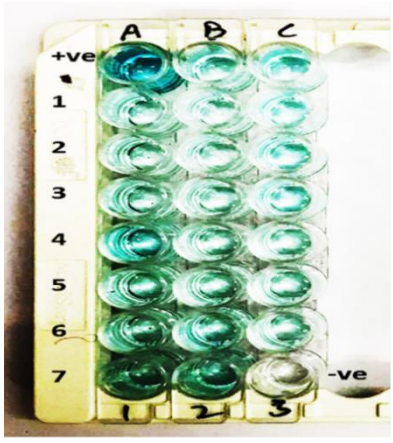


T_1 Generation Molecular Analysis (DNA/PCR based)

PCR analysis of T1 generation of three events (2 of Bittle 2016 and 1 of Noor 2013) in 12 plants (3 of event 1 (P1-P3) and 6 of event 2 (P4-P10) of Bittle 2016; 2 of event 3 (P11 and P12) of Noor 2013, +ve= Positive Vector Control, -ve= Negative Control



qPCR analysis of T_1 generation of three events



O.D	Samples	O.D	Samples	O.D	Samples
0.635	+ve	0.18	B-2016 P7	0.19	B-2016 P6 (R3)
0.14	B-2016 P1	0.19	B-2016 P9	0.17	B-2016 P7 (R4)
0.18	B-2016 P2	0.13	B-2016 P10	0.18	B-2016 P9 (R5)
0.17	B-2016 P4	0.16	N-2013 P11	0.15	B-2016 P10 (R6)
0.25	B-2016 P3	0.15	N-2013 P12	0.16	N-2013 P11 (R7)
0.16	B-2016 P5	0.13	B-2016 P1 (R1)	0.14	N-2013 P12 (R8)
0.20	B-2016 P6	0.19	B-2016 P2 (R2)	0.023	-ve

Event 1 (Bittle 2016-*invitro*)
Event 2 (Bittle 2016-*inplanta*)
Event 3 (Noor *2013-invitro*)

T_1 Generation Molecular Analysis (Protein based)

Positive ELISA Chickpea Samples O.D at 650 nm, +ve = Positive Control, -ve = Negative Control, P1-P3= Event 1 (yellow), P4, P5, P6, P7, P9 and P10 = Event 2 (green) of Bittle 2016, P11 and P12 = Event 3 of Noor 2013, R1-R7, R9, R10 replication of samples

Bioassay of transformed plants with non-transgenic control line

3.2.9: title of project: Collection and characterization of Phalsa (Grewia subinaequalis D.C.) landraces having high sugar and carotene contents		
Name of PI/Institute and designation:	**Dr. Kashif Razzaq** Assistant Professor, Department of Horticulture	
Postal Address:	Department of Horticulture, MNS-University of Agriculture, Multan Telephone:0345-7901236, Email: -kashif.razzaq@mnsuam.edu.pk	
Duration:	00.00.0000 to 00.00.0000	
Financial Status:	Total Cost: Funds Released: Funds Utilized:	Rs. 2.982 million Rs. 1.011 million Rs. 1.011 million

Objectives:

- Collection of different phalsa genotypes growing in different areas of Punjab
- Development of physico-chemical and nutritional profiling of phalsa genotypes
- Selection of genotypes having high sugar and carotene contents
- Conservation of selected genotype at research block of MNS-University of Agriculture, Multan (MNS-UAM)

Achievements:

- Total 170 phalsa genotypes from different phalsa growing areas of South Punjab including Multan, Khanewal, Vehari and Melsi were tagged on the basis of physical characteristics like Plant height, stem girth, fruit size, leaf length, leaf width leaf apex and leaf area.
- Difference in leaf on the basis of apex was observed from acute, acuminate, obtuse, and round. The leaf length varies from 12.35 cm to 23.3 cm while leaf difference in leaf width was recorded from 8.23 cm to 18.65 cm. A significant change in leaf area was also observed that varies from 109.23 cm to 235.98 cm.
- Among all genotypes, the plant height was varying from 5 feet to 13.4 feet and stem girth from 1.2 to 5 inches. The average fruit size differs from 10.12-15.6 mm.
- Biochemical characteristics including TSS ranges from (10-17.43°Brix), pH (2.19-3.67), TA (0.32-2.47%), Vitamin C (192-634.94 mg/100 ml, carotenoids (151.33-176.67 µg/g Fw), Ash (2.30-4.23%), crude fat (10.33%-23%), protein (14-29%), moisture (73.66-86.66%), total sugars (3.66-23%), reducing sugars (0.65-17.41%) and non-reducing sugars (1.50-8.16%).
- Establishment of research orchard of selected phalsa genotypes at research block of MNS-University of Agriculture, Multan (MNS-UAM)

Publication:

- Jamil, A., K. Razzaq, I.A. Rajwana, A. Naz, G. Akhtar, S. Ullah, H.N. Faried, S.B. Hussain, Y. Li, M. Amin, M.A. Sher, M.T. Altaf, H. Hassan, M.A.A. Ahmed, S. Alfarraj, M.J. Ansari. 2022. Characterization of indigenous Phalsa (Grewia subinequalis) genotypes using morphological traits and ISSR markers. Journal of King Saud University – Science (Minor revision submitted).

National / International Linkages Developed:

- Department of Food Science and Technology, MNS-University of Agriculture, Multan
- Department of Plant Breeding and Genetics, Bahauddin Zakariya University is located in Multan
- Phalsa Growers of South Punjab

Degrees Awarded under the project:

Three students completed their degree of MSc under this project.






3.2.10: Distribution pattern, race identification and tale based genetic diversity in Pakistani isolates of Xanthomonas oryzae pv. oryzae the causal organism of bacterial blight of rice		
Name of PI/Institute and designation:	**Dr. Muhammad Zakria** Principal Scientific Officer CDRI	
Postal Address:	CDRI, NARC, Park road, Islamabad Telephone: 3282 , Email: rmzakria@hotmail.com	
Duration:	01.07.2018 to 30.06.2021	
Financial Status:	Total Cost: Funds Released: Funds Utilized:	Rs. 9.182 million Rs. 7.389 million Rs. 7.250 million

Objectives:

- To determine the current status of bacterial blight (BB) disease in Pakistan
- To determine the race spectrum/pathoptyping of X. oryzae pv. oryzae (Xoo) in Pakistan.
- Xoo genotyping and the distribution of tale genes in X. oryzae pv. oryzae strains from Pakistan.
- Identification of source of resistance

Achievements:

- Status of BB in rice growing areas of Punjab, KPK and Sindh from 2019 & 2021 is available.
- More than 100 isolates of Xanthomonas oryzae pv. oryzae have been confirmed, verified and preserved. This is the largest collection of Xoo isolates in the country.
- Pathotype spectrum of 48 Xoo isolates based on NIL's is available. Among these 07 Pathotypes were identified. The pathotype 1 is the most dominant pathotype. Resistance gene xa13, Xa21 and Xa23 are effective. This is the first detailed pathotyping report from Pakistan. The information will help breeders in selecting resistance genes for breeding.
- Forty-eight Xoo isolates have been studied for the presence of tal genes by Southern blotting.
- Six tal genotypes have been identified so far. Genotype A, which is closely related to PXO99 from Philippines is the dominant one.
- Fourth eight Xoo isolates were studied using 11RAPD primers. Cluster analysis based on the similarity matrix, was performed using UPGMA (Unweighted Pair Group Method with Arithmetic mean) with NTSYSpc.v2.10e software. Eighteen monomorphic bands (10.23%) and 108 polymorphic bands (85.85%) for all the strains were found. Extensive

genetic variability was observed among the studied isolates. Isolates could be divided in 12 complex clusters.

- Three hundred and sixteen (316) lines were tested against BB. One thirty-eight (138) lines were found resistance against BB.

Publication:

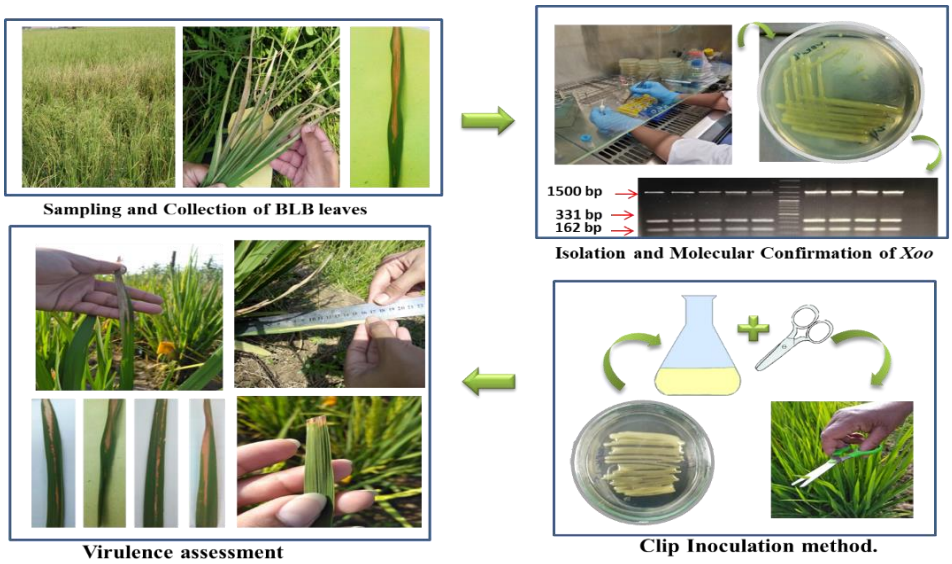
- Ahsan, R., S. Ullah, I. Yaseen, F.S. Fateh, M. Fayyaz, S. Asad, A. Jamal, M. Sufyan and M. Zakria. 2021. Assessment of bacterial leaf blight incidence and severity in rice growing areas of Pakistan. Pakistan Journal of Agricultural Research, 34(4): 693-699.
- Ahsan Rafia, Saif Ullah and Zakria Muhammad. 2022. Genetic variability among Xanthomonas oryzae pv. oryzae (Xoo) population in Pakistan by RAPD-PCR. Abstract submitted 14th International Conference on Plant Pathogenic Bacteria. Assisi (Italy) 3-8 July, 2022.

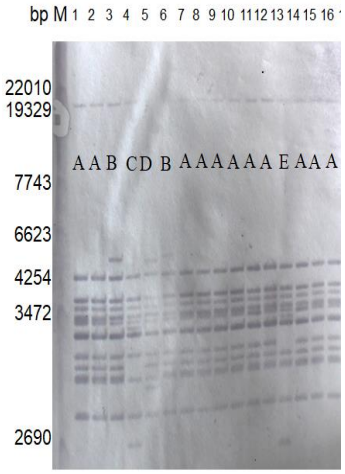
International Linkages Developed:

3-107 School of Agriculture and Biology, Shanghai, Jiaotong University, 800 Dongchuan Road, Shanghai, 200240, China. Tel: 0086-21-34205873 Fax: 0086-21-34205873 E-mail: gyouchen@sjtu.edu.cn

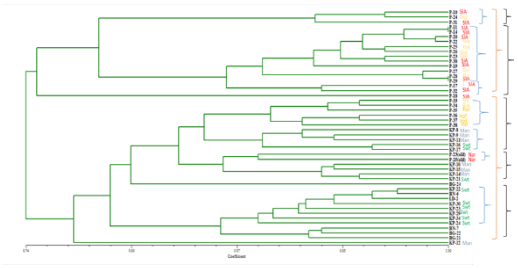
Degrees Awarded under the project:

- One PhD student from QAU (On-going)
- Six B.Sc (Hons.) students completed their internship





Tal effectors shown by Pakistani Xoo strains based on Southern blotting



Genetic relatedness and diversity of Xoo strains

3.2.11: Identification of parasitoids and ants complex associated with Psyllids on trees and management of olive psyllid in Pothwar and KPK.		
Name of PI/Institute and designation:	**Imran Bodlah** Assistant Professor	
Postal Address:	Department of Entomology, PMAS-Arid Agriculture University Rawalpindi Telephone: 03229758287, Email: imranbodlah@gmail.com	
Duration:	01.02.2018 to 31.01.2021	
Financial Status:	Total Cost: Funds Released: Funds Utilized:	Rs. 3.142 million Rs. 1.310 million Rs. 1.196 million

Objectives:

- Collection and identification of Psyllids.
- Collection and identification of ants associated with Psyllids round the year.
- Collection and identification of host plants of Psyllids.
- Collection and identification of parasitoids of Psyllids.
- To compare the toxicity and time of application of selective insecticides against Olive psyllid and its Bio-control agents in field conditions
- To find out the most effective insecticide for management of Olive psyllid and safety to bio-control agents.

Achievements:

- 23 species of psyllids including 17 species as new country records were added to insect fauna of Pakistan. Among these, Blastopsylla occidentalis Taylor, 1985, Platyobria biemani Burckhardt et al., 2014, Cornopsylla zanthoxylae Li, 1994, Yangus hyalinus (Mathur, 1975), Cacopsylla bidens (Šulc, 1907) and Cacopsylla zaicevi (Šulc, 1915) are invasive species recorded from Pakistan for the first time.
- 17 species of psyllid parasitoids in 9 genera were identified, including 5 species described as new to science Apocharips pakistanensis sp. n; Aprostocetus rawalpindiensis sp. n; Bracon punjabensis sp. n; Tamarixia bodlahae sp. n. and Tetrastichus mehrii sp. n. Moreover, 11 species belonging to different genera were recorded for the first time from Pakistan.
- 16 species of ants belonging to 10 genera were collected from various areas of study areas, all species are new record for Pakistan in association with different species of Psyllids. Plagiolepis jerdonii Forel, 1894 Crematogaster brunnea contemta (Mayr, 1879) and Crematogaster subnuda (Mayr, 1879) are recorded for the first time from Pakistan.

Lepisiota fraenfeldi (Mayer, 1855), Tapinoma melanocephalum (Fabricius, 1793), Crematogaster biroi (Mayr, 1897) and Trichomyrmex destructor (Jerdon, 1851) were recorded as invasive species in these studies.

- Five insecticides including diflubenzuron (Delphix 200ml/acre), imidacloprid (Imidacloprid 250ml/acre), chlorpyrifos (Helmet 400ml/Ltr), bifenthrin (Bifenthrin 250ml/acre) and clothianidin (Clothianidin 200ml/acre) were tested for the comparison of their toxicity their against olive psyllid and their parasitoids in laboratory and field conditions. Our studies concluded that the wise use of the insecticides in effective control of olive psyllids and clothianidin was the best insecticides to maintain psyllid population under threshold levels. Neonicotinoid insecticides may be used for their systemic nature to kill olive psyllids and comparatively bit safety to bio-control agents for their role in natural ways.

Publication:

- Rasheed, M. T., I. Bodlah, M. F. Nasi1 and T. Mahmood. 2021. New Record of Jumping Plant Lice, Trioza hirsuta (Hemiptera: Triozidae) and its Associated Parasitoid Psyllaephagus phylloplectae (Hymenoptera: Chalcidoidea: Encyrtidae) from Pakistan. International Journal of Agriculture and Biology 25(6):1383-1388.
- Imran Bodlah, Muhammad Tariq Rasheed, Muhammad Farooq Nasir, Tariq Mahmood and Muhammad Asif. 2021. Biology and distribution of Palaeolindbergiella simlae (Crawford, 1912) (Hemiptera: Psyllidae) along with its associations with ants from Pothwar region of Pakistan.P.J.Z.1-6.
- M. T. Rasheed, I. Bodlah1, M. F. Nasir, T. Mahmood, R. Zada and M. Asif. 2022. Addition to the fauna of psyllid (Hemiptera: Psylloidea) in Pothohar region of Pakistan. The J. Anim. Plant Sci., 32 (1). http://www.thejaps.org.pk/Volume/2022/32-01/32.php

National Linkages Developed:

Saighol Farm House Kallar Kahar was involved in our project activities related with olive Psyllids and related activities. Research collaboration was developed with Dr. Basit Rasheed (University of Peshwar) for ants identification confirmation. International research linkage was developed with the lab. of Professor Xiaolei Huang (China). Scientific relations were developed with various scientist from India and Turkey working on Pysilds and their parasitoids.

Degrees Awarded under the project:

- One Ph. D student completed research under this project.

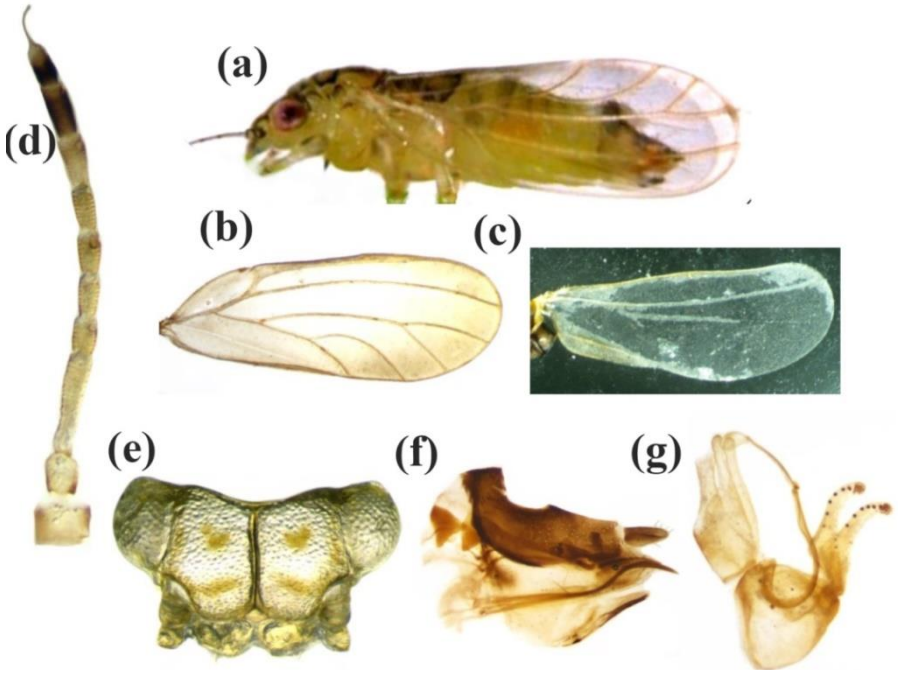


Figure 1: Blastopsylla occidentalis (a-g); (a) Habitus, female (lateral aspect) (b) Fore wing (c) Hind wing (d) Antenna (f) Female terminalia (lateral aspect) (g) Male terminalia (lateral aspect).

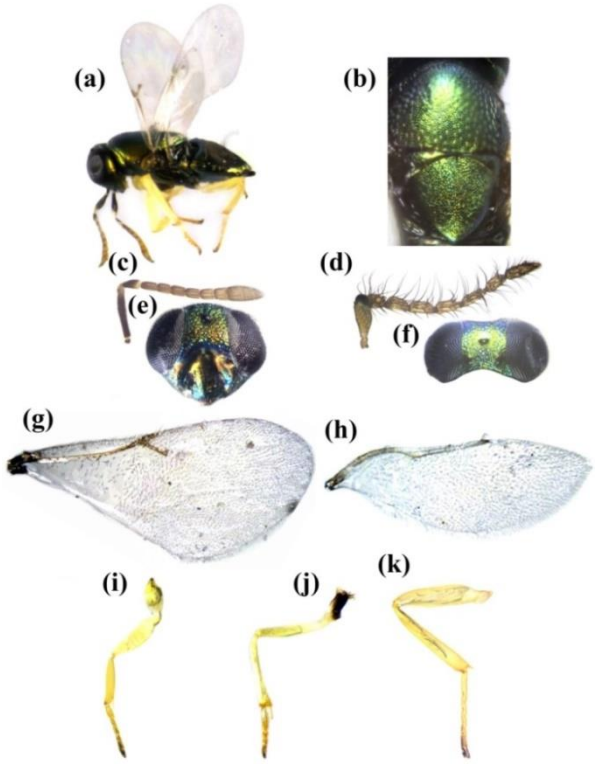


Figure 2: Psyllaephagus mesohomotoma (a-k) (a) Habitus, female (lateral view) (b) Mesosoma (dorsal view) (c) Antenna (female) (d) Antenna (male) (e) Head (frontal view) (f) Head (dorsal view) (g) Fore wing (h) Hind wing (i) Fore leg (j) Meso leg (k) Hind leg.

