IMPROVEMENT OF DROUGHT AND HEAT TOLERANCE IN WHEAT
(Triticum aestivum) USING INDUCED MUTAGENESIS

by

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Wheat Origin And Genome Organization

- Wheat (*Triticum* spp.) was domesticated in the Fertile Crescent >8000 years BC

- *T. aestivum* is an allohexaploid, with its three sub-genomes designated A, B, and D (*Progenitor species*)

- Diploid = (AA) 2n=14 example; Einkorn

- Tetraploid = (AABB) 2n=4x=28 example; Emmer & Durum

- Hexaploid = (AABBDD) 2n=6x=42 example; wheat bread 16.72 x 19^9 bp
THE ORIGIN OF WHEAT conti

**Triticum urartu**  \( \times \)  **Aegilops spp.**

\[
\begin{array}{c}
\text{AA} \\
(2n=14)
\end{array}
\begin{array}{c}
\text{BB} \\
(2n=14)
\end{array}
\]

**Triticum tauschii**  \( \times \)  **Triticum turgidum**

\[
\begin{array}{c}
\text{DD} \\
(2n=14)
\end{array}
\begin{array}{c}
\text{AABB} \\
(2n=4x=28)
\end{array}
\]

**Triticum aestivum**

\[
\text{AABBD} \\
(2n=6x=42)
\]
ECONOMIC IMPORTANCE OF BREAD WHEAT

- Second most produced cereal crop
- Its production is expected to reach 860 million tons per annum by 2030
Environmental stresses affecting wheat production

• Improving crop performance in moisture and temperature-stressed environments is among the most serious challenges facing global agriculture.

• Expected to worsen in Sub-Saharan Africa.

• Biotic stresses (pathogens and pests) impose great threats as well.

• Responses to moisture deficit include stomatal closure, reduced photosynthesis, reduced growth rate, and generation of toxic chemicals.

1.1: Breeding for high water use efficiency

- Irrigated agriculture (70% withdrawal)
- Remote Sensing: a tool in monitoring water use management
- RS provides information on land-use, irrigated area, crop type, biomass development, crop water requirements etc
- Crop water productivity derived remote sensing models for wheat
  - GEPIC
  - WATer PROductivity (WATPRO)
  - SEBAL etc
1.2: Induced Mutagenesis (Mutation Breeding)

- Enhancing the **genetic tolerance** of crops to Abiotic (drought) and Biotic stresses - crucial component for increasing crop production

- Mutation breeding - non-transgenic improvement of crops through the induction of mutations

- Base pair changes had been induced in a population of plants by treating seed with a **chemical** or **physical** mutagen (Mutagenesis)

- **Targeted Induced Lesions IN Genomes (TILLING)** - method for high-throughput reverse genetics
1.2.1: SUMOYLA TION

- SUMO (Small Ubiquitin-like Modifier) class of molecules drive the mechanism for target protein management

- Post-translational modifications of proteins plays role in cellular signaling processes (Targets for stress-responsive SUMOylation)

- SUMOylation improves plant growth during drought stress

- Key stress response regulators (CBF1, DREB1, ICE1 and AB15) have clear SUMO attachment sites
<table>
<thead>
<tr>
<th>Gene</th>
<th>Name</th>
<th>Refs</th>
<th>Gene</th>
<th>Name</th>
<th>Refs</th>
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<tbody>
<tr>
<td>DREB2A</td>
<td>Dehydration-responsive element binding protein 2</td>
<td>[10,91]</td>
<td>RBOHC &amp;</td>
<td>Respiratory burst oxidase</td>
<td>[27,91]</td>
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<td>DRIP1, 2</td>
<td>DREB2A-interacting proteins 1, 2</td>
<td>[56,91]</td>
<td>RBOHD</td>
<td>homologs C and D</td>
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<td>DSM1</td>
<td>Mitogen-activated protein kinase kinase kinase</td>
<td>[29]</td>
<td>RCA</td>
<td>Rubisco activase</td>
<td>[24]</td>
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<td>ERF</td>
<td>Ethylene response factor</td>
<td>[30,91]</td>
<td>RCA1</td>
<td>Short isoform of RCA</td>
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<td>FLC</td>
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<td>[18]</td>
<td>RD20</td>
<td>Responsive to desiccation 20</td>
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<td>FTA</td>
<td>$\alpha$-famesyltransferase</td>
<td>[53]</td>
<td>RD22</td>
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<td>GA2ox7</td>
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<td>Rma1H1</td>
<td>RING membrane-anchor 1 E3 ubiquitin ligase</td>
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<td>GH3</td>
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<td>ROF1</td>
<td>Rotase FK506 Binding Protein 1</td>
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<td>Regulatory particle non-ATPase 12a</td>
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<td>G protein</td>
<td>GTP-binding protein</td>
<td>[25,52]</td>
<td>SAL1</td>
<td>3’(2’), 5’-biphosphate nucleosidase</td>
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<td>GRP7</td>
<td>Glycine-rich protein 7</td>
<td>[72,91]</td>
<td>Ser 228</td>
<td>Autophosphorylation site of SOS2</td>
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<td>GSNOX</td>
<td>S-nitroso glutathione reductase</td>
<td>[63]</td>
<td>SODs</td>
<td>Superoxide dismutases</td>
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<td>HKT1;1</td>
<td>High affinity potassium transporter 1</td>
<td>[71]</td>
<td>SOR</td>
<td>Superoxide reductase</td>
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<td>HOS3</td>
<td>Hyper-osmotically sensitive gene</td>
<td>[73]</td>
<td>SOS2</td>
<td>Salt Overly Sensitive 2</td>
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<tr>
<td>HOT5</td>
<td>Sensitive to hot temperatures</td>
<td>[63]</td>
<td>TaSnRK2.4</td>
<td>T. aestivum serine/threonine protein kinase</td>
<td>[74]</td>
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<td>HPR1</td>
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<td>[53]</td>
<td>TdDHZN</td>
<td>T. durum dehydrins</td>
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<td>InsP5-ptase</td>
<td>Inositol polyphosphate 5-phosphatase</td>
<td>[51]</td>
<td>V-ATPase</td>
<td>Vacuolar H*-ATPase</td>
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<td>ISPS</td>
<td>Isoprene synthase</td>
<td>[58]</td>
<td>WLIP19</td>
<td>Wheat low-temperature induced protein 19</td>
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OBJECTIVES

