Genetic Engineering and Genome Editing: The Need for Legislation

Dr. Shahid Mansoor, *Sitara-e-Imtiaz*
HEC Distinguished National Professor
Fellow Pakistan Academy of Sciences

**DIRECTOR**
National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad, Pakistan
Agriculture and Pakistan

GDP Share  25%
Labour  50%
Export Earnings >80%

Agriculture dynamics
Food security
- Self sufficient in cereals, sugar, fruits, vegetables
- Importer of edible oil, pulses, cotton, tea, dry milk

Nutritional security
- Around 50% population suffers nutritional deficiency
- Nearly 44% children are stunted

Challenges
- Population
- Water
- Climate change
- Land
- Pest and diseases
- Salinity and water logging

Population Growth
1951  →  41 million
2012  →  185 million
2030  →  261 million

Need to enhance genetic gain by 50-70% for food security
Crop Improvement strategies

**Conventional Green revolution**
- Conventional breeding
  - Mutation breeding
    - High yielding varieties responsive to fertilizers and pesticides

**Biotechnological Gene revolution**
- Tissue Culture
- Genetic Engineering
- Marker-assisted selection
  - Crops with high yield and efficiency

**Plant Breeding**
- Gene Pool, Limited

**Genetic Engineering**
- Gene Pool, Unlimited
Crop Improvement strategies

Conventional Green revolution
- Conventional breeding
- Mutation breeding
- High yielding varieties responsive to fertilizers and pesticides

Biotechnological Gene revolution
- Tissue Culture
- Genetic Engineering
- Marker-assisted selection
- Genomics/genome editing
- Crops with high yield and efficiency

Plant Breeding
- Gene Pool, Limited

Genetic Engineering
- Gene Pool, Unlimited
PAEC in Agriculture and Biotechnology
Mandate of Agri & Biotech Division keeps evolving with time, according to requirements and with the advent of new technologies but remains within the fundamentally defined boundaries.

**Key goals of Agri & Biotech Centers:**
- Research Based Development of Agriculture Sector
- Socioeconomic Stability & Food Security
- Introduction / Transfer of Technology
- Analytical / Technical Services
- Human resource development
R&D goals are achieved through

1. Development of New Crop Varieties
   High yielding, better quality, abiotic and biotic stress tolerance, wider adaptability

2. Utilization of Marginal Lands
   Successive planting of salt tolerant grasses, shrubs and trees, utilization of biomass for goat farming, identification/selection and development of crops for drought prone areas

3. Improved Water and Fertilizer Management
   Application of various irrigation techniques, reduced / alternate application of fertilizers, removal of contaminants from water and soils

4. Pre and Post-Harvest Yield Losses
   Integrated pest management strategies for reduced losses to pathogen bio-control and male annihilation techniques, increased shelf life through irradiation and vacuum treatments
R&D goals are achieved through (cont)

5. **Animal Health and Production**
   Molecular disease diagnostics and treatment, vaccine production, feed supplements, synchronized breeding, animal genomics and breed improvement

6. **Biotechnology for Socio Economic Development**
   Biotechnology for variety development, Industrial enzymes, process improvement, nanotechnology, Soil & environment remediation, Human health and genetic diseases, prebiotics and probiotics

7. **Enhancing Exports/Analytical Services**
   Removal of contaminants from export commodities, analytical services DNA based testing & GMO testing
Cumulative Economic Impact of PAEC Varieties

Number of varieties

- Total: 1207
- NIA: 109
- NIBGE: 310
- NIFA: 122
- NIAB: 686

Economic impact (Rs. Billion)

- Total: 1207
- NIA: 1207
- NIBGE: 686
- NIFA: 122
- NIAB: 310

*Note: The data is represented in a bar chart showing the cumulative economic impact of various袍ecencies.*
Agriculture; crop improvement through genetic engineering and genomics tools

• Cotton, raw material for the largest industrial sector
• Rice, the largest export after textile
• Sugarcane, multipurpose crop
• Wheat, engine for food security
• Potato, huge potential for food security