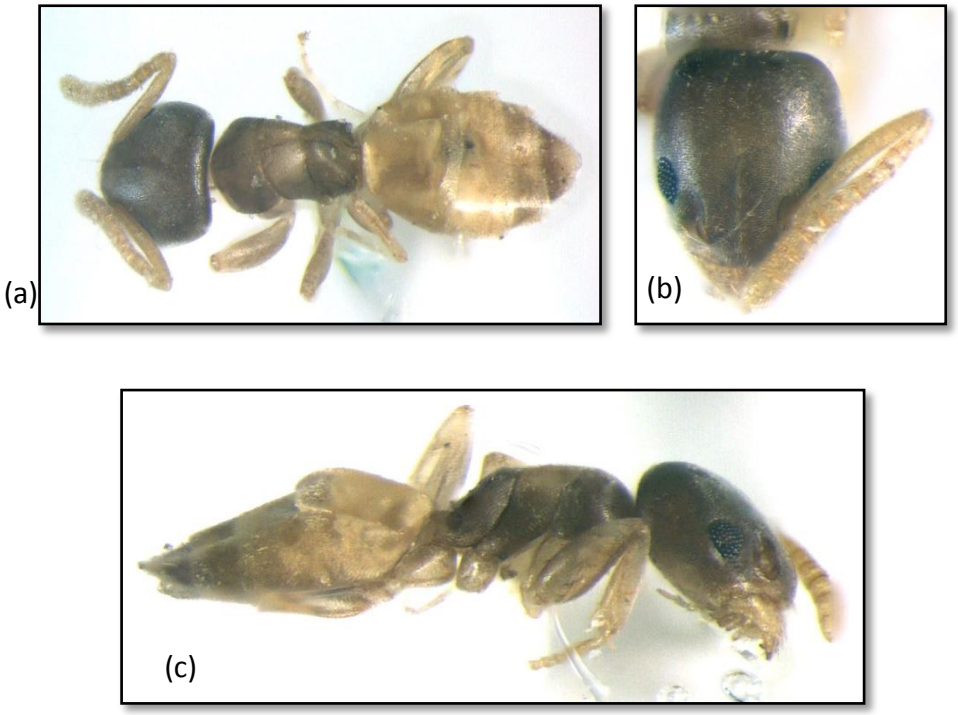


Fig. 3 External Morphology of Tapinoma melanosephalum (a) Frontal view(b) Dorsal view (c) Lateral view

3.2.12: Post-harvest grain losses management for food security		
Name of PI/Institute and designation:	**Dr. Waqas Waqil** Assistant Professor *Department of Entomology*	
Postal Address:	Department of Entomology, University of Agriculture, Faisalababad Telephone: 0333-5154955, Email: waqaswakeel@hotmail.com	
Duration:	15.11.2017 to 14.11.2020	
Financial Status:	Total Cost: Funds Released: Funds Utilized:	Rs. 3.918 million Rs. 3.390 million Rs. 0.057 million

Objectives:

- To determine the changes in the distribution of phosphine resistance in T. castaneum, R. dominica, T. granarium and S. granarius and screen various insect pathogenic fungal and B. thuringiensis isolates/strains and/or formulation/s against stored grain insect species (T. castaneum, R. domincia, T. granarium, S. granarius)
- To integrate different management practices (insect pathogenic fungi, B. thuringiensis, bio-rational new-chemistry insecticide/s and diatomaceous earth/s) in possible manner so to develop an effective and successful IPM program for the environment friendly management of stored grain insect species
- To study the residual efficacy of all control tactics (insect pathogenic fungi, B. thuringiensis, bio-rational new-chemistry insecticide/s and diatomaceous earth/s) in stored wheat against insect species
- To disseminate the techniques for the implementation of IPM module against stored grain insect species on their farms through production of printed extension material etc.

Achievements:

The long term and consistent use of phosphine fumigation for the control of stored grain insect pests lead to resistance development in insect pests. Limited studies are reported in Pakistan to determine the level of resistance among different coleopteran insect pests against phosphine. To determine the changes in the distribution of phosphine resistance in Rhyzopertha dominica, Tribolium castaneum, Trogoderma granarium and Sitophilus granarius, ten geographically different populations of these insect pests was subjected to phosphine gas to determine their resistance level. All of the 40 populations (ten populations of each species) were found resistant and Probit analysis estimated LC_{50} was 2.85, 1.90, 2.54 and 2.01 ppm for laboratory-susceptible populations of R. dominica, S. granarius, T. castaneum and T. granarium, respectively. The major insect pathogenic fungus (Beauveria bassiana and Metarhizium anisopliae) isolates were recovered from soil samples and from cadavers of different stored grain insect pests. Most potential isolate caused highest percentage of adult and larval mortality (almost 100%) after 21 days post-application at highest conidial concentration. To develop an effective and successful IPM program for the

environment friendly management of stored grain insect species, series of experiments were conducted to evaluate the insecticidal efficacy of different new-chemistry insecticides and diatomaceous alone and in combination with entomopathogenic fungi at three different temperature regimes 20°C, 25°C and 30°C. Among all the tested species, greater mortality was observed at 30°C followed by 25°C and 20°C either applied alone or in combination of all the control agents. Along with mortality, all the species significantly reduced the number of adults produced compared to untreated control group. The residual efficacy level of all treatments produced greater mortality at 0 day of post-exposure period while reduction in mortality was observed with the passage of time until last exposure (180 days). Under field conditions, all the treatments produced greatest mortality at initial exposure interval and their efficacy was found decreased with the passage of time but even at last exposure interval of post-treatment significant mortality was observed. The current research clearly suggested that stored grain insects have developed phosphine resistance, so there is need to develop alternative sustainable and environment friendly control strategies. Insect pathogenic fungi can be augmented with other control tactics for long term protection of stored-product commodities – the combination of fungi with diatomaceous and new-chemistry insecticides may provide higher mortality level and reduce progeny production compared to alone application of control agents against all tested stored product insect pests to ensure food security in Pakistan.

Publications:

- Wakil, W., N.G. Kavallieratos, E.P. Nika, M.A. Qayyum, T. Yaseen, M.U. Ghazanfar, M. Yasin. 2022. Combinations of Beauveria bassiana and spinetoram for the management of four important stored-product pests: laboratory and field trials. Environmental Science and Pollution Research. 29: 34912-34929 (IF 4.22) -Under Review-
- Wakil, W., N.G. Kavallieratos, M.U. Ghazanfar, M. Usman. 2022. Laboratory and field studies on the combined application of Beauveria bassiana and fipronil against four major stored-product coleopteran insect pests. Environmental Science and Pollution Research. 29: 34912-34929 (IF 4.22)
- Wakil, W., N.G. Kavallieratos, M.U. Ghazanfar, M. Usman, A. Habib, H.A.F. El-Shafie. 2021. Efficacy of different entomopathogenic fungal isolates against four key stored-grain beetle species. Journal of Stored Products Research. 93. https://doi.org/10.1016/j.jspr.2021.101845 (IF 2.64)
- Wakil, W., N.G. Kavallieratos, M. Usman, S. Gulzar, H.A.F. El-Shafie. 2021. Detection of phosphine resistance in field populations of four key stored-grain insect pests in Pakistan. Insects. 12: 288. https://doi.org/10.3390/insects12040288 (IF 2.76)

National / International Linkages Developed:

- We have established good scientific and a fruitful collaboration with Dr. Nickolas G. Kavallieratos and Erifili P. Nika at Laboratory of Agricultural Zoology and Entomology, Department of Crop Science, Agricultural University of Athens, Attica, Greece

- This project helped to strengthen the collaboration with Dr. Muhammad Sufian (Department of Entomology, University of Agriculture, Faisalabad), Dr. Muhammad Usman Ghazanfar (Department of Plant Pathology, University College of Agriculture, Sargodha University, Sargodha); Dr. Mirza Abdul Qayyum (Institute of Plant Protection, MNS University of Agriculture, Multan) and Dr. Muhammad Yasin (Department of Entomology, Faculty of Agriculture and Environment, The Islamia University, Bahawalpur), Pakistan

3.2.13: Molecular Characterization & Management of Citrus Greening Disease in Hazara Division of Khyber Pakhtunkhwa, Pakistan.		
Name of PI/Institute and designation:	**Dr. Maimoona Sabir** Assistant Professor *Department of Microbiology*	
Postal Address:	Department of Microbiology, The University of Haripur. Telephone: 0995-615075/ 0345-0733682, Email: monasabir@uoh.edu.PK	
Duration:	15.11.2017 to 15.11.2020	
Financial Status:	Total Cost: Funds Released: Funds Utilized:	Rs. 5.735 million Rs. 4.086 million Rs. 4.047 million

Objectives:

- Field Survey: Collection of samples to analyze the frequency of citrus greening disease in local citrus orchards. Identification of symptoms of disease in different varieties of citrus in the different area of Hazara Division, Khyber Pakhtunkhwa, Pakistan.
- Isolation and characterization of bacteria Candidatus Liberibacter, causative agent of Huanglongbing/citrus greening disease.
- Molecular characterization of pathogen by Polymerase Chain Reaction, PCR (using universal primer and specific primer), gel electrophoresis and direct sequencing.
- Management of disease-causing agents through awareness seminar among local growers of citrus.

Achievements:

- First Molecular biology lab was established in The University of Haripur, funded by ALP-PARC, which will benefit students of all department for their research activities at molecular level. Lab is equipped with all basic Molecular Biology equipment required for
- Survey helped and benefit citrus growers to made orchard disease free and samples were collected for molecular analysis and confirmation of disease.
- Results obtained from current study are under review in peer review journal of international repute.
- One M.Phil. scholar and one MSc student successfully completed their degree, and 8-10 departmental students successfully completed their degree by using laboratory equipments. No of departmental students are using equipments for practical classes.
- A public/farmer awareness campaigns were developed, including training materials, information pamphlets and posters on symptomology and

management. Awareness was also done via training video on HLB and its management. An awareness seminars were scheduled and conducted successfully dated 24 Dec, 2019 and series of seminar dated 20 January to 31 January 2020.

- Citrus farmers gained knowledge and developed skills to control the disease from these awareness seminars. That will also help in future to improve citrus production.

Publication:

- Naveed Shah, Faisal zaman, Sobia Nisa, Maimoona Sabir, Waqas ahmed, Sheikh Muhammad iqbal Incidence of Citrus greening in District Haripur, KP, Pakistan. (Submitted).
- Faisal zaman, Sobia Nisa, Maimoona Sabir, Waqas ahmed, Abdual Qayyum. Molecular Identification of Candidatus Liberibacter Causing Citrus Greening Disease in District Haripur, Pakistan (Submitted to Plos One).

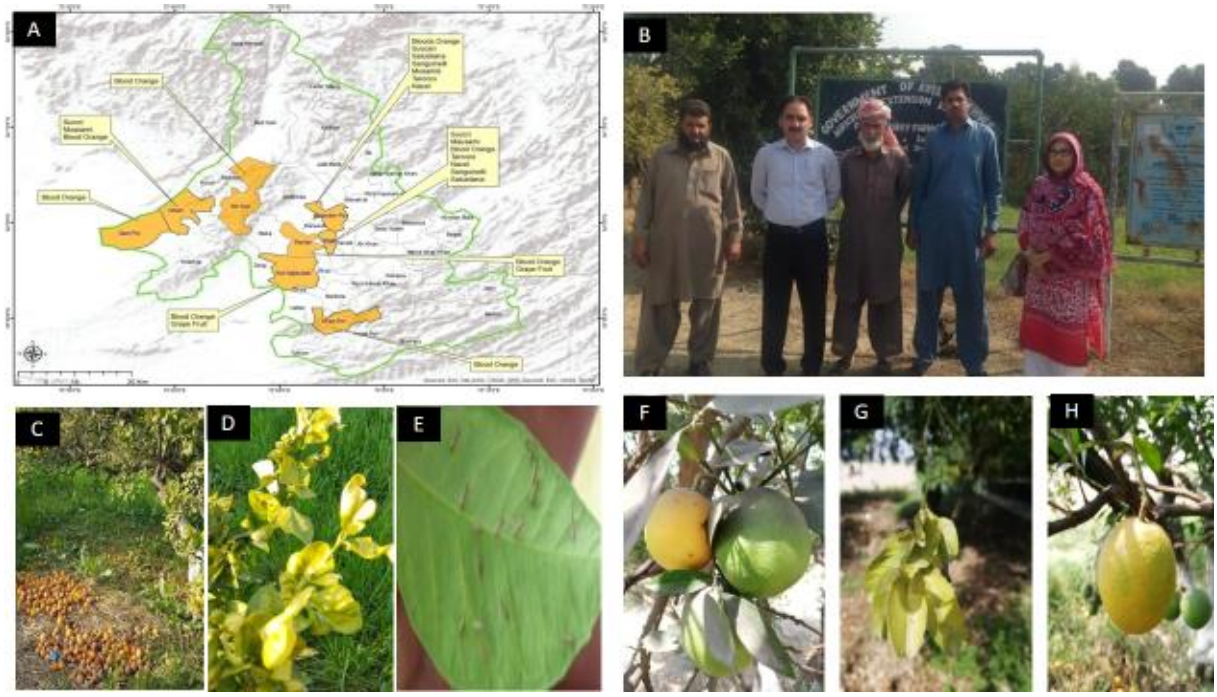
National / International Linkages Developed:

- Hazara University, Manshera
- NARC, Islamabad
- COMSATS, Abbottabad

Degrees Awarded under the project:

M.Phil. Scholar successfully completed his degree. Thesis Title, “Molecular Identification of Candidatus Liberibacter Causing Citrus Greening Disease in District Haripur”.

- MSc Student successfully completed his degree. Tile of his thesis was, “Isolation of Candidatus Liberibacter asiaticus causing citrus greening disease in Haripur”.
- PhD Scholars (In Progress).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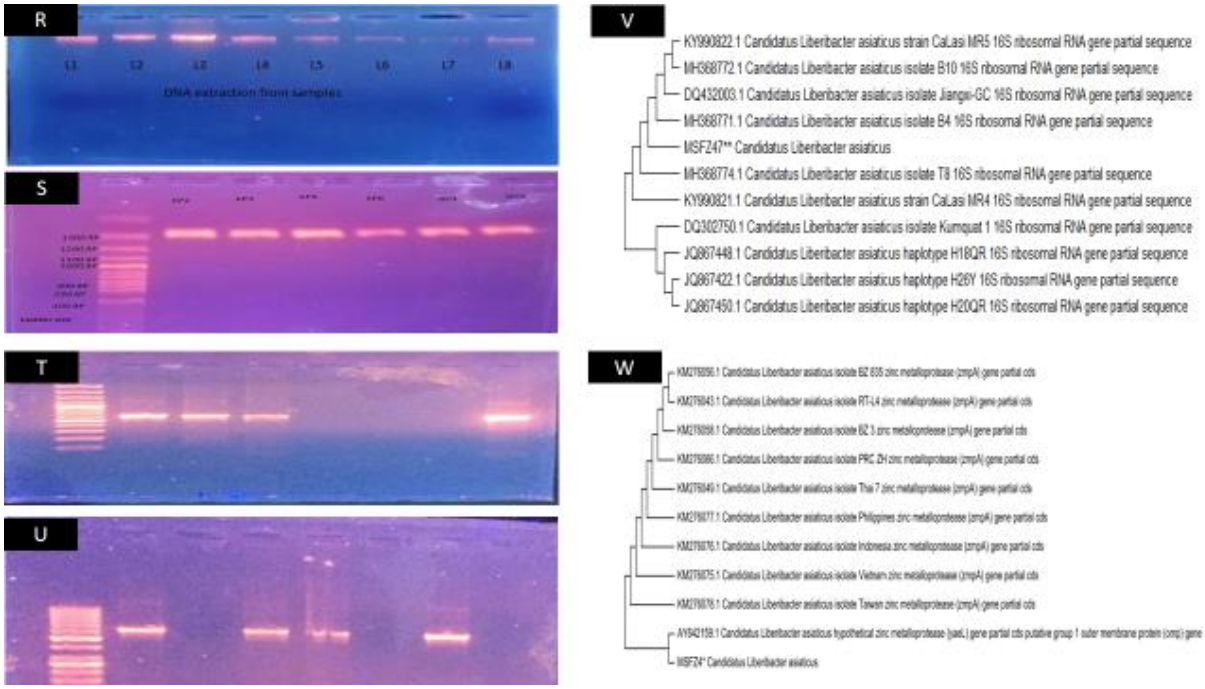

R
DNA extraction from samples
S
T
U
V
KY990822.1 Candidatus Liberibacter asiaticus strain CaLasi MR5 16S ribosomal RNA gene partial sequence
MH368772.1 Candidatus Liberibacter asiaticus isolate B10 16S ribosomal RNA gene partial sequence
DQ432003.1 Candidatus Liberibacter asiaticus isolate Jiangxi-GC 16S ribosomal RNA gene partial sequence
MH368771.1 Candidatus Liberibacter asiaticus isolate B4 16S ribosomal RNA gene partial sequence
MSF247** Candidatus Liberibacter asiaticus
MH368774.1 Candidatus Liberibacter asiaticus isolate T8 16S ribosomal RNA gene partial sequence
KY990821.1 Candidatus Liberibacter asiaticus strain CaLasi MR4 16S ribosomal RNA gene partial sequence
DQ302750.1 Candidatus Liberibacter asiaticus isolate Kumquat 1 16S ribosomal RNA gene partial sequence
JQ867448.1 Candidatus Liberibacter asiaticus haplotype H18QR 16S ribosomal RNA gene partial sequence
JQ867422.1 Candidatus Liberibacter asiaticus haplotype H26Y 16S ribosomal RNA gene partial sequence
JQ867450.1 Candidatus Liberibacter asiaticus haplotype H20QR 16S ribosomal RNA gene partial sequence
W

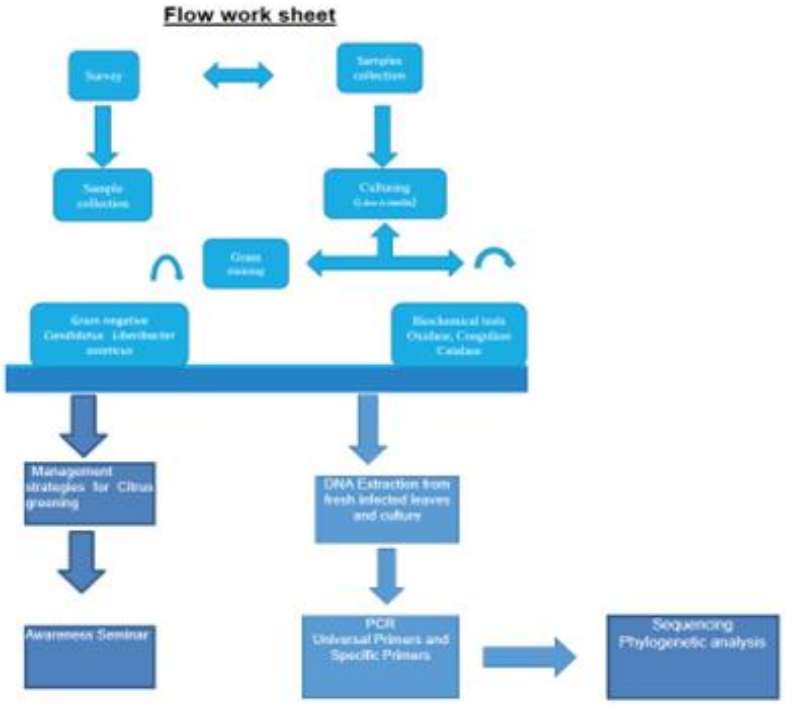


Figure A: Map of the region with different varieties of citrus (Using Arc GIS software), **Figure B:** Field visit of citrus orchids in haripur city, Dr. Samiullah Khan, Associate Professor Department of Agriculture (Entomology) and Dr. Maimoona Sabir Assistant Professor/PI Project Department of Microbiology University of Haripur with farmers and Agriculture department focal persons. **Figure C,D,F and G:** Showing symptoms of citrus greening disease like infected fruits and molted leaves shows symptoms of HLB disease, fruit drops & loss and molted leaves, **Figure E:** Vector psyllid on leaf, **Figure I, J K,L, M, N, O, P and Q :** Growers, farmers and workers were guided about the disease, symptoms and disease control measures, collection of samples and growing healthy plants. **Figure R:** Electrophoretogram of ethidium bromide stained bands of DNA isolated from disease sample. **Figure S:** Electrophoretogram of amplified product of PCR (1324) by using universal primer. **Figure T :** Electrophoretogram of amplified Omp gene (642 bp) along with 100 bp ladder. **Figurer U:** Electrophoretogram of amplified O1Ic gene (1132 bp) along with 100 bp ladder. **Figure V:** Phylogenetic tree of *C*las (16S rRNA OI1c) by maximum likelihood method, Our accession number is MSFZ47**. Submitted sequences in NCBI gene bank, (OL958579). **Figure V:** Phylogenetic tree of *C*las (Omp) by maximum likelihood method and Our accession number is MSFZ4**.