AIM: Improvement of drought and heat tolerance in wheat (*Triticum aestivum*) using chemical induced mutagenesis

1. **Develop** mutagenic wheat lines (SA) – Chemical induced mutagenesis

2. **Evaluate** the mutagenic lines & introduced lines under moisture-stress and control conditions

3. Evaluate the mutagenic lines & introduced lines for **pest resistance**

4. Conduct TILLING to **identify** induced mutations & natural sequence variation in candidate genes

5. Develop a **water-stress Map** (SA) using Remote Sensing
**MATERIALS AND METHODS**

**SA VARIETIES**

- Tugela Dn, Gamtoos Dn7 etc

**INDUCED MUTAGENESIS**

- Four independent treatments (for each chemical mutagen)
- Different concentrations & exposure times (in hours)
- Mutagenic control lines obtained (Using H₂O)
MATERIALS AND METHODS cont

1. SA VARIETIES

2. INDUCED MUTAGENESIS
   - Mutagenic lines Obtained
   - Control lines
   - Population development
   - EMS, Na₃N, NMU, MH

3. PHENOTYPIC SCREENING OF MUTAGENIC LINES
   - Drought stress conditions
   - Russian Wheat Aphids (RWA)
   - DNA extraction

- Weather data, Plant development, Plant height, Yield-related
- Grain filling duration (GFD) & Grain filling rate (GFR)
- Pest resistance (chlorosis scores, streaking, leaf rolling, virulence rating and aphid fecundity assessment)

Lee et al 2011
Initial Status of my project ....

NaN$_3$ treated

Untreated control

~ 85% $\text{H}_2\text{O}$ loss
Current Status of my project ....

4hr NaN₃ treatment
MATERIALS AND METHODS cont

1. SA VARIETIES

2. INDUCED MUTAGENESIS
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4. CYTOLOGY ANALYSIS
   - Chromosomal aberrations induced by Mutagens

Chromosomal rupture
- Anaphasic bridges
- Micronuclei

Non-disjunction
- Stekinesis

Marcano et al 2004
MATERIALS AND METHODS cont

1. SA VARIETIES

2. INDUCED MUTAGENESIS
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4. CYTOLOGY ANALYSIS
   - Chromosomal aberrations induced by Mutagens in root tips

5. TILLING
   - Identifies genetic variation at the nucleotide level
MATERIALS AND METHODS cont

1. **SA VARIETIES**

2. **INDUCED MUTAGENESIS**
   - Mutagenic lines Obtained
   - Control lines
   - Population development

3. **PHENOTYPIC SCREENING OF MUTAGENIC LINES**
   - Drought stress conditions
   - Russian Wheat Aphid (RWA)
   - DNA extraction

4. **CYTOLOGY ANALYSIS**
   - Chromosomal aberrations induced by Mutagens in root tips

5. **TILLING**
   - Identifies genetic variation at the nucleotide level

6. **SCREENING POPULATIONS**
   - Field trials
EXPECTED OUTCOMES

- The identification of key drought stress genes and related mutations

- Confirmation of the SUMO mechanism as a strategy for improving drought and heat stress tolerance in wheat

- Data on the agronomic adaptability of developed M₂ lines for drought and heat stress tolerance, including pest resistance

- Water-use and distribution map from Remote sensing data
POSSIBLE SOCIAL -ECONOMIC BENEFITS

• Benefits to smallholder agriculture of Sub-Saharan Africa where wheat is mostly grown under dryland conditions.

• Potential industry and/or innovation spin-off. The proposed project will be deliver wheat lines with good agronomic characteristics with enhanced tolerance to heat and drought.

• Water-use and water-distribution assessment in major wheat growing regions (SA)
HOW DOES THIS PROJECT ADVANCE FOOD SECURITY?

- Genomics (SU)
- Selection for improved drought and heat tolerance in cereal crops
- Products (Drought tolerant crop)
- Field release and trials
- Public acceptance
- Receptive
- Policies
- Regulations
- Food security
- Global concern
- Water security
- Global concern
- Water use efficiency
- Training
- Climate change
- Global concern
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2. Co-supervisor Dr. Leon van eck

3. Lab 239` colleagues

4. Department of Genetics

5. Parents
Questions?

GEORGE WEST
CHURCH OF CHRIST

BIBLE STUDY
9:30AM

SUNDAY WORSHIP
10:30AM & 6:00PM

WED. 6:00PM

PRAY FOR RAIN