Oil seed crops
Six billion Dollar import, suitable for dry areas
• Canola; dry and marginal lands
• Soybean; Pakistan Nil; India 10 million hectares
• Cotton; RNAi based reduction of gossypol and oil improvement
Pakistan is a party to the Cartagena Protocol on Biosafety (CPB)
National Biosafety Centre (NBC) was established as a project to cater the Obligation of Cartagena Protocol on Biosafety
The main objectives of the NBC are;

To provide safeguard against the undesirable effects of the Genetically Modified Organisms (GMOs)
Implementation of Pakistan Biosafety Rules and National Biosafety Guidelines as they provide the necessary management and regulatory framework for the GMOs.
Regulate the import, export, production, store, handle, sell or release the GMOs
To predict the effect of GMOs on the environment, plants, animals and human health.
Implementation Mechanism

- NBC
  - National Biosafety Committee

- TAC
  - Technical Advisory Committee

- IBC
  - Institutional Biosafety Committee
NIBGE efforts in developing Bt cotton varieties

**Total Approved cotton varieties**

1. NIBGE-2—2006
2. IR-NIBGE-1524—2010
3. IR-NIBGE-3701—2010
4. IR-NIBGE-901—2011
5. IR-NIBGE-3—2012
6. NIBGE-115—2012
7. NN-3—2013

**Time line**

- 2002: IR-FH-901, IR-448 & IR-443
- 2005: Improved IR-NIBGE-901
- 2006: IR-NIBGE-1524
- 2007: IR-NIBGE-3701
- 2010: IR-NIBGE-3
- 2011: IR-NIBGE-4
- 2013: Upcoming lines
- 2015: IR-NIBGE-5, IR-NIBGE-6

**Upcoming lines**

- Completed on year in NCBT
- Tolerance to lodging rotenning
- Approved in 2012
- Popular for spring cultivation
- National variety approved in 2010 and 2011
- 25%, 18% area in 2010, 2011, respectively
- 1-1.2 million bale advantage
- National variety approved in 2010 and 2011
- Drought prone area (2%)
- Approved for Sindh in 2011
- 20% (2009 & 2010)
- 25% in 2011
## Summary of the Impact

<table>
<thead>
<tr>
<th></th>
<th>Sindh (A)</th>
<th>Punjab (B)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Million bales</td>
<td>Million US$</td>
</tr>
<tr>
<td>IR-NIBGE-901</td>
<td>2.14</td>
<td>858.96</td>
</tr>
<tr>
<td>IR-NIBGE-1524</td>
<td>0.09</td>
<td>31.91</td>
</tr>
<tr>
<td>IR-NIBGE-3701</td>
<td>0.37</td>
<td>138.98</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>2.60</strong></td>
<td><strong>1029.84</strong></td>
</tr>
<tr>
<td></td>
<td>Million bales</td>
<td>Million US$</td>
</tr>
<tr>
<td><strong>Grand Total</strong></td>
<td><strong>8.37</strong></td>
<td><strong>3486</strong></td>
</tr>
</tbody>
</table>

Grand Total (A+B) = 8.37 Million bales
US$= 3486 Million or ~345 Billion
Development of double and triple gene construct for bollworms and weed control

Double gene construct: Cry1Ac+Cry2Ab+pGA482
- Cry1Ac+Cry2Ab cassettes = 6.3Kb
- pGA482 backbone = 13.2kb
- Cry2Ab full cassette (FMV-signal EPSPS-Cry2Ab-G7) = 2787bp
- Cry1AC full cassette (2X35S-Cry1Ac-35S) = 3474bp

Triple gene construct: Cry1Ac+Cry2Ab+EPSPS+pGA482
- Cry1Ac+Cry2Ab+EPSPS cassettes = 8.9Kb
- pGA482 backbone = 13.2kb
- EPSPS full cassette (CVM-EPSPS-E9) = 2598bp
- Cry2Ab full cassette (FMV-signal EPSPS-Cry2Ab-G7) = 2787bp
- Cry1AC full cassette (2X35S-Cry1Ac-35S) = 3474bp

Event characterization in cotton has been achieved
# Insect bioassay of transgenic Bt cotton using Armyworm *(Spodoptera litura)*