3.2.14: Selection/ Development of Citrus rootstock resistant against abiotic stresses (drought and Salinity)"		
Name of PI/Institute and designation:	**Dr. Umbreen Shahzad** Assistant Professor, Department of Horticulture	
Postal Address:	College of Agriculture Bahauddin Zakariya university Bahadur sub Campus Layyah Telephone: 03346927080-, Email: umbreenshahzad@bzu.edu.pk	
Duration:	15.02.2018 to 14.02.2021	
Financial Status:	Total Cost: Funds Released: Funds Utilized:	Rs. 2.962 million Rs. 1.782 million Rs. 1.546 million

Objectives:

- Development of True to type citrus rootstocks and bud/ graft wood source
- Establishment of citrus rootstocks mother block resistant against abiotic stresses and propagation protocol for commercial varieties.

Achievements:

Citrus is the main fruit tree crop in the world and therefore has a tremendous economic, social and cultural impact in our farmer society. Citrus is considered to be sensitive to abiotic stresses like salinity and drought. Citrus stands first in area and production among all the fruit crops in Pakistan. The citrus rootstocks has been established in the university research area as a rootstock source. Abiotic stress (salinity and drought) was given to the different rootstocks and results are described below.Citrus rootstocks, Volka Mariana, Benton, , Carrizo Citrange, Gada Dehi and Rough Lemon were collected from the Citrus research stations and were exposed to salinity 0, 30, 60 and 90 mM. Salinity showed the stunted growth and leaf started to drop.

The rootstocks of Gada Dehi, Benton, Co x Derine, Volka mariana, Rough Lemon, Baramasi, Rangpur Lime, C-35 US1, US2 and US3 were exposed to drought experiment as regular irrigation as a control, 10 days interval, 20 days interval and 30 days intervals. Many antioxidant enzymes such as SOD, CAT, and POD are endogenously produced in the plants to mitigate the cellular damage caused by the drought stress. The SOD, POD and CAT activities were higher in leaves of water stressed plants as compared with control plants. SOD is the primary enzyme which is produced in higher concentrations under stress conditions especially drought

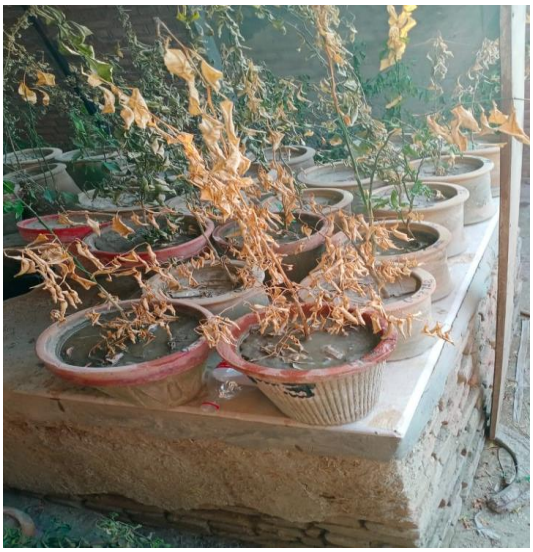
The maximum SOD in US3 was recorded at 20^{th} day of water stress. The maximum Leaf POD was recorded in C-35 at 10^{th} day of water stress. The maximum Leaf CAT activity was noted in US1 at 10^{th} day of water stress and the minimum. The maximum H_2O_2 content was recorded in rough lemon at 10^{th} day of water stress, whereas, the minimum H_2O_2 content was found in US3 at 20^{th} day of water stress. TSP content was recorded in C-35 at 30^{th} day of water stress and the minimum TSP content was recorded in control plants of Benton

rootstock. Among the rootstocks, Co x Derine showed maximum chlorophyll 'a' content at 30th day water stress and the minimum chlorophyll 'a' was noted in Benton at 30th day of water stress. Among the studied rootstocks, C-35 showed maximum chlorophyll 'b' content at 30th day water stress and the minimum chlorophyll 'a' was noted in Rangpur lime at 30th day of water stress

National Linkages Developed:

Linkages were developed with the Citrus Research Institute Sargodha. Citrus rootstocks were collected from CRI Sargodha. Covid -19 pandemic effected all the internationally linked institutes. So no international links was developed.

Different citrus rootstocks before starting abiotic stress Salinity effected plants of Citrus rootstocks



Drought effected plants of Citrus rootstocks

3.2.15: Utilization of Multiple Transcription Factor Genes for Enhancing Wheat Yield		
Name of PI/Institute and designation:	**Aftab Bashir,** Professor, Department of Biological Sciences	
Postal Address:	Forman Christian College University, Lahore. Telephone: 03206687953, Email: aftabbashir@fccollege.edu.pk	
Duration:	01-07-2018 to 30-06-2021	
Financial Status:	Total Cost: Funds Released: Funds Utilized:	Rs. 6.608 million Rs. 4.9 million Rs. 4.9 million

Objectives:

- Construction of single and double gene cassettes for the transcription factors TaNFYB4 NAC2-5A and Bht
- Development of Wheat transgenics using the three transcription factors
- Evaluation of the transgenics for transgene expression and agronomic traits

Achievements:

The project's main objective was to enhance wheat yield by improving transgenic wheat's nitrogen and carbon assimilation efficiency. We selected two transcription factors TaNAC2-5A and TaNFYB4, to accomplish the tasks, respectively. Additionally, the spike differentiation gene (Bht/mrs) was also selected to place an effort to enhance spikelet numbers. The cloning of the given genes/transcription factors and the development of wheat transformation vectors having the expression cassettes of the single and double gene cassettes was accomplished in a cereal transformation vector (pSB219) during the project's first year. The IBC, TAC, and NBC approval for carrying the genetic manipulation work at the lab level was obtained.

The second year of the project was focused mainly on the transformation work in wheat and transgene analysis. Three wheat lines were tested for their regeneration and transformation efficiency. Two of the tested lines, NARC-2011 and Borlaug were selected for further transformation work based on their higher transformation efficiencies. The transformation of the selected rust tolerant wheat lines was initiated in the middle of the second year per project activities and was continued till April 2021. The double gene cassettes in three combinations developed during year one of the project were transformed into the two selected wheat varieties.

The T_0 transgenics were selected on BASTA and were further verified by PCR and RT-PCR. The T1 seeds were obtained from the double gene transformants and analyzed by PCR, RT-PCR, and qRT-PCR. Upon maturity of the T_1 transgenics, the agronomic traits like plant height, 1000 seed weight, and the number of tillers were determined and compared with the parent lines. It was estimated that single gene transgenics having TaNaC2-5A or TaNF-YB4 could increase the seed weight by 5% compared to the non-transgenic controls. The analysis of the double gene cassettes for agronomic traits are being performed. However,

the double gene transgenics expression for TaNaC2-5A and TaNF-YB4 was identified to be two-fold to six-fold higher among different transgenic lines. The transgenics having Bht gene as a single cassette or in combination with TaNaC2-5A or TaNF-YB4 did not show multiple spiking or any variations in spike development. It is concluded that transgenics having single or double gene cassettes of TaNaC2-5A and TaNF-YB4 yield expression levels up to sixfold. These transgenic lines have the potential to increase the seed weight by 5% under lab conditions. It is expected that the performance of these transgenics may be higher under field conditions. Our future studies will focus on getting the field trial permission from NBC, developing the homozygous lines up to T_6 generation, and evaluating the transgenic lines under field conditions.

Publication:

- The qRT-PCR data on the T3 transgenic lines will be completed by the end of July. The agronomic traits of the T3 generation are also being obtained. We are expecting to complete the first manuscript by the end of this year (2022). A PhD student was involved in this work. She is completing her thesis and compiling the data for publication very soon.

International Linkages Developed:

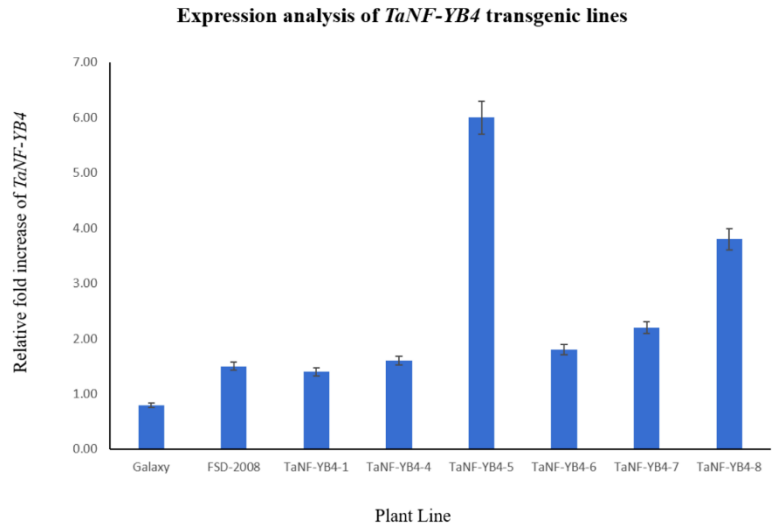
- Dr. Eduardo Blumwald, University of California, Davis, USA

Degrees Awarded under the project:

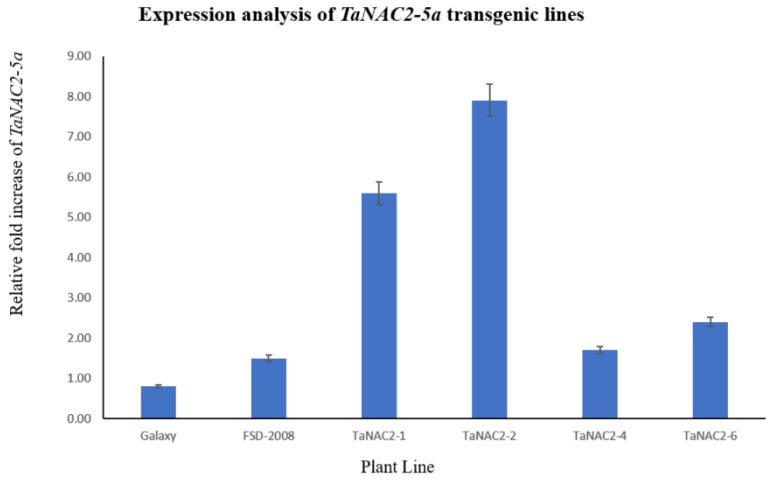
One PhD thesis completed his research under this project.

The T_1 Transgenics of *TaNF-YB4* and *TaNAC2-5A* in Net House

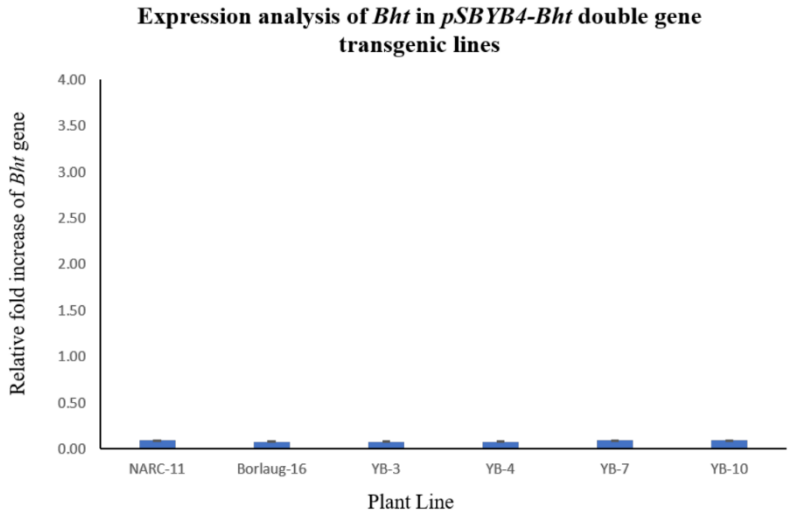
1. *The T_1 seeds of wheat transgenics for TaNF-YB4 and TaNAC2-5A were sown on November 23, 2020. The leaves were collected. Total RNA was extracted from the leaves and cDNA was synthesized. The cDNA was analyzed by RT-PCR for the selection of lines positive for TaNF-YB4 and TaNAC2-5A expression. The cDNA from positive lines was later tested for quantitative real time gene expression.*



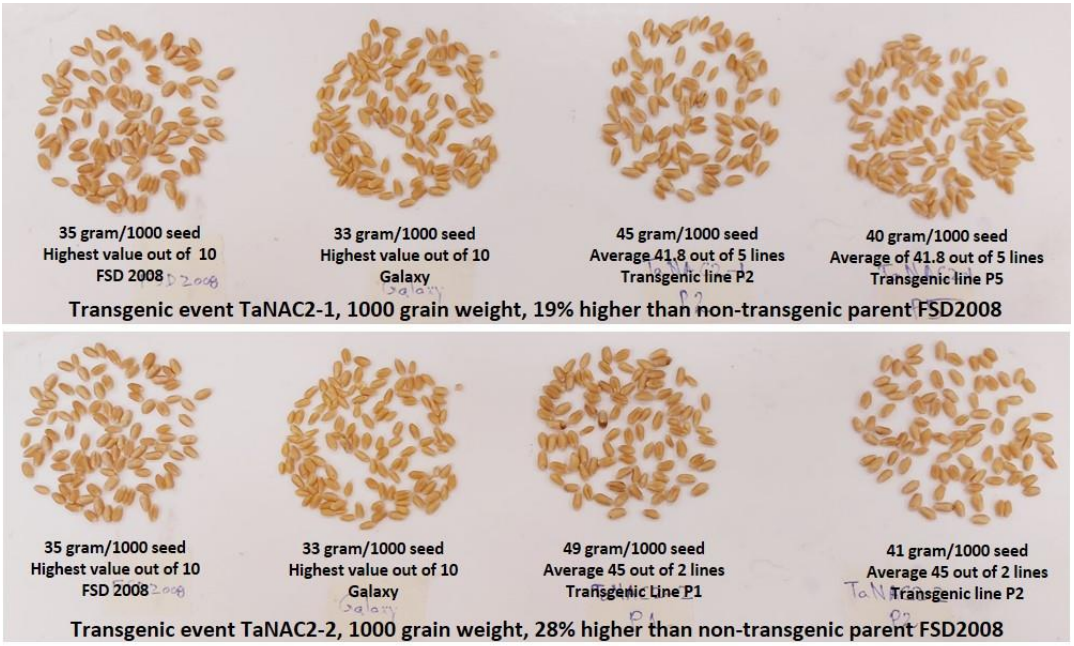
2. *The qRT-PCR analysis of selected RT-PCR positive lines of TaNF-YB4 transgenics. The transgenic lines TaNF-YB4-5, TaNF-YB4-7 and TaNF-YB4-8 were identified to overexpress the TaNF-YB4 transcription factor. The line TaNF-YB4-7 showed the minimum overexpression of about 0.5-fold higher than the parent line FSD-2008, while the line TaNF-YB4-5 showed the highest (4.5-fold) expression. The line TaNF-YB4-8 showed about 2.5-fold higher expression than FSD-2008.*



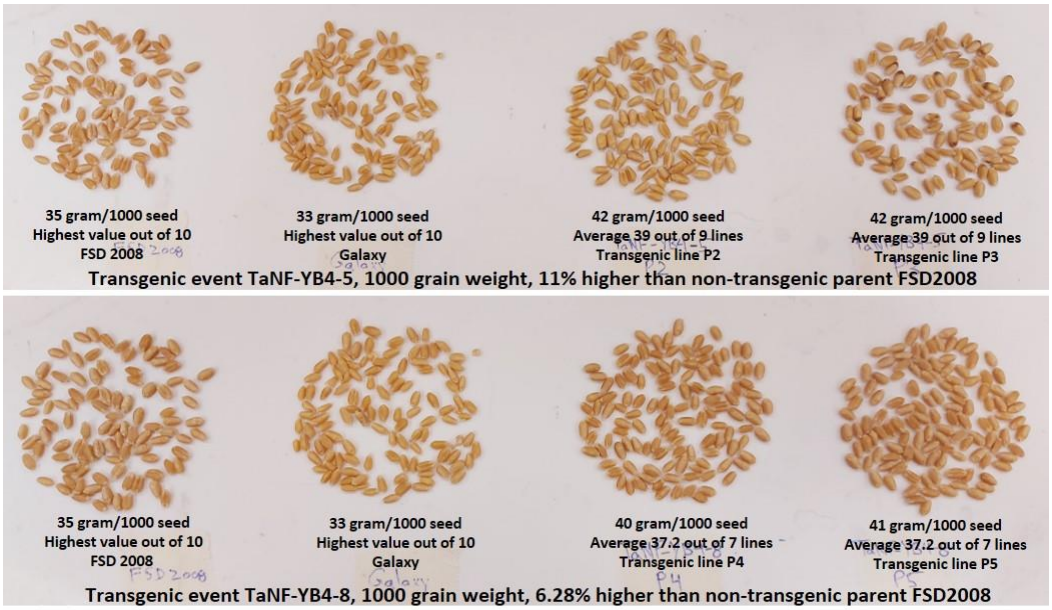
3. *The qRT-PCR analysis of selected RT-PCR positive lines of TaNAC2-5A transgenics. The transgenic line TaNAC2-1 showed about 4-fold higher expression of TaNAC2-5A, while the expression of this gene in the transgenic line TaNAC2-2 was 6-fold higher than the parent line FSD-2008.*



***4.** The qRT-PCR analysis of selected RT-PCR positive lines of YB4-Bht double gene transgenics. None of the transgenic lines was identified to express the Bht transcription factor including the parent line NARC-11.*



***5.** The grain size comparison of the T1 generation TaNAC2-5A transgenic lines. The transgenic event TaNAC2-1 resulted in 19% increase in 1000 grain weight of the lines P2 and P5. The transgenic event TaNAC2-2 showed 28% increase in 1000 grain weight of the lines P1 and P2.*



6. *The grain size comparison of the TaNF-YB4 transgenic lines. The transgenic event TaNF-YB4-5 resulted in 11% increase in 1000 grain weight of the lines P2 and P3. The transgenic event TaNF-YB4-8 showed 6.28% increase in 1000 grain weight of the lines P4 and P5.*

3.2.16: Management of Dusky Cotton Bug (Oxycarenus Sp; Lygaeidae: Hemiptera): An Emerging Threat to Cotton Crop In Pakistan		
Name of PI/Institute and designation:	**Dr. Muhammad Tahir Jan** Officer In-Charge/Cotton Botanist Central Cotton Research Institute (CCRI)	
Postal Address:	ARI, Model Town A, Gulberg Road, Old Sujabad Road, Bahawalpur. Telephone: 03339356987, Email: tahirjanmuhammad@gmail.com	
Duration:	01-02-2018 to 01-02-2021	
Financial Status:	Total Cost: Funds Released: Funds Utilized:	Rs. 5.300 million Rs. 4.128 million Rs. 3.966 million

Objectives

- Agro- Ecosystem analysis (AESA) of dusky cotton bug
- Taxonomy and Biology of the Pest & its natural enemies
- Ecology and Economic Threshold levels
- Monitoring of insecticide resistance in Dusky cotton bugs against the field tested insecticides
- Development of mass rearing technique for potential natural enemies and insecticide resistance studies
- Chemical Control Measures
- Development of Integrated Pest management strategies for the sustainable control of Dusky cotton bug
- Dissemination of management strategies to the extension system, academia and farmer community.