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Gene</th>
<th>Event or Cotton Line</th>
<th>Plants</th>
<th>Leaves</th>
<th>Larvae (1st instar larvae per leaf)</th>
<th>Insect Died</th>
<th>Insect Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cry1Ac+Cry2Ab+EPSPs</td>
<td>C3-E2-P4</td>
<td>1</td>
<td>3</td>
<td>15</td>
<td>9</td>
<td>60</td>
</tr>
<tr>
<td>2</td>
<td>Cry1Ac+Cry2Ab+EPSPs</td>
<td>C3-E2-P1</td>
<td>1</td>
<td>3</td>
<td>15</td>
<td>4</td>
<td>27</td>
</tr>
<tr>
<td>3</td>
<td>Cry1Ac+Cry2Ab+EPSPs</td>
<td>C3-E20-P1</td>
<td>1</td>
<td>3</td>
<td>15</td>
<td>11</td>
<td>73</td>
</tr>
<tr>
<td>4</td>
<td>Cry1Ac+Cry2Ab+EPSPs</td>
<td>C3-E2-P5</td>
<td>1</td>
<td>3</td>
<td>15</td>
<td>8</td>
<td>53</td>
</tr>
<tr>
<td>5</td>
<td>Cry1Ac+Cry2Ab+EPSPs</td>
<td><strong>C3-E2-P2</strong></td>
<td>1</td>
<td>3</td>
<td>15</td>
<td><strong>15</strong></td>
<td><strong>100</strong></td>
</tr>
<tr>
<td>6</td>
<td>Cry1Ac+Cry2Ab+EPSPs</td>
<td>C3-E20-P2</td>
<td>1</td>
<td>3</td>
<td>15</td>
<td>9</td>
<td>60</td>
</tr>
<tr>
<td>7</td>
<td>Cry1Ac+Cry2Ab</td>
<td>E49-P3</td>
<td>1</td>
<td>3</td>
<td>15</td>
<td>2</td>
<td>13</td>
</tr>
<tr>
<td>8</td>
<td>Cry1Ac+Cry2Ab</td>
<td>E36-P1</td>
<td>1</td>
<td>3</td>
<td>15</td>
<td>14</td>
<td>93</td>
</tr>
<tr>
<td>9</td>
<td>Cry1Ac+Cry2Ab</td>
<td>E49-P2</td>
<td>1</td>
<td>3</td>
<td>15</td>
<td>4</td>
<td>27</td>
</tr>
<tr>
<td>10</td>
<td>Cry1Ac+Cry2Ab</td>
<td>E16-P1</td>
<td>1</td>
<td>3</td>
<td>15</td>
<td>11</td>
<td>73</td>
</tr>
<tr>
<td>11</td>
<td>Cry1Ac+Cry2Ab</td>
<td>E45-P1</td>
<td>1</td>
<td>3</td>
<td>15</td>
<td>12</td>
<td>80</td>
</tr>
<tr>
<td>12</td>
<td>Cry1Ac+Cry2Ab</td>
<td>E35-P1</td>
<td>1</td>
<td>3</td>
<td>15</td>
<td>11</td>
<td>73</td>
</tr>
<tr>
<td>13</td>
<td>Cry1Ac+Cry2Ab</td>
<td>E39-P1</td>
<td>1</td>
<td>3</td>
<td>15</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>14</td>
<td>Coker-312 (Control)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Challenges

• Regulatory framework

• Trade issues

• Public acceptance
  Perception from Europe vs North America/South America

The way out for developing world

• Education of policy makers/masses

• Genomics assisted breeding

• New breeding technologies
New breeding technologies

New breeding technologies (NBTs) include

1) Site-directed nucleases such as
   a) zinc finger nucleases,
   b) transcriptional activator-like nucleases
   c) clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated Cas9 systems

4) oligonucleotide-directed mutagenesis
3) cisgenesis
4) RNA-dependent DNA methylation,
5) grafting (non-transgenic scion on transgenic rootstock),
6) reverse breeding
7) agro-infiltration
Genomics; a silent revolution

Major improvements in sequencing technologies

All major crops/livestock have either been sequenced or will be sequenced in the next few years

- DNA based marker technologies
- Genotyping by sequencing (GBS)
- Automated phenotyping
- Bioinformatics; Our ability to handle genomic data

Applications
- Technology platform for genomic selection in livestock and plants
- Genome sequencing of microbes selected as microbial factories
- Technology platform for inherited diseases and metabolic disorders
New initiatives for food security; Livestock and poultry