Achievements:

The pest showed trends either toward susceptibility or development of resistance. The study reflects the farmer's choice of the insecticides for the control of Dusky Cotton Bugs (DCB), Oxycarenus spp. (Lygaeidae: Hemiptera). Due to pest ability to develop resistance and instability of the resistance of the insecticides against the pest, it is suggested not to spray the single insecticides repeatedly and use those insecticides which are more toxic having low LC50 values. It will reduce the cost of insecticides as well as the insecticide resistance against DCB.

Although no evidence is available for the management of DCB from the past literature, efforts in the present study reveal that that actual infestation was initiated with 10 pairs per fruiting part in all the fruiting parts. Above this pest population level, the damage especially shedding in squares and small bolls, losses in seed cotton weight and reduction in germination were becoming alarming and control measures became necessary against the DCB. Therefore, in the present study, ETL for the DCB is suggested as 5-10 pairs per fruiting

part for better control of DCB. From the results it could be concluded the pest can easily be reared on sacked cotton fuzzy seeds, fresh young leaves as well as old leaves of China rose.

Cotton has the enormous value in the Pakistan foreign income by corporation in terms of the cloths material and oil production.

This exercise of spray regime reveals that when the insecticides used individual (single) as group A against dusky cotton bugs the efficacy increased followed by group B in which the order of the insecticides were changed in the fourth coming sprays. However, when each insecticide in the treatments compared, the efficacy was not found stable. The results therefore reveal instability among the insecticides whether these are used as single entity, as alternate or in combination.

To raise the production, new Bt cotton verities was screened at Central cotton research institute of the Bahawalpur, Pakistan. These were eight Bt. Cotton verities CIM-632, CIM-602, CIM-663, CIM- 573, CIM-600, CIM-598, CIM-595 and CIM-599. The verities having the minimum population of the dusky cotton bug have high yield and the best cotton lint that ultimately have the better fiber length and also to the good quality seed with highest per seed weight. The CIM-602 verity performed best in this experiment because of the good heat tolerance and also to better stand with bollworms resistant although other verities were also Bt but in the pest prevalence CIM-602 was having good height with better yield also present that it can better stand against the dusky cotton bug in the area of the Bahawalpur, Pakistan. The overall low performance was from the CIM-598 in many aspects as the pest population was high on it and also it was not at standard height with very low yield. CIM-600 was performed in between the all verities at standard height and also moderate pest prevalence was seen on this verity but it was performing in the sprayed plot to equal with the CIM-632 which also was near to the performance of the CIM-595 having the good yield but not satisfactory about the fiber length in the sprayed or in the unsprayed. Among all the verities the CIM-663 shown that it is a good verity in terms all parameters by not going to the least and not come ever on the top of the verities, it just remain moderate due to its behavior to the season when the rain was high all the verities got damaged but he CIM-663 was standing high as for the CIM-602 which have long height but was highly affected by the weather changes as the rainfall can damage the open bolls and deteriorated the seed cotton but CIM-602 was on top of the list due to its better characteristics for regaining the nutrients at full peak again as the weather was suitable for the pest and the prevalence was high when the weather gets cool and moist. CIM-602 was below in the list of the pest population, it remain on the top with other parameters under sprayed and unsprayed plots but there was also a significant difference among the sprayed and unsprayed plants of the CIM-602 but the treatment did not effects on the plant height of the all verities and also for the CIM-602. The plant height was significance among the verities but not significance when seeing the interaction between the treatment and verities, when all verities were given equal nutrients and other requirements are fulfilled at time with no delay, the verity with better characteristics will perform better from the other with not better genetic makeup. Sprayed and unsprayed plots have the ultimately the different affect as the unsprayed plots allow to enhance the population of the pest with no control but the sprayed plot have not such liabilities to spread the population on the crop when the pest reaches the ETL, controlled through the pesticides and the population comes to the point where it would not further damages the crops. Sprayed plot showed best

results as pre and post-harvest for the plant population, pest population, plant height, yield of the verities, Ginning out turn, seed soaking, and seed germination in the laboratory, fiber length and the weight of the 100 seed. Unsprayed plot was not going to meet the minimum requirement for suggesting the plant to depend only on the verities as they performed below the average of the sprayed plants. There is the fact that no one can completely relay on the sprayed but there must be relativity of the verity, not just the verity but the verity with best desirable characteristics as for the yield and the best quality of the lint is concerned

Cotton crop (Gossypium hirsutum L.) is considered economy crop worldwide especially in Pakistan. Cotton plays strong role in proportion of GDP and foreign exchange earnings. In Pakistan, around 55% cotton is exporting fiber commodity and 15 % area of cultivation is devoted to cotton crop especially Bt cotton. However, Bt cotton suppress the chewing pests but give a favorable niche to sucking pests. Among suck- ing pests, the Oxycarenus hyalinipennis Costa is emerging sucking pest in Bt cotton, and damaging the more than 40 different plants. Study regarding preference of seed and two-sex life table traits of O. hyalin- ipennis is necessary for its eco-friendly management. Therefore, this explain the preference of cotton seeds e.g., CIM-620 (non-Bt variety), CIM-717 (non-Bt variety), Bt-CIM-632 and Bt-Cyto-179 by O. hyalin- ipennis, as well as its fitness on these seeds under laboratory condition. Results indicated that both males and females preferred Bt-Cyto-179 for ovipositing as compared to other three varieties. The developmen- tal time from eggs to males (176.85 ± 7.43) and females (184.94 ± 7.85) were significantly longer on other verities as compared to Bt-Cyto-179. The O. hyalinipennis females produce significantly more eggs on Bt-Cyto-179 (215 ± 2.34 eggs female-1) than those on other three seeds e.g., CIM-620, CIM 717, and Bt-CIM-632. In conclusion, Bt-Cyto-179 was the more suitable varieties to attract and rear O. hyalinipennis than CIM 632, and CIM 717 seeds. And our study suggests that Bt-Cyto-179 can be used as a bait crop to control O. hyalinipennis and rearing purpose for molecular studies.

Publication

Rabia Saeed, Zahid Mahmood, Syed Ishfaq Ali Shah, Waqar Jaleel, Jam Nazir Ahmad, Tassawar Hussain Malik, Muhammad Tahir Jan, Hamed A. Ghramh, Zubair Ahmad and Khalid Ali Khan. 2022. Using two-sex life table tools to compare the population parameters of Oxycarenus hyalinipennis Costa (Lygaeidae: Hemiptera) when fed on Bt and non-Bt cotton seeds. Journal of King Saud University – Science (34) 1- 6

Degrees Awarded under the project:

Two M.Sc. Hons. Completed research in this project.

3.2.17: Value addition of citrus waste as a dietary fiber source in extrusion-based food products		
Name of PI/Institute and designation:	**Muhammad Kashif Iqbal Khan** Associate Professor *Department of Food Engineering*	
Postal Address:	Department of Food Engineering, University of Agriculture, Faisalabad. Telephone: 0333-6112737, Email: kashif.khan@uaf.edu.pk	
Duration:	01.03.2019 to 31.01.2021	
Financial Status:	Total Cost: Funds Released: Funds Utilized:	Rs. 3.54 million Rs. 1.195 million Rs. 1.188 million

Objectives:

- Utilization of citrus waste as a valuable dietary fiber source to develop the extruded products with high nutritional values
- Characterization of citrus pomace-based food products
- Optimization of protein and fiber contents in extruded products and commercialization of the products

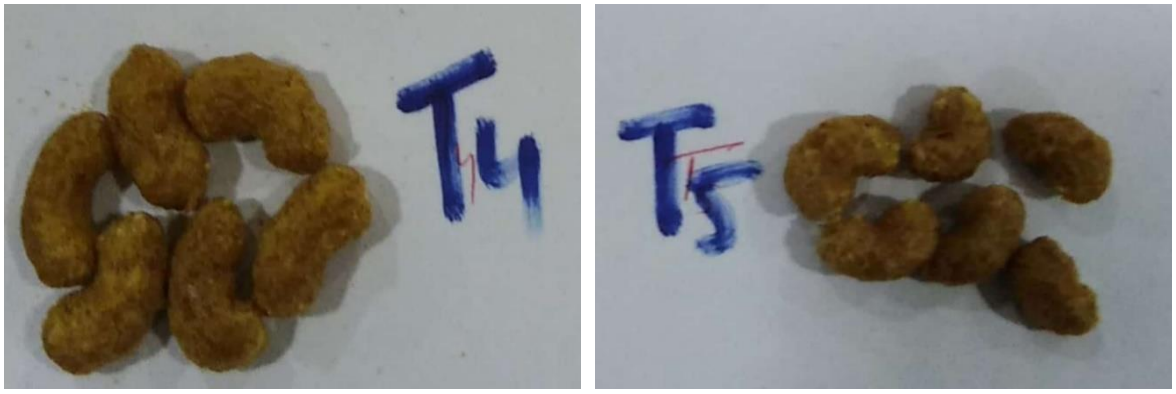
Achievements:

In first year study, citrus pomace was successfully dried with hot air oven and its physicochemical analysis indicated that pomace contained moisture (8.13%), fat (3.10%), protein (5.50%) ash (3.80%), fibre (4.22%) and carbohydrates (75.25%).The physiochemical analysis indicated that extrude contains moisture (8.33%), fat(3.30%), protein (5.50%), ash (3.80%), fibre (2.80%) and carbohydrates (76.6%).Besides, the expansion ratio of extrudates decreased (from 4.6-2.5) with the increase in citrus pomace concentration (0.30%). While bulk density increase (from 0.73 to 1.91 g/cm^3) with increasing pomace concentration in extrudates. The color changed from bright yellow to dark brown with a change in citrus pomace concentration. The crispiness of extrudates was increased with increasing pomace concentration.

During the second year of project, protein contents in the extrudates were increased by using lentil as a source protein. The previous year results indicated 5% citrus pomace was best addition in extrudates regarding fibre contents and organoleptic properties. Thus, citrus pomace and lentil contents were optimized to produced protein and fibre enrinched extrusion products. Total phenolic and dietary fibre contents were significantly influenced by the addition of fibre and protein in the formulation. Based on organoleptic results, the overall acceptability source favored the T_1 for the best treatment. Thus, 5% fibre and protein each in extrudates had control like properties.

Grinding of dry pomace

Packaging of pomace in airtight polythene bags



A comparison of control sample with varying citrus pomace concentration on various attributes of extrudates

T_0 T_1

T_2 T_3

A comparison of control sample with varying citrus and lentil pomace concentration sensory attributes of extrudates

3.2.18: Research for Productivity Enhancement of Drought Tolerant and shattering Resistant Cultivars of Sesame in Rainfed Areas of Punjab, Pakistan		
Name of PI/Institute and designation:	**Dr. Ghulam Qadir (Associate Professor)** Associate Professor Department of Agronomy	
Postal Address:	*Department of Agronomy, PMAS, Rawalpindi*. Telephone: 051-9292143 , Email: *qadir@uaar.edu.pk*	
Duration:	01.04.2018 to 03.03.2021	
Financial Status:	Total Cost: Funds Released: Funds Utilized:	Rs. 5.080 million Rs. 4.126 million Rs. 4.126 million

Objectives:

- Screening and evaluation of local and exotic line/varieties of sesame for drought tolerant and shattering free cultivars under changing climate.
- Efficient use of inputs; Seed, NM & IPM
- Motivational activities to grow more sesame, Comparison of farmer practices vs improved package of technology.
- Promotion of shatter less and drought resistant cultivars through participatory approach

Achievements:

- Split nitrogen application method and incremental dose was optimized for increased sesame yield.
- Paclobutrazol (PGR) application @ 250 mg L^{-1}significantly impacted yield and reduced shattering losses in sesame.
- Exotic sesame germplasm from USDA showed fair adaptability under indigenous climate conditions.
- Selected germplasm showed consistent results for grain yield, shattering resistance and disease incidence
- Optimum sowing date for sesame cultivation was assumed to be better fit for higher production with 6.5 kg ha^{-1} seed rate.
- Integration of farmyard manure along with recommended dose of fertilizer resulted in higher sesame grain yield.

Publication:

a) Mehmood, M. Z., Qadir, G., Afzal, O., Din, A. M. U., Raza, M. A., Khan, I., & Ahmed, M. (2021). Paclobutrazol improves sesame yield by increasing dry matter accumulation and reducing seed shattering under rainfed conditions. *International Journal of Plant Production*, 1-13.

b) Mehmood, M. Z., Afzal, O., Ahmed, M., Qadir, G., Kheir, A. M., Aslam, M. A., .& Ahmad, S. (2021). Can sulphur improve the nutrient uptake, partitioning, and seed yield of sesame? *Arabian Journal of Geosciences*, *14*(10), 1-15.

Degrees Awarded under the project:

Two M. Sc students and one PhD student completed their degrees in this project.






SECTION-4

ANIMAL SCIENCES SECTOR

4. Animal Sciences Sector

Under ALP, eight (08) projects were completed during 2020-21, out of which four (04) projects were completed in Punjab Province with total cost of Rs. 14.651 million and one each in Federal (PARC/NARC), Other Federal, Baluchistan and in KPK with total cost of Rs. 5.72, 2.87, 3.071 and 2.35 million respectively. While nine new projects have been started in the reported period with total cost of 74.169 million rupees. Thirteen (13) projects were on-going with total cost of Rs. 70.113 million of this sector. Total eleven (11) projects were presented in three meetings of Technical Advisory Committee (TAC), out of these nine (09) projects were recommended by TAC for further approval from BOD. While one meeting of BOD were held in 2020-21, in which six (06) projects were presented for approval. BOD approved only five (05) projects of Animal Sciences Sector for funding under ALP.

4.1 On-Going Projects

The progress of ongoing projects was monitored during the reporting period on the basis of technical and financial reports by ALP Secretariat as well as Technical and Finance Division of PARC. Region wise detail of on-going projects is given below in table.

Table: Region wise Animal Sciences Sector projects and approved cost of on-going projects (2020-21)

Region/Sector	No. of Projects	Total cost (million Rs.)
Federal (PARC/NARC)	04	22.915
Other Federal	01	3.574
Punjab	05	25.278
Khyber Pakhtunkhwa	01	9.555
AJK	02	8.791
Total	**13**	**70.113**

4.2 Achievements of completed Projects

Under ALP, eight (08) projects were completed of Animal Sciences Sector during 2020-21 in various universities, agricultural research centres, institutes and stations. The completed projects have been contributed in molecular biotyping of Brrucella abortus strains, preparation of Heterotypic Septicaemia (HS) vaccine, isolation of lactic acid bacteria for bio-preservatives production, effect of optimum feeding in Lassi Camels and pregnancy rate in Nili-Ravi Buffaloes etc. The salient achievements/progress of Eight completed projects of Animal Sciences Sector are given below.

4.2.1: One Health Approach for Molecular Diagnosis, Epidemiology and control of brucellosis in Pakistan		
Name of PI/Institute and designation:	**Dr. Raheela Akhtar** University of Veterinary and Animal Sciences	
Postal Address:	Department of Pathology, University of Veterinary and Animal Sciences, Lahore. Telephone: 042-9211449 Ext 332, Email: raheela.akhtar@uvas.edu.pk	
Duration:	01.05.2018 to 31.12.2020	
Financial Status:	Total Cost: Funds Released: Funds Utilized:	Rs. 3.6 million Rs. 3.32 million Rs. 3.31 million

Objectives:

- This study would help to determine molecular biotyping of Brucella abortus strains/biovars in different main districts of Punjab and KPK.
- The present project will add to the knowledge of distribution of Brucella strains among bovines (cattle & buffaloes) and humans of selected districts in Punjab and KPK.
- In a long run this exact determination of Brucella strains may assist in the formulation of effective vaccines against field strains of Brucella abortus. This would not only decrease treatment cost but would also help in better and timely control of brucellosis in selected areas.

Achievements:

Occurrence of brucellosis is higher in district Bannu as compare to district Mardan and Brucellosis is more in bovines as compared to humans. All bovines from Bannu and Mardan are affected by B. abortus. And in district Bannu both B. abortus and B. melitensis are existent in the blood of humans. The strain of B. abortus isolated from bovines of Bannu is similar to the stain isolated in Europe (island). While the strain of B. abortus isolated from humans of Mardan similar to the strain detected from West Africa (Togo). Most similar strain of B. abortus is isolated from Bovines of Bannu were detected Amrica and West Africa (Togo). Most similar strain of B. melitensis is isolated from humans of Mardan were detected from United State of America. Prevalence of brucellosis is higher in district Lahore as compared to district Islamabad. Brucellosis is more in animals as compared to humans. All animals from Lahore and Islamabad are affected by Brucella abortus. Humans from Islamabad are affected by Brucella abortus but in Lahore both Brucella melitensis and Brucella abortus are present in human blood. The prevalence of Brucellosis is higher in district Peshawar as compared to district Swat. The disease brucellosis is more in humans as compared to Bovines in Swat but in Peshawar the disease is more in animals as compared to Humans. All animals from Swat and Peshawar are affected by Brucella abortus while the Humans are affected by Brucella melitensis. The strain of Brucella Melitensis bv 1 isolated from humans were observed and it were closely resembled with the strains B. melitensis bv1_BCCN#00-18 identified in India at 2000. This strain was also resembled with B.

melitensis bv3_SC63-13_Ovine_Sichuan, China and B. melitensis_BwIM_IRN_37_Human, Iran. The incidence of bovine brucellosis in Jhang district is higher than in Sheikhupura district. As compared to bovines in Sheikhupura. Our study is the first comprehensive genotyping and genetic analysis of B.abortus and B.melitensis in eight districts of Pakistan. It is believed that this study will help to improve the effectiveness of brucellosis control programs. Due to the presence of some common strains in different districts it was suggested that a lack of control of animal movement between different districts or the circulation of contaminated animal products in the market may be the reason for disease spread.