• Livestock constitute 58.33% of agriculture
• 2.3 trillion Dollar Halal market
• Potential to control nutritional deficiency and stunting
• Low milk productivity of local breeds

Solution

• High milk producing foreign breeds
• High quality semen of foreign breeds for crossed animals
• Cross-bred animals by using semen of foreign breeds
• Genetic improvement in yield of local breeds of cows and buffalo
Collaborations in Livestock Genomics

- Center of Excellence in Bovine Genetics (CEBG), RVFC
- Research Center for Conservation of Sahiwal Cattle (RCCSC)
- Jamilur Rehman Institute for Genomics, Karachi
- Beijing Genomics Institute (BGI), Shenzhen, China

Objectives

- Improved quality milk availability in the country for food security and health
- Provision of genomic selection facilities to CEBG and others
- Development of DNA chip for local breeds

Total import of Holstein/Friesian cattle and semen in Pakistan

Estimated number x cost of animal
100,000 x 250,000 = 25 billion
Expected to increase to 250 billion in next five years
Genomic selection of Holstein/Friesian bulls and heifers
Whole genome sequencing of cattle and buffalo breeds of Pakistan

- Whole genome sequencing of cattle breeds
- Sequencing data of buffalo breeds obtained

Applications
- ARM-PCR test for A1A2 and services to RVFC provided
- Data on genomic selection of nine bulls to RVFC

Future
- Use of available SNP chip on Sahiwal cattle
- A new DNA chip design for local breeds
- Genomic selection for meat
Speed breeding; fast forward technology for genetic gain for food, nutritional and feed security

- Progress in productivity is slow
- Need to enhance ‘genetic gain’ by 30-100% by 2050
- **Speed breeding to increase the number of breeding cycles;** homozygous progenies
- Adopted globally by the developed countries and multi-national companies
- ‘Uses extended photoperiods and controlled temperature regimes
- Fully enclosed growth rooms or glasshouses
- 4-7 generations per year can be achieved in six crop species
  - Spring wheat, durum wheat, barley, chickpea, pea, oat, lentils and oilseed brassica
  - A similar approach can be used in maize, and rice, cotton, soybean and mungbean
What is genome editing?
The modification of specific genomic sequences in living cells

For determining, changing, or expanding their function(s).

Typically, GE occurs
After delivering sequence-specific designer nucleases (e.g., ZFNs, TALENs, and CRISPR/Cas9) and donor DNA constructs into target cells

These designer nucleases can generate gene knockouts or gene knock-ins when applied alone or in combination with donor DNA templates, respectively
• Zinc finger nucleases
• Transcription activator-like effector nucleases
  • Expensive and time-consuming
• A recent and more innovative process..
Clustered regularly interspaced short palindromic repeats (CRISPRs) and CRISPR-associated (Cas) proteins
CRISPR

A powerful gene-editing technology is the biggest game changer to hit biology since PCR. But with its huge potential come pressing concerns.
To initiate gene modification

sgRNA
(single guide RNA)

+ 

Cas9 nuclease

Cas9 complex
Gene of Interest

Protospacer Adjacent Motif

Target Sequence (PAM)

Gene of Interest
Non-Homologous End Joining (NHEJ) DNA repair pathway
Summation: crispr/Cas system

sgRNA/
Cas9 complex
binds to
gene

Cas breaks
gene
(double
strand
breaks)

Induced
mutation in
gene
sequence

altered gene sequence
Genome edited products to hit market very soon without undergoing biosafety trials.

DuPont Pioneer’s high amyllopectin corn is the first CRISPR-edited plant likely to bypass USDA oversight.