Publications/Patent applications

The research article "MLVA genotyping of Brucella strains isolated from humans and cattle of Different geographical regions of Pakistan: One health diagnostic approach" has been resubmitted after revision in **Electronic Journal of Biotechnology JCR impact factor 2.894.** Ms. Ref. No.: EJBT-D-20-00303

International linkages developed

- Escola de Veterinária, Universidade Federal de Minas Gerais, 31270-901 - Belo Horizonte - MG - Brazil
- Pathogen and Microbiome Institute, Northern Arizona University
- Institute for Medical Research (IMR), National Institutes of Health (NIH), Malaysia

National Linkages

Department of Paediatric and Child Health, Agha Khan University, Karachi

4.2.2: Evaluation and protective efficacy of different stabilizers on shelf life of live heterotypic Haemorrhagic Septicaemia Vaccine (AS 142)		
Name of PI/Institute and designation:	**Dr. Hamid Irshad** Scientific Officer, ASI	
Postal Address:	Animal Health Program, ASI, NARC, Islamabad Telephone: 051-9255029, Email: hamidirshad@hotmail.com	
Duration:	01-04-2017 to 30-06-2021	
Financial Status:	Total Cost: Funds Released: Funds Utilized:	Rs. 5.72 million Rs. 4.17 million Rs. 4.01 million

Objectives:

- To prepare live aerosol HS vaccine.
- To evaluate the effect of different stabilizers and temperatures on the shelf life of live heterotypic HS vaccine.
- To test the safety and efficacy of live heterotypic vaccine in experimental animals.
- To transfer technology through PATCO

Achievements:

Lyophilized Pasteurella multocida B:3,4 present in culture bank of Animal health Program, Animal Sciences Institute, NARC was revived by mice inoculation method. Swiss albino mice (n=2) were inoculated intraperitoneally with P. multocida B: 3, 4. After 24 hours of inoculation mice died. The dead mice were dissected and spleen and heart were collected aseptically and streaked onto blood agar. After incubation, the resultant colonies were identified by Gram's staining, biochemical tests using RapID NF Plus kit and PCR. Three stabilizers, trehalose, lactalbumin and skimmed milk were selected to evaluate their effect on viability of P. multocida B:3,4. To prepare stabilizer media at 5%, 10%, 15% and 20% concentrations, desired quantity of stabilizers namely skimmed milk, lactalbumin and trehalose were suspended in distilled water. Skimmed milk and lactalbumin were sterilized by autoclaving before mixing with the P. multocida B:3,4 whereas trehalose was sterilized by filtration using microfilter having pore size 0.20µm. Four different concentrations of trehalose, skimmed milk and lactalbumin were used to prepare P. multocida B: 3,4 vaccine. Colony forming unit (CFU)/ml in the vaccine before lyophilization were enumerated using Mile's and Misra method. After that vaccines prepared with three different stabilizers were dispensed into vials for lyophilization. The vaccine vials were lyophilized using 48 hours lyophilization cycle. The viability of lyophilized P. multocida B: 3,4 was determined using Miles and Misra Method. The stabilizers demonstrated maximum viability after freeze drying at the concentration of 15% using 48 hours' duration of lyophilization. Live aerosol HS vaccine vials were prepared with trehalose (15%), skimmed milk (15%) and lactalbumin (15%) as stabilizers. These vials were stored at -20 ºC, 4ºC, 25ºC and 37ºC to evaluate their thermostability at various temperatures. The study indicated that most effective stabilizer for lyophiliztion of P. multocida B:3,4 was trehalose at 15% concentration and -20^{o}C was

most suitable temperature for storage of lyophilized P. multocida B:3,4. Safety of the HS vaccine prepared with 15% trehalose as stabilizer was tested in cattle (n=2) and buffalo (n=2) calves of age more than six months by intra-nasal inoculation of $2x10^9$ organisms of P. multocida B:3,4. The efficacy of the live aerosol HS vaccine was evaluated by vaccinating cattle (n=97) and buffaloes (n=64) at various farms in Quetta and Muzaffarabad with live aerosol HS vaccine (82 animals; 44 cattle and 38 buffaloes) and commercially available killed HS vaccine (79 animals; 53 cattle and 26 buffaloes). Blood samples were collected from vaccinated animals and serum was extracted to evaluate the presence of antibodies against HS vaccine using indirect haemagglutination assay (IHA). The efficacy of the vaccine was further evaluated using challenge protection test. Two healthy calves were challenged ten months post vaccination by sub-cutaneously injecting $2x10^7$ P. multocida B:2 organisms while two un-vaccinated calves aging 6-8 months were used as control. The results indicated that peak IHA values in buffaloes with live HS vaccine and killed HS vaccine were observed four months post vaccination in experimental animals maintained at Quetta. The peak IHA value was higher with live aerosol HS vaccine compared to killed HS vaccine in buffaloes. In cattle the highest IHA values with live aerosol HS vaccine were observed 3 months post vaccination whereas peak IHA values with killed HS vaccine were observed 5 months post vaccination in experimental animals at Quetta. In case of cattle vaccinated with live aerosol vaccine at Muzaffarabad, the peak IHA values were observed 2 months post vaccination whereas peak IHA values with killed vaccine were observed 6 months post vaccination. The IHA values with live aerosol vaccine remained higher than killed vaccine even after 12 months of vaccination. The vaccinated animals (n=2) that were challenged 10 months post vaccination withstood challenge and did not show any un-desirable reaction. Live aerosol P. multocida B:3,4 vaccine prepared using 15% trehalose was found safe and efficacious against HS in experimental animals. A two days training workshop was carried out live aerosol HS vaccine production technology was disseminated to scientists (n=6) of different vaccine producing institutes in Pakistan.

Publication:

- Effect of various stabilizers on viability of lyophilized Pasteurella multocida B:3,4 for use as hemorrhagic septicemia vaccine. International Journal of Agriculture and Biology, 25 (3): 567-574.
- An application to get patent (provisional) for the process of lyophilization of P. multocida B: 3, 4 has been submitted.

National Linkages

- PMAS Arid Agriculture University, Rawalpindi
- Livestock and Dairy Development Department, AJK
- CASVAB, Baluchistan University, Quetta

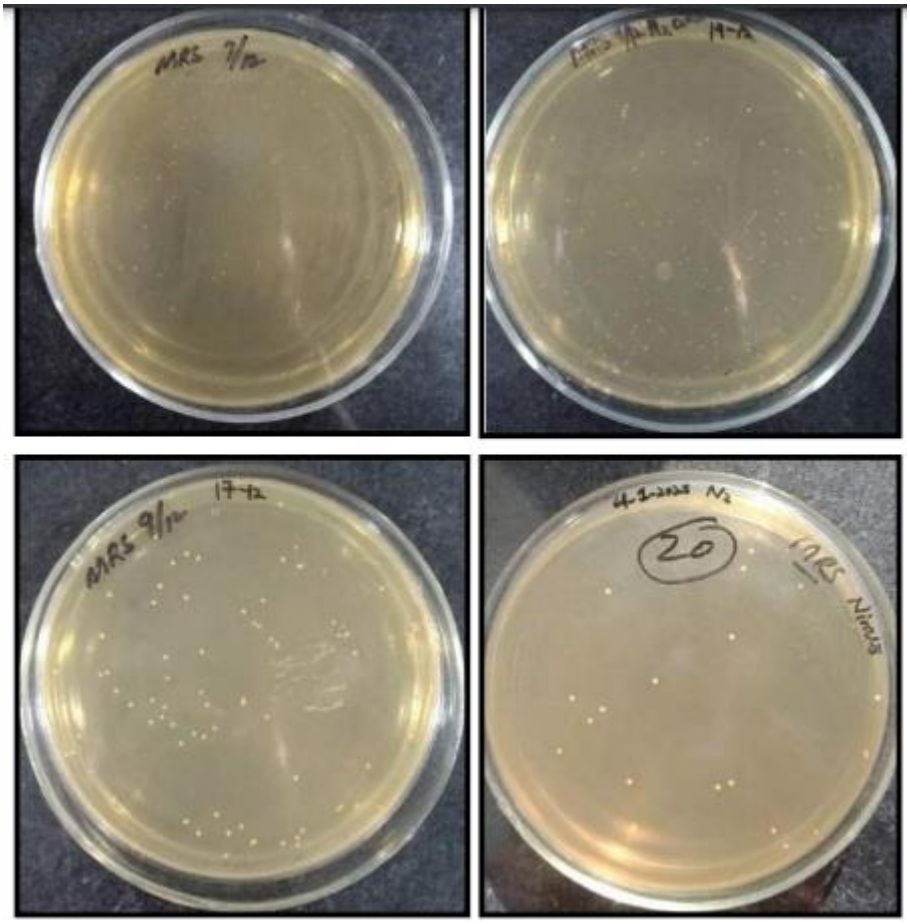
4.2.3: Production of indigenous food bio-preservatives from the micro flora isolated from the fermented dairy products (AS-221)		
Name of PI/Institute and designation:	**Dr. Talat Mahmood** Nuclear Institute for Food and Agriculture NIFA	
Postal Address:	Nuclear Institute for Food and Agriculture NIFA, Peshawar Telephone: 03009852130, Fax: 091-2964059 Email: talat204@hotmail.com	
Duration:	01.07.2018 to 10.01.2021	
Financial Status:	Total Cost: Funds Released: Funds Utilized:	**Rs. 2.87 million** **Rs. 2.87 million** **Rs. 2.62 million**

Objectives:

- Isolation and characterization of lactic acid bacteria for bio-preservative production (bacteriocin)
- Suitability of characterized bacteriocin as bio-preservatives and their application in various food systems.

Achievements:

In the present investigation 26 lactic acid bacterial strains were collected from indigenous fermented Foods. Amongst them, six were characterized as potential producer for biopreservative and probiotic traits. Amongst these isolate, three strains of Enterococcus faecium (NIFA-1), E. faecium (NIFA-2NA-1) and E. lactic (NIFA-3) were reported internationally in NCBI. Bio-peptides (bacteriocin) of NIFA-3 was strong inhibitor of food pathogens which was coagulated by 60 % ammonium sulphate, purified by dialysis tubbing and fractions were eluted by Sephodex G-75 in 1.5 x 30 cm column @0.5ml/min with 0.1 M Tris-HCl buffer. The purified bacteriocin was used as bio-preservative on guava fruit and extends shelf life upto 12 days at room temperature storage. These strains were further characterized for probiotic potential having good acid-bile tolerance, survival against GIT enzymes. NIFA-2NA-1 was used as probiotic culture to make probiotic yoghurt. Similarly, NIFA-1 was used to make lactic cheese. In conclusion, fermented food of this region has very good source of probiotic lactic acid bacteria, especially Enterococcus faecium and Enterococcus lactis have a great potential to be used in food industry.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4.2.4: Effect of Feeding Different Energy and Protein Level Supplements on Milk Yield and Composition in Lassi Camels		
Name of PI/Institute and designation:	**Prof. Dr. Illahi Bakhsh Marghazani** Department of Animal Nutrition, Faculty of Veterinary & Animal Sciences	
Postal Address:	Department of Animal Nutrition Faculty of Veterinary & Animal Sciences Lasbela University of Agriculture, Water & Marine Sciences, Uthal, Balochistan Telephone: 03337896071, Email: marghazani76@yahoo.com	
Duration:	07.03.2019 to 28.02.2021	
Financial Status:	Total Cost: Funds Released: Funds Utilized:	Rs. 3.071 million Rs. 2.799 million Rs. 2.684 million

Objectives:

- To know the Lassi camel farmers prevailing farming and feeding practices, indigenous feed resources and their nutrient profile, milk yield, milk composition and milk utilization of Lassi camels.
- To know the optimum energy and protein levels in supplements fed to early lactating Lassi camels under management circumstances of Lassi camel farmers.
- To educate/aware Lassi camel farmers about optimum feeding, production, reproduction and health management, religious misconceptions, milk marketing potentials and recent trends in camel farming.

Achievements:

This research project was comprised of three phases. In first phase of the project, study was conducted to know the prevailing farming and feeding practices, milk yield, milk composition and socio-economic status of camel farmers in Lasbela region. For this purpose, survey questionnaire was designed and one hundred camel farmers from different areas of Lassi camel habitat (Lasbela region) were visited and randomly interviewed. During survey study, camel preferred rangeland species and milk samples were also collected and nutritionally analyzed. Data collected were statistically analyzed using frequency distribution. Capital items of the project were also purchased during this reporting year. In second phase, collaborative meetings were held with different groups of Lassi camel farmers to coordinate in feeding experiment that designed to be conducted in farmers conventional camel management circumstances. Finally, one group of Lassi farmers agreed to coordinate in field Lassi camels feeding experiment on certain terms. Adaptation of Lassi camel to experimental supplements was also hard task as these camels were semi-wild in nature and were being kept free in grazing areas/forests and were never fed any kind of supplement feeding. However, based on observing day to day feeding behavior and implementing further strategies for improvement, these were successfully adopted to their respective supplements in thirty days' duration. Results showed encouraging potential of

milk production in Lassi camel breed. All supplemented groups showed significantly higher milk yield than control group. Among supplemented groups, animals fed high energy (2.5 Mcal/kg) level supplement with either low CP (10 %) or high CP (14%) showed significantly maximum milk yield. In phase-3, capacity building of Lassi camel farmers (n=70) were carried out by conducting field workshops/ schools. Field awareness/education workshops for Lassi camel farmers were conducted in two different areas (Sukkan and Winder) of district Lasbela. Both field workshops were consisted of three days each. The Day-1 was consisted of interactive sessions on optimum feeding management, reproductive management, health care, religious misconceptions of milk marketing, and feasible milk marketing model. Day-2 was comprised of practical use of spraying/drenching and other field treatment activities. Day-3 was fixed for exposure to university, practical demonstration of supplement preparation, value addition of camel milk and video sessions on recent trends of camel farming and marketing of milk and milk products at international level. In second sub-part of phase-3, camel milk value added products i.e., camel milk ice cream and camel milk Kroot were also prepared.

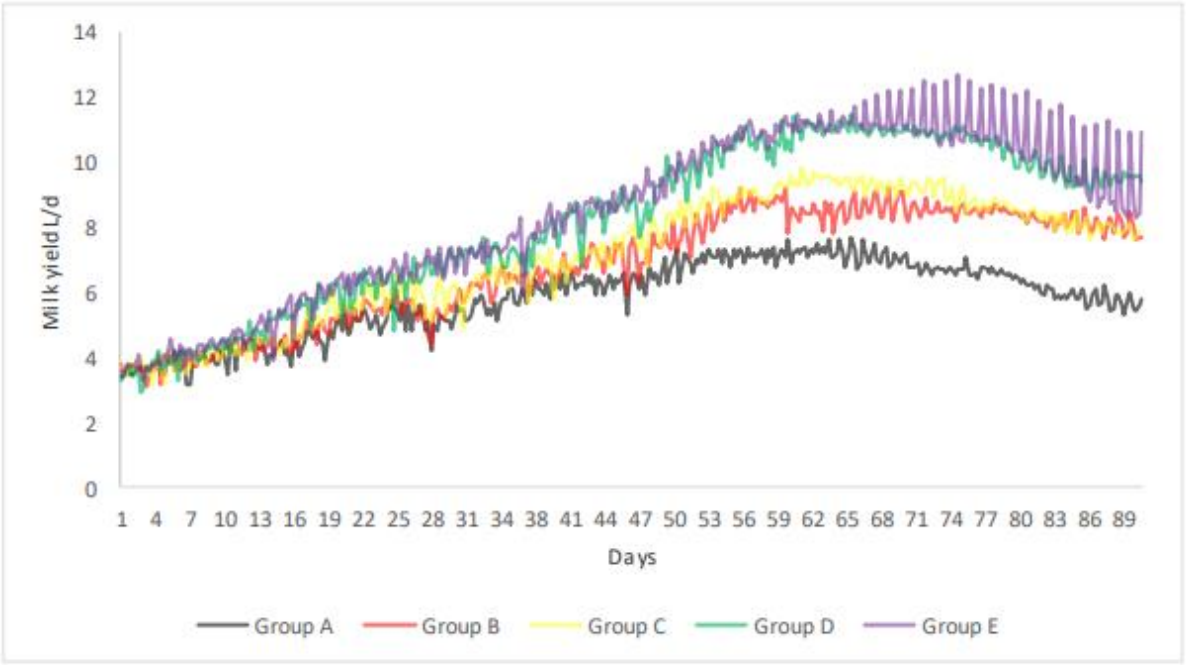


Figure 1: Milk production trend (daily basis) in early lactating Lassi camels fed different energy and protein level supplements

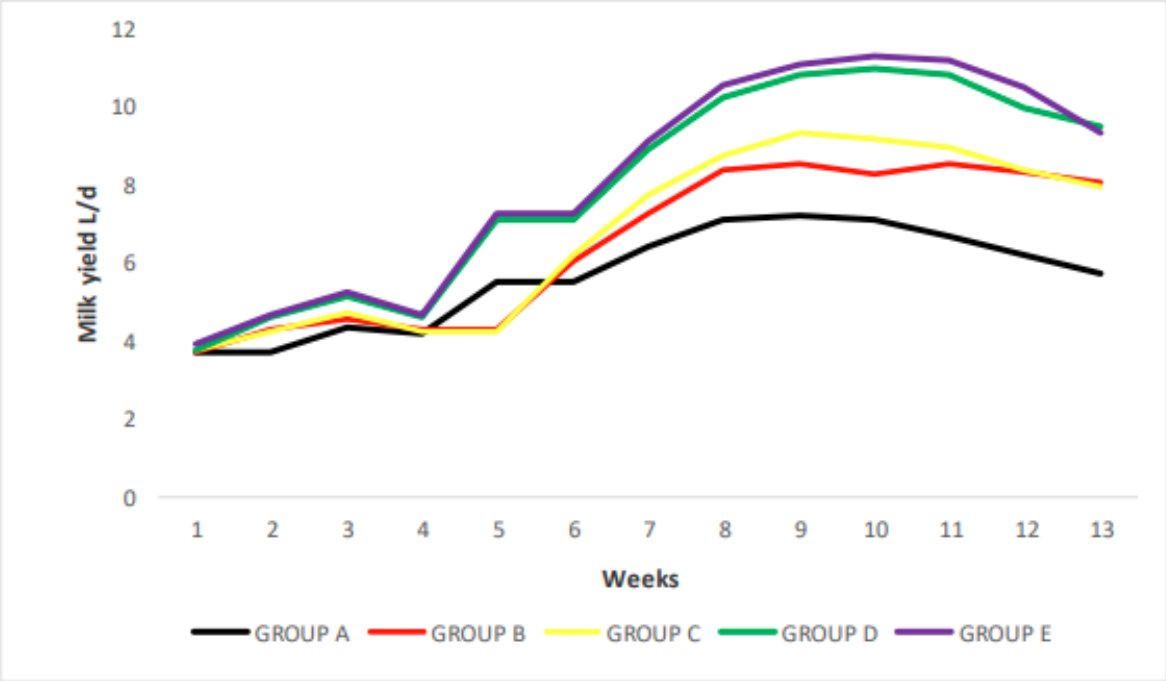


Figure 2: Milk production trend (weekly basis) in early lactating Lassi camels fed different energy and protein level supplements

4.2.5: The effect of season and semen quality on invitro fertilization and developmental competency of Nili-Ravi buffalo oocytes.		
Name of PI/Institute and designation:	**Dr. Amjad Riaz** Assistant Professor Department of Theriogenology	
Postal Address:	Department of Theriogenology, University of Veterinary and Animal Sciences, Outfall Road Lahore Telephone: 042-9213659, Fax: 041-9213659 E-mail: dramjadriaz@uvas.edu.pk	
Duration:	19.09.2018 to 18.03.2021	
Financial Status:	Total Cost: Funds Released: Funds Utilized:	Rs. 3.589 million Rs. 3.412 million Rs. 3.410 million

Objectives:

- Effect of high breeding (September-December) and low breeding (May-Jul) season on harvested oocyte quality, in-vitro maturation and fertilization of Nili-Ravi buffalo oocyte.
- Effect of semen cryopreserved during high breeding vs. low breeding season on in-vitro fertilization and developmental competency of Nili-Ravi buffalo embryo.

Achievements:

Livestock is largest subsector in agriculture sector of Pakistani economy and it has a big share in economic development of Pakistan. It contributed around 60.6 percent to the agriculture sector and 11.7 percent to the overall GDP during the 2019-20 period. Among livestock, buffalo has a significant role in the agricultural economy of many developing countries by providing milk, meat and draught power. The world's population of buffalo is estimated to be around 199 million (FAOSTAT, 2012) with more than 96% of the population located in Asia including 16.4% from Pakistan. The population of buffalo in Pakistan stands at around 41.2 million (Economic survey of Pakistan 2019-2020). Pakistan is blessed to have best genetics of native buffalo breed NiliRavi (black gold of Pakistan). This performs well in harsh tropical and subtropical conditions and is a best converter of low-quality roughages. Nili-Ravi buffalo is a major dairy animal in Pakistan contributing more than 60.4% of total milk produced in the country (Economic survey of Pakistan, 2019-20). It is also used for meat and draught purposes. Despite being major contributor of dairy industry, the availability of better germplasm is a major limitation. Reproductive efficiency in buffalo is low due to delayed puberty and maturity, seasonality of breeding, silent ovulations, less number of follicles, increased follicular atresia, high rate of early embryonic mortality, poor semen freeze ability and less tolerance to heat stress (summer anestrus). The use of traditional reproductive techniques like AI, MOET and estrus synchronization to overcome these problems has given poor results in buffaloes, as compared to the cattle. In-vitro embryo production technique (IVEP) is the best tool to exploit the superior maternal genetic potential in breeding programs. The world has already moved towards IVEP in commercial

set-ups for exploiting superior genetics. Therefore, the project was planned with following objectives: 1) to determine the effect of season (high breeding vs. low-breeding season) on oocyte quality, in-vitro maturation and embryo production, 2) to 7 evaluate the effect of semen cryopreserved during high breeding vs. low breeding season on invitro fertilization and developmental competency of Nili-Ravi buffalo embryo. To achieve our objectives, we planned two separate experiments, each consisting of further two parts.

Publications/patent application

Based on results obtained from experiment no. 1, a research article entitled "Photoperiodicity affects follicular population, oocytes quality, and in-vitro nuclear maturation of oocytes in Nili- 24 Ravi buffaloes" has been submitted in journal "Animal Reproduction" with IF 0.916 and is under process.

Graduate students/ degrees

Two students have completed their M.Phil research in the current project

4.2.6: Spatial Distribution, Population Density, Threats And Food Habits Of Barking Deer (Muntiacus Muntjak) In Murree-Kahuta-Kotli Satiyan National Park, Pakistan		
Name of PI/Institute and designation:	**Dr Ume Habiba** Lecturer, Department of Forestry and Wildlife Management	
Postal Address:	Department of Forestry and Wildlife Management, The University of Haripur, Haripur Telephone: 0333-5889670, Email: habiba8@uoh.edu.pk	
Duration:	15-12-2017 to 14-12-2020	
Financial Status:	Total Cost: Funds Released: Funds Utilized:	Rs. 2.350 million Rs. 0.188 million Rs. 0.188 million

Objectives:

- Determine the range and status of Barking Deer habitat in Murree,Kotli Sattain and Kahuta National Park.
- Estimate population density of Barking Deer in the study area.
- Analyze the composition of diet and food preference of Barking Deer.
- Identify the major threats to this species and recommend measure for its conservation.

Achievements:

As barking deer is endangered and key stone species of Murree-Kotli sattian and Kahuta national parks, so it is need of hours to take steps to conserve this shy species. At the international level, trophy trade is controlled by the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). The purpose of the Convention is to establish global control over trade of endangered wildlife and their products. The Punjab government is now paying more attention to wildlife protection. It needs to develop laws and regulations to protect animals and their habitats through Punjab Wildlife Protection Act, 2007. Community involvement in the protection of Barking Deer is also enhanced by community based guides. The department of wildlife and Parks is participating in a pilot project with the communities of monitoring resources. We believe that the results of this study also highlighted as much as possible the consideration of local ecological knowledge as a standard tool. Park management should declare potential habitat of Barking Deer as a grazing free zone to expand its distribution in the park. Its habitat range and movement patterns need to be further studied in future.

4.2.7: Pregnancy Rates in Anestrus Nili-Ravi Buffaloes Treated with Human Chorionic Gonadotropin Hormone and Flunixine Meglumine in a Timed Artificial Insemination Protocol During Low Breeding Season		
Name of PI/Institute and designation:	**Dr. Muhammad Saleem Akhtar** Department of Clinical Sciences Faculty of Veterinary Sciences	
Postal Address:	Department of Clinical Sciences, Faculty of Veterinary Sciences, Bahauddin Zakariya University, Multan Telephone: 0314-7890797, Email: drsaleemakhtar@bzu.edu.pk	
Duration:	19-08-2019 to 10-03-2021	
Financial Status:	Total Cost: Funds Released: Funds Utilized:	Rs. 2.551 million Rs. 2.077 million Rs. 2.071 million

Objectives:

- To know the effect of human chorionic gonadotropin (HCG) administration on day 7 after TAI on conception rate in postpartum Nili-Ravi buffaloes
- To know the effect of flunixine meglumine (FM) administration on day 14-15 after TAI on conception rate in postpartum Nili-Ravi buffaloes
- To know the combined effect of hCG administration on day 7 and flunixine meglumine (FM) on day 14-15 after TAI on conception rate in postpartum Nili-Ravi buffaloes

Achievements:

Pakistan is blessed with 41.1 million buffaloes producing more than 65% of the total milk produced in the country. The majority of buffaloes in Pakistan are on small farms with holdings of less than 5 per head. Buffalo is the most favored milk animal in Pakistan, as most people prefer buffalo milk to cow milk because of its white color, sweet taste and high fat percentage. The buffalo peak breeding season starts from October to February and low breeding season starts from May to August. The gestation period of buffalo is 10 months. The animals which become pregnant during peak breeding season (NovemberDecember) calve in August-September. The buffalo milk scarcity period occur during May-July. If we develop a method to bring animals in heat in low breeding season (May-July) and then get these animals pregnant, the parturition in these animals will occur in March-April. Therefore, the animals which will become pregnant in low breeding season will be lactating during period of shortage of milk in the market. Therefore, the farmer will get more benefit of his animals during May-July. In the present research, a method was developed through which we grought animals in to heat and get pregnant during low breeding season. In experiment-1, Sixty multiparous buffaloes were divided into 4 groups and were given different hormonal treatment in a scientific way. In T1 Group, animals were given intramuscular injections of human chorionic gonadotropin on day 7 after TAI. In T2 Group, on day 14-15 after TAI, animals were given intramuscular injection of flunixine meglumine at

an interval of 12 hours for 4 times. In T3 Group, animals were given intramuscular injections of hCG on day 7 and, on day 14-15 after TAI, animals were given injection of flunixine meglumine. In Control Group, a saline injections was at day 7, day 14-15 after TAI. In Experiment-2, forty four buffalo heifers were divided into four groups. In HT1 Group (n=11), heifers were injected with 3000IU of human chorionic gonadotropin IM on day 7 after TAI. In HT2 Group (n=11), on day 14-15 after TAI, animals were injected with 1.1 mg/kg IM flunixine meglumine at an interval of 12 hours for 4 times. In HT3 Group (n=11), heifers were given T1 and T2 protocol. In Control Group (n= 11), a saline injections was at day 7, day 14-15 after TAI. Their blood samples were collected and ultrasonography was done to monitor different structures on ovary. Data of different parameters was recorded. The results of present study revealed that treatment of lactating buffaloes and heifers with hCG on day 7 after AI during low breeding season effectively increased the corpus luteum size and serum progesterone concentrations. The pregnancy rates in Nili-Ravi buffaloes can be improved by the use of 3300 IU of hCG on day 7 after AI during low breeding season. The present research drawn a method of improving reproductive efficiency of buffaloes in connection with milk and meat production/supplies from Punjab to the whole country and across the border exports. This research helps farmer to overcome the cause of buffalo poor conception rate that will ultimately be beneficial to the farmers and result in poverty alleviation of rural (farmer) life.

4.2.7: Pathobiology and Molecular Epidemiology of Chicken Infectious Anemia and Infectious Bronchitis (IB) in Commercial Poultry and Immunopathogenesis of IB Variants		
Name of PI/Institute and designation:	Dr. Muhammad Kashif Saleemi, Associate Professor, Faculty of Veterinary Science	
Postal Address:	*Department of Pathology, University of Agriculture Faisalabad Pakistan.* Telephone: *0419201766*, Email: *drkashif313@gmail.com*	
Duration:	01-07-2017 to 30-06-2021	
Financial Status:	Total Cost: Funds Released: Funds Utilized:	Rs. 4.911 million Rs. 4.844 million Rs. 4.794 million

Objectives:

I. Molecular epidemiology and pathology of Chicken infectious anemia in commercial poultry

II. II. Molecular epidemiology, identification, and characterization of Infectious Bronchitis Variants circulating in commercial poultry in Pakistan

III. III. Immunopathogenesis of IB variant viruses when directly inoculated into the oviduct of growing and mature female chickens. Achievements:

Achievements:

Infectious Bronchitis (IB) was prevalent both in broilers and layers confirmed through RTPCR. Total no of samples 2720 Samples were processed and out of these, 625 (22.97%) were found positive for IB through RT-PCR (Nested). In Layer birds 1005 Samples were processed and out of these 255 (25%) samples were positive. In broiler birds 1620 Samples were processed and out of these 330 (20.37%) samples were positive. In non-descript birds 85 Samples were processed and out of these 35 (41%) samples were positive. In Layer breeder birds 10 Samples were processed and out of these 5 (50%) samples were positive. In case of chicken infectious anemia, 17.29% farms were found positive for CIA through PCR. In Layers 22.67%, broilers 11.69% and in non-descriptive 22.22% samples were for positive for CIA. It indicates Chicken infectious anemia and infectious bronchitis are endemic in the country. Chicken infectious anemia is leading to heavy economic losses due to immunosupression and heavy mortality. Further 10 samples of IBV were sequenced and two selected samples were sequenced for full S1 gene. Phylogenetic analysis revealed that the isolated strains belonged to G1 L24. The G1-L24 IBVs identified in current study are closely associated with Indian IBVs from the same lineage. It has been reported before that G1-L24 is only reported from India. This is the first time G1-L24 is isolated and genetically characterized from commercial poultry in Pakistan. The present work also highlights the epidemiological connection between Indian and Pakistani IBVs. But this is unclear if there has been an independent induction of IBVs from across the border or if migratory birds are playing a role in the transmission of IBVs. Therefore, current study also highlights the importance of surveillance and effective control of IBV in the region and especially in Pakistan The new IBV variants which are evolutionarily distant from vaccinal strains are still emerging within the Pakistan chicken flocks and are mostly associated with acute tubular

nephrosis syndrome, ciliostasis and 13 reproductive disorders in laying hens. In the trachea of IB infected birds necrotic changes were observed in the epithelial cells and cilia were disappeared from epithelium of IBV infected birds. There has been standard IBV vaccines used in Pakistan since last 2 decades and over the years. However, IBV outbreaks in commercial broiler and layer birds have been reported in Pakistan continuously. Based on the deduced amino acid sequence of the S1 gene of IBV isolates showed around 78 % similarity to the vaccine strains currently being used in Pakistan. This could possibly explain why there is a pattern of disease outbreaks of IBV due to incomplete protection. The deduced amino acid sequence of the IBV isolates in current study showed 78% similarity to the vaccines strains and 94% similarity to the Indian isolates and 78% similar/close to some other wild IBV isolates. These isolates showed highest similarity with the Mass type vaccine (78 %) and only 72 % with 4/91 vaccine type of IBV. There is a immunity gap of 22% between vaccinal strains and field virus of infectious bronchitis. The best option is to develop indigenous vaccine of both IB and CIA from local isolates of this study for proper control of both diseases.

Publications/patent application

Fayyaz, A., M. Kashif Saleemi, S. Tehseen Gul, Mashkoor Mohsin and Hamid Irshad, 2021. Seroepidemiology and Pathology of Infectious bronchitis in commercial poultry from Faisalabad division Published in Pakistan Veterinary Journal.

Graduate students/ degrees

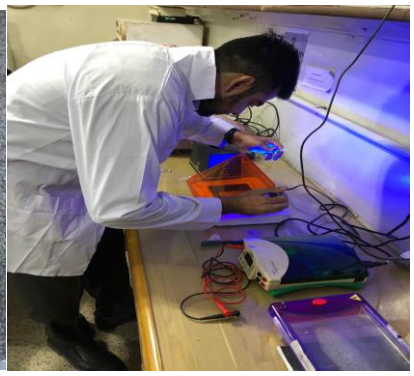
Two PhD and six M.Phil students have completed their degrees in the current project.

International Linkages Developed:

Dr. Kannan Ganapathy DVM, PhD, Diplomat European College of Poultry Veterinarian. RCVS & European recognized specialist in poultry medicine Infectious diseases (Avian virology). University of Liverpool United Kingdom (UK). Department of Infectious Disease Biology, University of Liverpool, United Kingdom, UK. Telephone: +44 1517946019 Fax: E-mail: K.Ganapathy@liverpool.ac.uk

National Linkages Developed

Dr. Arfan Ahmed UVAS Lahore, Dr. Najam ul Islam, L& DD and Dr. Hamid Irshad NARC



SECTION-5

NATURAL RESOURCES SECTOR

5. NATURAL RESOURCES SECTOR

Three (03) projects of Natural Resources Sector were completed during 2020-21 under ALP. One project each in PARC/NARC, Balochistan and KPK with total cost of Rs. 9.127, 6.171, 4.717 million respectively. While only one (01) project was started of Rs. 5.991 million in the reported period and Sixteen (16) projects are on-going with total cost of Rs. 86.805 million of this Sector. Six (06) projects were presented in three meetings of Technical Advisory Committee (TAC) of Natural Resources Sector, out of these only two projects were recommended by TAC for further approval from Boar of Directors (BOD). One meeting of BOD held in 2020-21, in which two (02) projects were presented for approval. BOD approved all the projects of Natural Resources Sector for funding under ALP.

5.1 On-Going Projects

The progress of ongoing projects was monitored during the reporting period on the basis of technical and financial reports by ALP Secretariat as well as Technical and Finance Division of PARC. Region wise detail of on-going projects is given below in table.

Table: Region wise Natural Resource Sector projects and approved cost of on-going projects (2020-21)

Region/Sector	No. of Projects	Total cost (million Rs.)
Federal (PARC/NARC)	*06*	*47.029*
Other Federal	*01*	*2.876*
Punjab	*07*	*32.834*
AJ&K	*01*	*4.066*
Total	***16***	***86.805***

5.2 Achievements of completed Projects

Under ALP, three (03) projects were completed of Natural Resources Sector during 2020-21 in various universities, agricultural research centres, institutes and stations. The completed projects of Natural Resources Sector has been contributed to explore the Carbon sequestration potential of benchmark soils, under different cropping systems, evaluation of sustainable soil in Baluchistan, effect of potassium and zinc on crops productivity etc. The salient achievements/progress of three completed projects of Natural Resources Sector are given below.

5.2.1: Actual, Attainable and Maximum Carbon Sequestration Potential of Benchmark Soils under Different Cropping Systems (NR-01)		
Name of PI/Institute and designation:	**Munazza Yousra** Scientific Officer, LRRI	
Postal Address:	Land Resources Research Institute, NARC Telephone: +92 51 90733127 Email: munzkhan04@gmail.com	
Duration:	01.02.2018 to 30.06.2021	
Financial Status:	Total Cost: Funds Released: Funds Utilized:	Rs. 9.127 million Rs. 8.646 million Rs. 8.646 million

Objectives:

- Estimate the present status of carbon sequestrated in benchmark soils under different land uses and management
- Explore the carbon sequestration potential of benchmark soils under different cropping systems
- Validate the carbon sequestration model for local soils and conditions

Achievements:

Soil series maps developed by Soil Survey of Pakistan were digitized using ArcGIS software. A detailed field survey sampling was done using selected benchmark soils of Pakistan. The sampled benchmark soils under different cropping systems (Rice-Wheat, Cotton-Wheat, Mix and Rainfed cropping) were found non-saline, alkaline calcareous in nature and varied from coarse to fine in texture. The intensity and frequency of plough-pan prevalence was found higher in Rice-Wheat cropping system than those of other cropping systems. Overall, most of the soil series had total organic carbon (TOC) deficiency in surface soils (<0.75%). The TOC in surface (0-15 cm) soil of Cotton Wheat was found highest which was followed by Mix, Rice-Wheat and Rainfed cropping area. In all the cropping systems, the soil having high clay content, had generally larger non-labile carbon pool than those of having less clay content. The non-labile carbon pool was found more in Cotton-Wheat cropping system than the Mix-, Rice-Wheat and Rainfed cropping area. The nutrient stoichiometry decreased the CO2 emission with passing days and increased the non-labile pool which ultimately improved the attainable carbon in soil. The extent of SOC stock (Mg ha-1) in different cropping systems was found in the order of Rice-wheat>Cotton-wheat>Mix>Wheat-Fallow cropping systems. The CENTURY model has been validated using twenty eight years long-term experimental data. The R2 values of measured vs. modelled data showed a reasonably good fit for model. The simulated carbon sequestration potential was found in the order of heavy>medium>light texture soils under two scenarios i.e., Farmer Practice (FP) and Best Management Practice (BMP). In all the cropping systems, the simulated TOC and carbon fractions were reduced from 2019 to 2099 under the FP scenario while the BMP scenario showed a marked increase in TOC and other carbon fractions till 2099.

Publication:

Publication Title	Type	Year	Status
Carbon Sequestration: An Important Strategy to Mitigate Carbon Emissions	Brochure	2021	Published
Soil organic carbon pools in benchmark soils of Punjab, Pakistan	Research Article	2021	Submitted (Communications in Soil Science ad Plant Analysis)

National Linkages Developed:

- Ayub Agriculture Research Institute, Faisalabad
- PMAS Arid Agriculture University, Rawalpindi

Degrees Awarded under the project:

Eight students of B.Sc. (Hons.) completed their Internship under this project.