The common white button mushroom (Agaricus bisporus) has been modified to resist browning.
New breeding technologies; Applications in food and fibre crops

Rice; yield, better grain, nutritional value, herbicide tolerance

Potato; virus resistance, sweetening control, stress tolerance, late blight resistance

Wheat; yield, disease resistance, nutritional value

Cotton; disease resistance, better quality, nutritional value

Oilseed crops; higher yield, better quality, nutritional value
### Grand regional/global challenges where new plant breeding technologies can provide immediate solutions

<table>
<thead>
<tr>
<th>Food crop</th>
<th>Target region / community</th>
<th>Food security issue</th>
<th>Yield losses</th>
<th>How New Breeding Technologies (NPBTs) can help</th>
</tr>
</thead>
</table>
| Wheat     | West Asia, North Africa   | Powdery mildew     | Up to 40% on average, 100% in case of early infection | • Genome editing of MLO \(^1\) (encoding a membrane-associated protein that is required for fungal penetration of host) or/and TaEDR1 \(^2\) (encoding a Raf-like mitogen-activated protein kinase kinase kinase (MAPKKK)) in stably transformed local cultivars  
• Speed breeding for rapid introgression of MLO/TaEDR1 resistance alleles \(^3\) into local cultivars |
| Maize     | South and East Africa     | Drought             |              | • Genome editing of a gene encoding negative regulator of ethylene responses ARGOS8 \(^4\) in stably transformed local cultivars |
| Rice      | South-East Asia           | Rice blast (Magnaporthe oryzae) | 10-30% loss in annual production | • Genome editing of an ethylene responsive factor gene OsERF922 \(^5\) in stably transformed local cultivars |
|           |                            | Bacterial leaf blight (Xanthomonas oryzae) | Up to 70% under favorable conditions | • Genome editing of a sucrose transporter gene OsSWEET14 \(^6\) in stably transformed local cultivars |
|           |                            | Drought             | Up to 30% loss in rainfed production zones | • Genome editing of genes in the large effect QTL qDTY12.1 \(^7\) especially the gene encoding a recently characterized amidohydrolase for root architecture (IRRI, unpublished results) |
| Cassava   | East and Central Africa   | Cassava brown streak disease | Annual losses worth US$1billion | • Genome editing of elongation factor eIF4E \(^8\) in stably transformed local cultivars |
| Banana    | West and Central Africa   | Fusarium wilt       | 40-60%, responsible for Panama disease epidemic | • Replacement of RGA2, a nucleotide-binding and leucine-rich repeat (NB-LRR)-type resistance gene \(^9\) using CRISPR/Cas RNP in banana |
Poultry, fish and cattle become target of genome editing
Editing humanity
A new technique for manipulating genes holds great promise—but rules are needed to govern its use
The Economist Aug 22nd 2015
Conclusions

- Genome editing by CRISPR-Cas has emerged as a powerful tool due to ease, specificity and low cost
- Diverse applications
- No markers to check for editing
- Raises ethical concerns
Thematic group; Priority areas

**Poultry vaccines**
- Salmonella conjugate vaccines
- Vector based multiple vaccines
- Epitope focused vaccines

**Feed**
- Feed enzymes
- Soybean meal
- Aflatoxin free cotton seed cake
- Probiotics

**Genetic improvement**
- Sahiwal cow genome initiative; breeds for milk and meat
- Nilli-Ravi buffalo genome sequencing initiative
- Genotyping by sequencing (GBS); cows and buffalos
Genetic improvement of livestock breeds

Whole genome sequencing of Sahiwal cattle in collaboration

Center for Conservation of Sahiwal Cattle
Jamil-ur-Rehman Institute, HEJ, Karachi,
Beijing Genomic Institute, China
CAAS, Beijing, China

Center for Bovine Genetics, RVFC
UMT Enterprises and Neogen, USA
Project Workflow

1. Sampling
2. DNA extraction
3. DNA fragmentation
4. Library preparation
5. Enrichment
6. Sequencing by ABI SOLiD
7. Emulsion PCR
8. Tertiary Data processing
9. Secondary Data processing
10. Primary Data processing

Steps:
- Sampling
- DNA extraction
- DNA fragmentation
- Library preparation
- Enrichment
- Sequencing by ABI SOLiD
- Emulsion PCR
- Tertiary Data processing
- Secondary Data processing
- Primary Data processing
## Summary of the mapping statistics