5.2.2: Evaluation of Sustainable Soil and Crop Management Practices to Combat Land Degradation in Balochistan		
Name of PI/Institute and designation:	**Dr. Muhammad Sharif** Assistant Professor/HoD Department of Soil Sciences	
Postal Address:	Department of Soil Sciences, Balochistan Agriculture College, Quetta Telephone: 0323-8329158, Email: sharifbaloch84@yahoo.com	
Duration:	15.03.2018 to 15.03.2021	
Financial Status:	Total Cost: Funds Released: Funds Utilized:	Rs. 6.171 million Rs. 5.29 million Rs. 5.28 million

Objectives:

- To investigate the impact of different tillage systems, crop residues and legumes on soil organic carbon and its different fractions under upland cropping system of Balochistan.
- To assess the effect of different soil and crop management practices on structural stability and fertility status of soil.
- To evaluate the alternative leguminous crop production in comparison with cereal
- crop production under conservation and conventional practices

Achievements:

Soil samples were collected from 25 different sites in upland of Balochistan from farmer field, analysed and results reported to farmers and researchers under ongoing project. The samples were collected from different field under conventionally doing farmer practices in the upland of Balochistan that includes i.e. Ghazabund, Kuchlak, Bostan, Yaroo, Pishin, Khanozai, Bostan (Rangeland), Ziarat (Forest Area), Ziarat (Orchard Area), Nohsar, Noshki Road Mastung, Panjpai, Noshki, Mastung, Jangal Barah Bagh, Jangal Barah Bagh (Kalla kalo wala hisa), Adda Mashadi, Adda Kalla Kalo, Jangal Barash Bagh, Baleli, Jaffar Abad, Hana Lake. Samples were analyzed for texture, soil pH, electrical conductivity, Total Organic Carbon (TOC), Microbial Biomass Carbon (MBC), Particulate Organic Carbon (POC), Mineral Associated Organic Carbon (MAOC), Nitrate Nitrogen, available P and extractable K samples were taken through the soil auger. After soils analysis it were observed that the soils of the different sites were low contents of organic matters, low contents of nitrate-N, high pH value with alkaline calcareous.

Experimental treatments were installed during July 2018 and repeated 2nd year that include different tillage's and crop practices at Bostan, Quetta. The treatments include conventional tillage (CT), zero tillage (ZT), minimum tillage (MT) and four crops maize, guar, wheat and gram. Maize and guar crops were sown and harvested in summer while wheat and gram crop were sown in winter and the crop is still under observation in field. During this period germination of leguminous crop was remained a serious problem while soil properties were

gradually improved under conservation tillage and statistically remained the same under all treatments.

Under the on-going project different national level seminars were conducted at different locations in Balochistan that are:-

- Seminar was conducted on “Balanced fertilization for Sustainable Soil and Crop Management” on 19th Jun, 2018 at Pashin.
- Seminar was conducted on “Opportunities of Conservation Agriculture to Combat Land Degradation” at Gulistan on 10th February, 2021.
- One day national level seminar was conducted on “Land Degradation in Balochistan: A Serious Threat to Environment and Food Security” on 8th October, 2018 at Press Club Quetta. The seminar was attended by farmers, public representatives, students, academia, administrator and experts of related field.
- Another special seminar was conducted under ongoing project on “Balanced fertilization for Sustainable Agriculture” on 6th August, 2019 at Loralai, Balochistan. The seminar was attended by different stakeholder’s i.e researcher, academia and farmers.
- Special seminar was conducted on “Stop soil erosion save our future” on 5th December, 2019 at the auditorium of Balochistan Agriculture College, Quetta. The seminar was attended by massive no of farmers, academia, researchers and students.

The PI of the project presented the research activities and results of different treatments on different international/ national forum and acknowledged Agriculture Linkage Program (ALP) of Pakistan Agriculture Research Council (PARC).

- The PI of this project was presented at 1st International Conference on “Sustainable Agriculture: Food Security and Changing Climate Scenarios” hold on April 3-5, 2019 at Ghazi University D. G Khan. Title of the article was “Opportunities of Conservation Agriculture to Combat Land Degradation and Changing Climate”.
- The PI of this project was presented at international conference on 26-27 November, 2019 on “Climate Smart Agriculture: The Way Toward Sustainability” at Muhammad Nawaz Sharif Agriculture University, Multan. The title of research article was “Soil Organic Carbon Dynamic and Structural Stability under Conservation Tillage in Upland of Balochistan”
- The PI of the project was also presented on 11-13 February, 2020 on “Wise Soil Management Ensure Better Environment and Livelihood” at Sindh Agriclture University, Tandojam. Title of the research article was “Soil Properties and Crop Production under Conventional and Conservation Tillage in Upland of Balochistan”

Publication:

- Performance of leguminous crops under different tillage practices in silty-loam soil of Balochistan. International Journal of Plant & Soil Science, 33 (24):627-639, 2021.
- Assessment of soil characteristics and maize crop production under conservation tillage in upland of Balochistan. (Under Progress and near to publication in Pakistan Journal of Agriculture Sciences)
- Evaluation of soil fertility and water dynamics under different tillage practices in loess degraded soil of Balochistan. (Under Progress)

National Linkages Developed:

- Balochistan Agriculture College
- The Islamia University Bahawalpur Pakistan

Degrees Awarded under the project:

S.No	Name of Student	Title	Status
1	Shafiq-ur-Rehman (17-BAC-907)	Soil Aggregates Size Distribution and Associated Organic Carbon Under Different Land Management Practices In Upland of Balochistan.	Completed
2	Muhammad Ayaz (17-BAC-9)	Water Dynamic and Nutrient Concentration Under Different Tillage Practices In Upland of Balochistan	Completed
3	Mr. Misbahullah 18-BAC-05/SS	Performance of Leguminous crops under different management practices in silty-loam soil of Balochistan.	Completed
4	Mr. Asmatullah 18-bac-06/SS	Performance of Cereal crops under different tillage management practices in upland of Balochistan.	Completed

5.2.3: Investigating the Effect of Potassium and Zinc to increase Potato and Maize Productivity in FATA Kurram Agency		
Name of PI/Institute and designation:	**Iqrar Hussain** Senior Research Officer Agriculture Research, Soil & Water Testing Lab	
Postal Address:	Agriculture Research, Soil & Water Testing Lab, Malana Road, Tribal District Kurram, Parachinar Telephone: 0926-312298, 0303-8894888, Email: iqrarpcr1@gmail.com	
Duration:	01.08.2017 to 31.07.2020	
Financial Status:	Total Cost: Funds Released: Funds Utilized:	Rs. 4.717 million Rs. 4.294 million Rs. 4.294 million

Objectives:

- To study the nature, extent and severity of nutrient disorder in potato-maize growing areas of Kurram Agency.
- To investigate the effects of varying levels of potassium and zinc on the yield of potato and maize in the prevailing condition of FATA Kurram Agency.
- To capacitate farming community in potato and maize production techniques through demonstration plots, field days, trainings and brochures/pamphlets

Achievements:

Three hundred soil samples were collected analyzed for the below given characteristics.

Table 1. Soil fertility status of the soils of Tribal District Kurram

Parameter/property	Unit	Range	Average
pH (1:5)	-------	6.2-8.9	7.6
E.C (1:5)	dSm^{-1}	0.05-0.35	0.19
Organic Matter	%	0.4-2.4	0.88
Calcium Carbonate	%	4.1-26.0	13.5
Nitrogen	%	0.02-0.12	0.04
Phosphorus	($mg\ kg^{-1}$)	2.3-20.2	8.7
Potassium	($mg\ kg^{-1}$)	41-192	103
AB-DTPA Extractable-Zn	($mg\ kg^{-1}$)	0.13 – 6.30	0.74

Table 2. Fertility Status based on % of total samples

Parameter	Low	Medium	Adequate
Organic Matter (%)	53	26	21
Phosphorus (mg kg^{-1})	43	32	25
Potassium (mg kg^{-1})	50	22	28
AB-DTPA Extractable-Zn	41	26	33

Realization developed in farmers regarding soil sampling for analysis and determination of soil fertility. Based on the result of soil analysis, fertilizers recommendations were extended to farmers.

Experiments were conducted to investigate the effect of potassium and zinc on the yield of maize and potato. Maize was sown on 5 m × 3 m plots on flat beds. Row to row and plant to plant distance was kept 75 and 30 cm respectively through thinning. Data were recorded on number of grains per cob, 1000 grain weight and grain yield.

In case of potato, the plot size was 3.35 × 4.26 m for potato. The row to row and plant to plant distance for potato was 2 ft (60.96cm) and 1 ft (30.48 cm) respectively. Cut potatoes were sown. Nitrogen and phosphorus were applied at the rate of 250, 125 kg ha^{-1} in the form of urea, FYM (50:50 ratio) and DAP respectively.

Lay out: Randomized Complete Block Design in two factorial arrangements with three replications.

Factor A: Potassium levels (kg ha^{-1}) $K_1 = 0$, $K_2 = 60$, $K_3 = 90$, $K_4 = 120$

Factor B: Zinc levels (kg ha^{-1}) $Zn_1 = 0$, $Zn_2 = 5$, $Zn_3 = 10$, $Zn_4 = 15$

The experiments showed that highest average grain maize and potato yield was recorded in treatments receiving potassium and zinc at the rate of 120 and 15 kg ha^{-1} followed by treatment receiving potassium and zinc @90 and 10 kg ha^{-1}.

Maize yield was increased up to 53 % by the application of potassium and zinc @ 120 and 15 followed by 45 % by the application of 90 and 10 kg ha^{-1} respectively. Potato yield was increased up to 41.2% by the application of potassium and zinc @ 120 and 15 followed by 39.4 % by the application of 90 and 10 kg ha^{-1} respectively.

Cost Benefit Ratio of 2.6with highest net return of Rs. 162135 for maize crop by the application of potassium and zinc @90 and 10 kg ha^{-1} indicated economic significance for profitable maize production and is recommended for maize crop in Tribal District Kurram. Cost Benefit Ratio of 7.5 with highest net return of Rs. 897900 for potato crop by the application of potassium and zinc @90 and 10 kg ha^{-1} indicated economic significance for profitable potato production and is recommended for potato crop in Tribal District Kurram.

Data shows that application of either potassium or a zinc fertilizer is not advisable and combined application is inevitable. Potassium and zinc application were introduced for the first time in maize and potato crops in Erstwhile FATA.

Two trainings (each with 30 participants) were organized for farmers and field assistants of Agricultural Extension Department Kurram at Agricultural Research Kurram. Sixty persons

participated in the trainings. The participants were trained on the production technology of potato and maize crops with special focus on fertilizers application.

Publication:

One pamphlet was developed on the production technology of Maize and another pamphlet was developed on the production technology of Potato. Nine hundreds copies of each pamphlet were distributed among the farmers involved in growing of maize and potato.

National Linkages Developed:

- University of Agriculture, Peshawar
- National Agriculture Research Center Islamabad

Degrees Awarded under the project:

One student completed his degree of M. Sc (Hons) under this project.

Soil sample collection

Field preparation for potato

Sowing of potato

Potato crop

Maize trial at Shanai Kurram Tribal District

Maize crop being inspected for insects

SECTION-6

SOCIAL SCIENCES SECTOR

6. SOCIAL SCIENCES SECTOR

One project of Social Sciences Sector was completed during 2020-21 in Punjab region with total cost of Rs. 3.53 million under ALP. Two projects were ongoing of this Sector during 2020-21, one each in Federal (PARC/NARC) and at PARC outstation with total cost of Rs. 3.1939 and Rs. 4.530 million. While one new projects have been started in the reported period with total cost of Rs. 4.387 million of this Sector.

6.1 On-Going Projects

The progress of ongoing projects was monitored during the reporting period on the basis of technical and financial reports through Technical and Finance Division of PARC. Region wise detail of on-going projects is given below in table.

Table: Region wise Social Sciences Sector projects and approved cost of on-going projects (2020-21)

Region/Sector	No. of Projects	Total cost (million Rs.
Federal (PARC/NARC)	*01*	*3.1939*
PARC Outstaion	*01*	*4.530*
Total	***02***	***7.7239***

6.2 Achievements of completed Projects

Under ALP, two project were ongoing of Social Sciences Sector during 2020-21 at NARC, Islamabad and at SSRI Faisalabad. The ongoing projects will contribute in the capacity building of NARS Scientists in advance analytical techniques and in crop livestock integrated farming systems at marginal and small farms level in irrigated Punjab.

6.2.1: Controlling Adulteration in Milk Marketing Systems of Punjab by Developing Testing Technologies for Whey Powder and Vegetable Fat		
Name of PI/Institute and designation:	Dr. Muhammad Nadeem	
Postal Address:	*University of Veterinary and Animal Sciences* *Lower Mall Out Fall Road, Lahore* Telephone: *0345-8882047* Email: *muhammad.nadeem@uvas.edu.pk*	
Duration:	02-01-2019 to 01-01-2021	
Financial Status:	Total Cost: Funds Released: Funds Utilized:	Rs. 3.530 million Rs. 1.565 million Rs. 1.565 million

Objectives:

- To develop a rapid and cost-effective method for the determination of adulteration of whey powder in milk and method for the determination of vegetable fat in milk butter and desi ghee and developing milk adulteration kit for major adulterants;
- Determination of the types and extent of presence of different adulterations, particularly the whey powder and vegetable fat, in the supply and value chains of milk in mega cities of Lahore and Faisalabad;
- Determination of cost of production of such adulterated milk and some of its products vis-à-vis the prices charged for these products by various stakeholders in milk supply and value chains in Lahore and Faisalabad cities;
- Gathering information about the sources of supply of various adulterants to the stakeholders in both the cities;
- To get feedback of the stakeholders after sharing the developed kits about the diffusion potentials of these kits in two mega cities of Punjab; and
- Suggesting further research and policy guidelines to control adulteration in milk in the country.

Achievements:

Milk Adulteration Kit for the Detection of Caustic Soda, Neutralizers, Salt, Sugar, Starch, Urea and Synthetic Milk/ Fabricated Milk was developed in Food Chemistry Lab. Milk Adulteration Kit available in the market can detect the adulterants in milk at higher concentrations above 1%. Newly developed kit is easy to use and can detect adulterants at 0.05% level. First ever Milk Adulteration Kit for the Detection of adulteration in value added dairy products and milk based traditional sweets was developed. Dairy industries of Pakistan do not have any method for the determination of adulteration of whey powder in milk.Development of Rapid Methods for the Determination of Adulteration of Whey Powder in milk (New Discovery, method does not exist in the literature). Method for the determination of adulteration of whey powder in milk and dairy products was validated by

SDS-PAGE. Rapid, accurate and reliable method for the determination of adulteration of vegetable fat in milk, butter and desi ghee was developed and shared with all stakeholders, industries and Punjab Food Authority and method was evaluated by measuring fatty acids composition on GC-MS in our Lab. Samples of milk collected from different stakeholders i.e. milk producers, milk shops, de-creamers and *Gawala* (milk men) of different locations of Lahore and Faisalabad were analyzed for composition, adulterants and fatty acids composition. Newly developed methods and Milk Adulteration Testing Kit was highly effective in detection of various adulterants. With the aid of Newly developed methods and Milk Adulteration Testing Kit, adulteration can be controlled at all points in milk value chain from producer to consumer. This analytical work will be highly useful to know the route of milk adulteration and to know the magnitude of adulteration at different levels. Completed Surveys of all stakeholders of milk value chain in Lahore and Faisalabad Cities were also conducted. Survey revealed that quality of milk is deteriorated at each stage in value chain and every player makes his contribution in adulteration of milk.

Publication / Patent Applications

Drafting of two patents i.e. Development of a method for the determination of Adulteration of Vegetable Fat in Milk, Butter and Desi Ghee and Detection of Adulteration of Whey Powder is in Progress. Research Paper is under process for Publication in Food Additives and Contaminants Part B.

International Linkages Developed

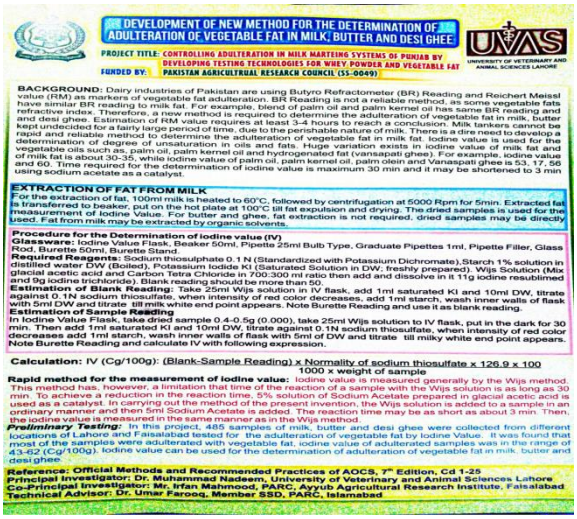
Queens University Belfast, UK
Michigan State University, USA
University of Kentucky, USA

National Linkages Developed

University of Agriculture Faisalabad, University of Sargodha, Ayuub Agricultural Research Institute, Punjab Food Authority, Haleeb Foods, Shakrganj Food Prodcts, Engro Foods Limited
Kary International, Acchha Foods, Lahore

Graduate Degrees:

Two students completed their degree of M-Phil under this project.



DEVELOPMENT OF NEW METHOD FOR THE DETERMINATION OF ADULTERATION OF VEGETABLE FAT IN MILK, BUTTER AND DESI GHEE

PROJECT TITLE: CONTROLLING ADULTERATION IN MILK MARTEING SYSTEMS OF PUNJAB BY DEVELOPING TESTING TECHNOLOGIES FOR WHEY POWDER AND VEGETABLE FAT

FUNDED BY: PAKISTAN AGRICULTURAL RESEARCH COUNCIL (S-5049)

UVAS UNIVERSITY OF VETERINARY AND ANIMAL SCIENCES LAHORE

BACKGROUND: Dairy industries of Pakistan are using Butyro Refractometer (BR) Reading and Reichert Meissl value (RM) as markers of vegetable fat adulteration. BR Reading is not a reliable method, as some vegetable fats have similar BR reading to milk fat. For example, blend of palm oil and palm kernel oil has same BR reading and and desi ghee. Estimation of RM value requires at least 3-4 hours to reach a conclusion. Milk tankers cannot be kept undecided for a fairly large period of time, due to the perishable nature of milk. There is a dire need to develop a rapid and reliable method to determine the adulteration of vegetable fat in milk fat. Iodine value is used for the determination of degree of unsaturation in oils and fats. Huge variation exists in iodine value of milk fat and vegetable oils such as, palm oil, palm kernel oil and hydrogenated fat (vansapati ghee). For example, iodine value of milk fat is about 30-35, while iodine value of palm oil, palm kernel oil, palm olein and Vanaspati ghee is 53, 17, 56 and 60. Time required for the determination of iodine value is maximum 30 min and it may be shortened to 3 min using sodium acetate as a catalyst.

EXTRACTION OF FAT FROM MILK

For the extraction of fat, 100ml milk is heated to 60°C, followed by centrifugation at 5000 Rpm for 5min. Extracted fat is transferred to beaker, put on the hot plate at 100°C till fat expulsion and drying. The dried samples is used for the measurement of Iodine Value. For butter and ghee, fat extraction is not required, dried samples may be directly used. Fat from milk may be extracted by organic solvents.

Procedure for the Determination of iodine value (IV)

Glassware: Iodine Value Flask, Beaker 50ml, Pipette 25ml Bulb Type, Graduate Pipettes 1ml, Pipette Filler, Glass Rod, Burette 50ml, Burette Stand.