<table>
<thead>
<tr>
<th>Animal ID</th>
<th>Number of total mapped reads</th>
<th>Number of mapped reads</th>
<th>Number of bases in mapped reads (Gbs)</th>
<th>Mean depth</th>
<th>Genome Coverage (in %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sunny</td>
<td>1022539078</td>
<td>1022539078</td>
<td>59.90</td>
<td>21x</td>
<td>77%</td>
</tr>
</tbody>
</table>
Per chromosome depth coverage

x Coverage

% Width Coverage
Other breeds being sequenced with BGI

- Cholistani cattle
- Cholistani cattle
- Achai Cattle
- Achai Cattle
- Bhagnari
- Bhagnari
- Bhagnari
- Dajal
- Gabraali Cattle
- Gabraali Cattle
- Hisar Hiryana
- Hisar Hiryana
- Sahiwal
- Tharparkar
- Dhanni
- Dhanni
- Dhanni
- In pipeline
- Red Sindhi
- Lohani
Genetic improvement of livestock breeds

Whole genome sequencing of Sahiwal cattle in collaboration with University of Karachi, Karachi and Research Center for Conservation of Sahiwal Cattle (RCCSC) Jhang.
Degradation of Bispyribac-Na, a widely used wheat herbicide by the bacterial consortium BDAM in vegetated soil (pot experiment)
BioPower Production

>150,000 bags, were produced (2010-13) July 2014 to-date 38392 bags = Rs 3.33 M

Technology transfer process in progress

Future: Next generation Biofertilizers

Value additions, e.g., biopesticides, growth promoting hormones, P-solubilization, insecticide and herbicide degradation, new carrier material

Field testing of BioPower
## Wheat-mungbean-rice cropping system

### Wheat
- 20% saving of Urea & DAP fertilizers
- 6% yield increase i.e. 99 kg/ acre
- @Rs. 1280/ 40 kg
- Rs. 2000/ acre
- Rs. 3168/ acre

### Mungbean
- Yield 200 kg/acre @ Rs. 4500/ 40 kg
- Rs. 22500/-
- (Yield potential can be upto 1200 kg/ acre)

### Rice
- 20% saving of Urea & DAP fertilizers
- 3.8% yield increase i.e. 65 kg/ acre
- @Rs. 1500/ 40 kg
- Rs. 2000/-
- Rs. 2438/-

**Total saving from Wheat-Mungbean-Rice cropping system Rs. 32,106/ acres**

*If 10% area= more than Rs. 10 Rs. Billion/year*
Take home message

Beyond impact factor; impact factor to impact

Identification of key impact areas

Optimal use of manpower

Greater collaboration to enhance discovery and applications

Road map and timeline for key activities
Thanks
## Drought Tolerant Transgenic Sugarcane Expressing AVP1 Gene

Field evaluation of transgenic sugarcane for drought tolerance at SRI, AARI, Faisalabad

<table>
<thead>
<tr>
<th>Irrigation</th>
<th>Treatments</th>
<th>Cane yield (t/ha)</th>
<th>% increase</th>
<th>Expected Production (tons)</th>
<th>Economic Gain (Rs.)@ 10% Adoption</th>
</tr>
</thead>
<tbody>
<tr>
<td>100%</td>
<td>Control</td>
<td>118.75</td>
<td>4.42</td>
<td>448818.70</td>
<td>13.73 billion</td>
</tr>
<tr>
<td></td>
<td>Transgenic</td>
<td>124.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60%</td>
<td>Control</td>
<td>94.25</td>
<td>7.43</td>
<td>4617563.70</td>
<td>22.78 billion</td>
</tr>
<tr>
<td></td>
<td>Transgenic</td>
<td>101.25</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Salt Tolerant Sugarcane Expressing AtNHX1 Gene

Field evaluation of transgenic sugarcane for drought tolerance at SSRI, Pindi Bhattian

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Cane yield (t/ha)</th>
<th>% increase</th>
<th>Expected Production (tons)</th>
<th>Economic Gain (Rs.) @ 10% Adoption</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>173.65</td>
<td>10.04</td>
<td>4729746.9</td>
<td>25.28 billion</td>
</tr>
<tr>
<td>Transgenic</td>
<td>191.09</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
## Field evaluation of transgenic sugarcane for drought tolerance at SRI, AARI, Faisalabad

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Cane yield (t/ha)</th>
<th>% increase</th>
<th>Expected Production (tons)</th>
<th>Economic Gain (Rs.) @ 10% Adoption</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>114.25</td>
<td>6.96%</td>
<td>4580169.38</td>
<td>20.61 billion</td>
</tr>
<tr>
<td>Transgenic</td>
<td>122.25</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>