Required Reagents: Sodium thiosulphate 0.1 N (Standardized with Potassium Dichromate),Starch 1% solution in distilled water DW (Boiled), Potassium Iodide KI (Saturated Solution in DW; freshly prepared). Wijs Solution (Mix glacial acetic acid and Carbon Tetra Chloride in 700:300 ml ratio then add and dissolve in it 11g iodine resublimed and 9g iodine trichloride). Blank reading should be more than 50.

Estimation of Blank Reading: Take 25ml Wijs solution in IV flask, add 1ml saturated KI and 10ml DW, titrate against 0.1N sodium thiosulfate, when intensity of red color decreases, add 1ml starch, wash inner walls of flask with 5ml DW and titrate till milk white end point appears. Note Burette Reading and use it as blank reading.

Estimation of Sample Reading

In Iodine Value Flask, take dried sample 0.4-0.5g (0.000), take 25ml Wijs solution to IV flask, put in the dark for 30 min. Then add 1ml saturated KI and 10ml DW, titrate against 0.1N sodium thiosulfate, when intensity of red color decreases add 1ml starch, wash inner walls of flask with 5ml of DW and titrate till milky white end point appears. Note Burette Reading and calculate IV with following expression.

Calculation: IV (Cg/100g): (Blank-Sample Reading) x Normality of sodium thiosulfate x 126.9 x 100 / 1000 x weight of sample

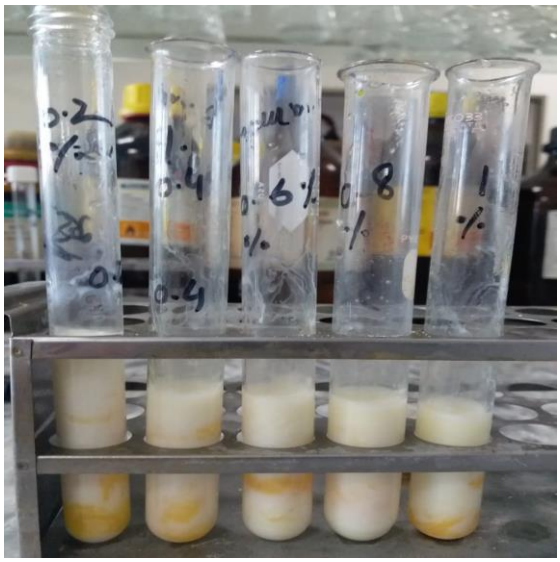
Rapid method for the measurement of iodine value: Iodine value is measured generally by the Wijs method. This method has, however, a limitation that time of the reaction of a sample with the Wijs solution is as long as 30 min. To achieve a reduction in the reaction time, 5% solution of Sodium Acetate prepared in glacial acetic acid is used as a catalyst. In carrying out the method of the present invention, the Wijs solution is added to a sample in an ordinary manner and then 5ml Sodium Acetate is added. The reaction time may be as short as about 3 min. Then, the iodine value is measured in the same manner as in the Wijs method.

Preliminary Testing: In this project, 485 samples of milk, butter and desi ghee were collected from different locations of Lahore and Faisalabad tested for the adulteration of vegetable fat by Iodine Value. It was found that most of the samples were adulterated with vegetable fat, iodine value of adulterated samples was in the range of 43-62 (Cg/100g). Iodine value can be used for the determination of adulteration of vegetable fat in milk, butter and desi ghee.

Reference: Official Methods and Recommended Practices of AOCS, 7th Edition, Cd 1-25

Principal Investigator: Dr. Muhammad Nadeem, University of Veterinary and Animal Sciences Lahore
Co-Principal Investigator: Mr. Irfan Mahmood, PARC, Ayyub Agricultural Research Institute, Faisalabad
Technical Advisor: Dr. Umar Farooq, Member SSD, PARC, Islamabad

Milk Adulteration Testing Kit



Whey Powder Test

Samples of *Barfi*

SECTION-7

AGRICULTURAL ENGINEERING SECTOR

7. AGRICULTURAL ENGINEERING SECTOR

Three (03) project of Agricultural Engineering Sector were completed under ALP during 2020-21 in federal (PARC/NARC). One new project has been started in the reported period while nine projects are on-going with total cost of Rs. 52.143 million of this Sector. Eight projects were presented in altogether three meetings of Technical Advisory Committee (TAC) of Agricultural Engineering Sector, TAC recommended all seven projects for further approval from Board of Directors (BOD), while one project was deferred. One meeting of BOD held in 2020-21, in which 04 projects were presented for approval. BOD approved all four projects of Agricultural Engineering Sector for funding under ALP.

7.1 On-Going Projects

The progress of ongoing projects was monitored during the reporting period on the basis of technical and financial reports by ALP Secretariat as well as Technical and Finance Division of PARC. Region wise detail of on-going projects is given below in table.

Table: Region wise Agricultural Engineering Sector projects and approved cost of on-going projects (2020-21)

Region/Sector	No. of Projects	Total cost (million Rs.)
Federal (PARC/NARC)	*05*	*40.204*
PARC (Outstation)	*01*	*6.331*
Other Federal	*01*	***5.608***
Total	***07***	***52.143***

7.2 Achievements of completed Projects

Under ALP, one project was completed of Agricultural Engineering Sector during 2020-21 at NARC, Islamabad. The completed project had contributed in adaptation of ispaghol and Kalongi processing technologies and to evaluate developed processing technologies for ispaghol and Kalongi. The salient achievements/progress of completed projects of Agricultural Engineering Sector is given below:

7.2.1: Smart Phone-Based On-tree Mango Fruit Maturity and Quality Estimation using Near-Infrared Spectroscopy and Machine Vision		
Name of PI/Institute and designation:	**Dr. Waqar Shahid Qureshi,** Assistant Professor	
Postal Address:	Department of Mechatronics, NUST College of Electrical and Mechanical Engineering, Rawalpindi Telephone: 051-54444450, Email: waqar.shahid@alumni.ait.asia	
Duration:		
Financial Status:	Total Cost: Funds Released: Funds Utilized:	Rs. 5.283 million Rs. 3.934 million Rs. 3.670 million

Objectives:

To reduce fruit loss and ensure quality, harvest timing and load information are critical to farm management (labour and packing consumables). Early harvest brings poor eating quality fruit to the market, while late-harvest decreases the available shelf life of fruit. These factors drive the need for quantitative tools for fruit maturity and quality testing. The assessment of harvest time is generally based on time (number of days from flowering) and physical features (size, shape and surface characteristics, firmness and pulp colour). The assessment of these physical features is subjective and requires experience labour. The current quality inspection methods in Pakistan include weight-based segregation and packaging. Therefore, the quality of each fruit is not traceable. A few value chains have set standards for fruit DM content at harvest assessed non-destructively via NIRS (e.g. Australian Mango Industry Association). The Pakistani supply chain also needs to adopt a traceability and visibility system to each sample within fruit packs. Two devices are proposed, one a LED-based technology and one utilizing a low-cost micromirror array-based spectrometer.

Achievements:

Overall, this project is completed in six phases. A brief description of these stages is as follows:

a. The procurement of a commercially available NIR-based instrument, i.e., Felix F-750 Produce Quality Meter (we have the first and the only fruit quality produce meter in Pakistan).
b. In the first phase, we performed calibration and infield testing of the Felix F-750 on local export mango varieties.
c. In the second phase, we designed and developed a colour-based mango maturity device and decision support software.
d. We designed anddeveloped a NIRmicromirror array-based spectrometer based mango maturity meter and decision support intelligent software. (1st device)

e. We designed and developed a NIR-LED-based mango maturity device and decision support software. (2nd device)
f. Finally, we tested the two developed devices (d and e) in-field (Mango Orchards), i.e., NIR-LED and shortwave NIR spectrometer-based fruit maturity device prototypes.

Degrees awarded

Three master's students and one PhD student have done their research around the equipment purchased through this funding and the devices developed through these funds. In the field, testing was done with the help of the mango growers group Pakistan. The prototype d is ready for market and commercialization. The following are the details of the published work.

Publication

Three journal publications in an internationally recognized journal have been published; the fourth one is under the second phase of review.

1. **Title:** Mango maturity classification instead of maturity index estimation: A new approach towards handheld NIR spectroscopy

Journal name: Elsevier Infrared Physics and technology
Print ISSN: 1350-4495, **Volume:** 115, **Date of Publication:** 29 Mar 2021

2. **Title:** Towards fruit maturity estimation using NIR spectroscopy

Journal name: Elsevier Infrared Physics and technology
Print ISSN: 1350-4495, **Volume:** 111, **Date of Publication:** 04 Sep 2020

3. **Title:** Is this melon sweet? A quantitative classification for near-infrared spectroscopy

Journal name: Elsevier Infrared Physics and technology
Print ISSN: 1350-4 **Volume:** 114, **Date of Publication:** 04 May 2021

National Linkage:

Prof. Aman Malik from the University of Agriculture Faisalabad
Dr Amin from Bahauddin Zakriya University Bahawalpur;
Mango growers group Pakistan.
Robot Design and Development, NCRA

International Linkage:

Prof. Kerry Walsh from Central Queensland, Australia,
Dr Hassan Raza, USA


MATFruit

7.2.2: Design, development and computational modeling of a solar assisted cold storage system for the preservation of perishables agricultural products		
Name of PI/Institute and designation:	**Dr. Waseem Amjad** Assistant Professor Department of Energy Systems Engineering	
Postal Address:	*Department of Energy Systems Engineering, Faculty of Agricultural Engineering and Technology, University of Agriculture Faisalabad* Telephone: *0345-2900234* Email: *waseem_amjad@uaf.edu.pk*	
Duration:	01.07.2018 to 30.06.2021	
Financial Status:	Total Cost: Funds Released: Funds Utilized:	Rs. 5.448 million Rs. 4.743 million Rs. 4.657 million

Main Objectives:

The main objective of the project was to design and develop a complete system for the storage of freshly harvested perishable agri. produce using solar energy in the form of thermal and power applications.

Specific Objectives:

- Design and development of an energy efficient solar cold storage unit vapor compression refrigeration system to reduce spoilage rate in perishable products.
- Technology transfer and capacity building of students and stakeholders for community development.
- Demonstration of thermal applications of solar energy to tackle food security issues and use of computer technology in agriculture.

Achievements:

Food processes are complex due to interaction of numerous factors to get optimum operating conditions and processing quality. In this context a computer-based approach (ANSYS-Fluent) used for design optimization with respect to air distribution and temperature exchange. Physically, the cold storage chamber had been developed with a roof to hold solar panels. A vapor compression refrigeration system was installed with invertor technology to eliminate the torque load which is a big hurdle in using solar PV systems. Refrigeration capacity was calculated by taking the respirations (kJ/kg/d) of the product to be stored and basic calculations were made to design a required solar PV size. For experimental phase, the system was equipped with data logger, thermocouples and Pyranometer for the continuous control and data monitoring helpful in the development of algorithm to make a program for remote control of the cooling conditions. Solar irradiance profile, relationships of cold storage temperature with humidity and cooling pads were developed and effect of storage condition on the quality of stored product was also

assessed. Outcomes of Computational Fluid Dynamics (CFD) analysis for airflow and temperature distribution were compared with that of experimental for validation and model development. Preliminary experimental correlation for energy distribution and losses were developed which are important to assess the energy flow in the system. For this, a detailed thermal analysis was performed required to analyze the cooling process for energy saving and optimum conditions. Development of a control mechanism for online monitoring of temperature and humidity was also developed to make the system monitoring and operation user-friendly. Payback period (years) was calculated 2.48 with batteries backup and 2.19 without batteries backup. The outcomes were presented at various forum and published as well.The developed cold storage is especially beneficial for decentralized application in remote areas where no grid electricity is available. The system can play a vital role in addressing the decentralized storage of various agricultural products to reduce losses with minimum energy requirements for the value addition and income generation. The outreach and commercialization would be convincible by employing renewable energy source as cooling is an energy intensive unit operation. All the components of the systems can be developed using indigenous resources which will help to reduce the manufacturing cost and dependency on high cost cool chain units.

Publications

Amjad W, Akram F, Rehman S, Munir A, Manzoor O. Thermal Analysis of a Solar Assisted Cold Storage Unit for the Storage of Agricultural Perishables Produce. Engineering Proceedings. 2021; 12(1):24. https://doi.org/10.3390/engproc2021012024

Research Assistant worked in this project presented project outcomes in an International Conference on “Green Energy Technologies: Opportunities and Challenges” held on 29-30 October 2019 at UAF.

National / International linkages developed

POWER PACK Energy (Pvt) Ltd.Office No. 03, Rehman Centre-2, Near DHA Phase underpass, Service Lane, Lahore, Pakistan

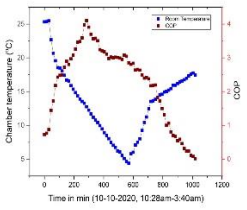
Dr. M. ImtiazHussain, Green Energy Technology Research Center, Kongju National University, South Korea); imtiaz@kangwon.ac.kr

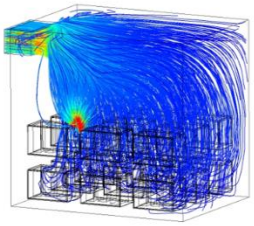
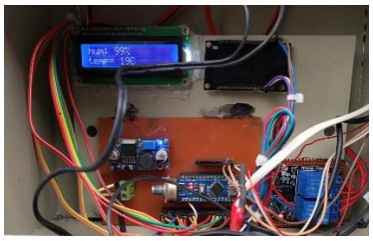
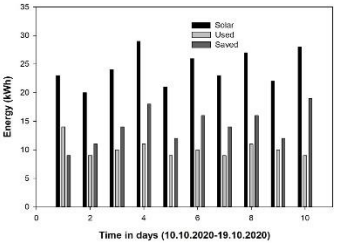
Degree awarded under the project

Four students successfully completed their M.Sc. (Hons.) Energy Systems Engineering







Chamber temperature (°C)
COP
Time in min (10-10-2020, 10:28am-3:40am)

Velocity
3.00
2.67
2.33
2.00
1.67
1.33
1.00
0.67
0.33
0.00
[m s^-1]



Solar
Used
Saved
Energy (kWh)
Time in days (10.10.2020-19.10.2020)


7.2.3: Design and Development of a Tractor Operated Carrot Harvester and Trimmer		
Name of PI/Institute and designation:	**Dr. Muhammad Ashraf** Agriculture Engineer	
Postal Address:	AMRI (Division Faisalabad)/Field Wing (Agriculture Deptt.) Agricultural Engineering Workshops Altaf Ghunj chowk Jhang Road Faisalabad Telephone: *+92 41 9201661 Mob. No. +9203336517932* Email: *drashraf289@gmail.com*	
Duration:	*01-07-2018 to 30-06-2021*	
Financial Status:	Total Cost: Funds Released: Funds Utilized:	Rs. 5.195 million Rs. 4.644 million Rs. 4.601 million

Specific Objectives:

1. To study on design parameter influencing mechanical harvesting of carrot.
2. To develop and evaluate a carrot harvester (Two Row) based on design parameter.
3. To study economic analysis and field demonstrations

Achievements:

The development of machine with the collaboration of Pvt. Manufacturersis not only brought a technological advancement in carrot crop by providing a complete mechanized harvesting system but also strengthen the Private manufacturers by intervention of local industry in latest technology. In addition to this, the mechanized harvesting of carrot with the help of carrot harvester cum trimmer is efficient and economically viable operation with the 63.04% and 86.4 % saving in cost and labor reduction as compared to traditional method. At the forward speed of 2.5 km/hr, belt speed of 0.75 m/s, rake angle of 25 degree and belt inclination angle of 24 degree is best combination to achieve maximum field and lifting efficiency as well as minimum un-lift carrot root losses. At the same combination performance efficiency of machine is 72.97% with combination of Lifting efficiency of 88 %, Conveying Efficiency of 93.9%, Belt losses of 6.1 %, Trimming Efficiency of 87%, damage percentage of 11 % and carrot root quality of 89% at the recommended operational parameters of machine. The field test shows that the carrot harvester runs smoothly, but needs improvement in picking unit and trimming unit to improve harvesting efficacy and carrot root quality. The performance of carrot harvester will be improved by single line sowing of carrots on ridges rather than the broadcasting method, wherein seeds are broadcasted and ridges are made.

Publication

A paper on the Development and economic evaluation of carrot harvester cum trimmer is submitted to Journal of Agriculture Research Faisalabad.

Graduate Student/Degree

One PhD scholar of University of Agriculture Faisalabad was engaged in this project.

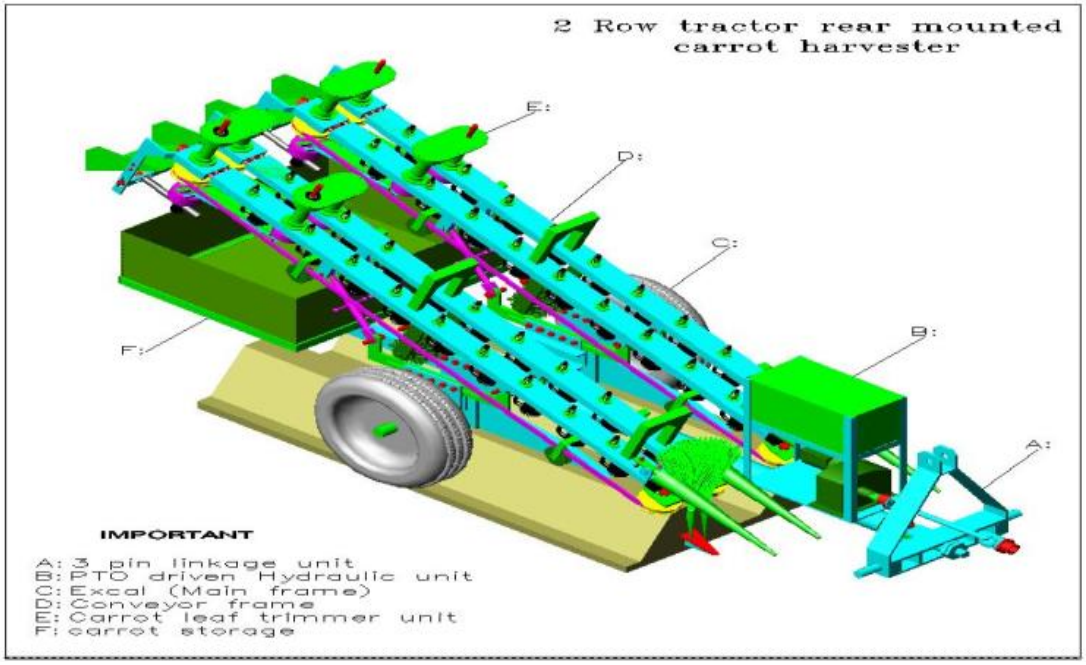


Figure 1: 3D Drawings of Two Row Tractor Operated Carrot Harvester and Trimmer




Manpower of ALP Secretariat

All manpower of ALP secreratiat is being provided by PARC main budget.

Sr.#	Name	Designation
1.	Mr. Muhammad Asif	Executive Director (ALP)
2.	Mr. Tahir Zahoor Chohan	Director (CG-ALP)
3.	Mrs. Naheed Zahra	Senior Scientific Officer
4.	Mrs. Ya.Sakina	Scientific Officer
5.	Mr. Mushahid Raza	Assistant Director
6.	Mr. Mudassar Hussain Satti	Office Assistant
7.	Mr. Safeer Ahmed Sabri	Assistant Private Secretary
8.	Ms. Aamash Haqqani	Senior Scientific Assistant

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