Current Trends in Genomics and Phenomics in Wheat Breeding

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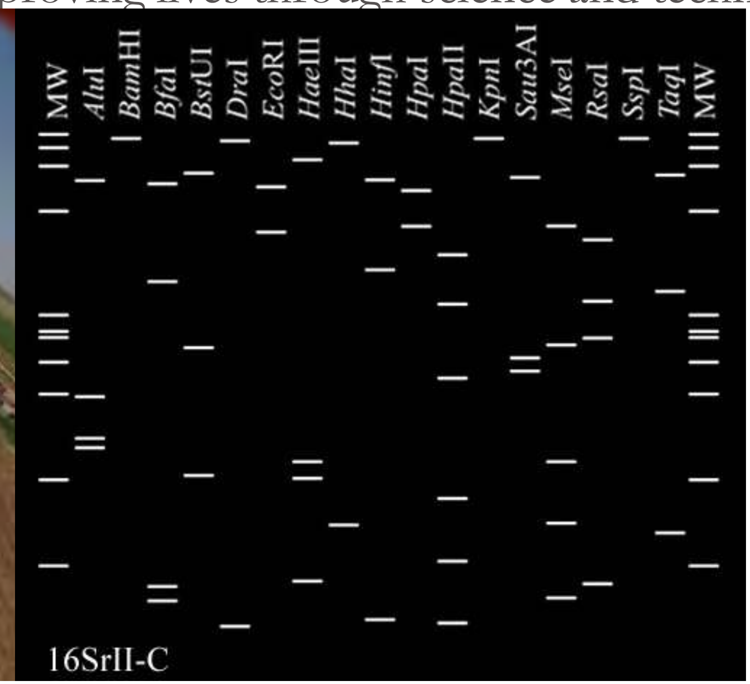
Improving lives through science and technology



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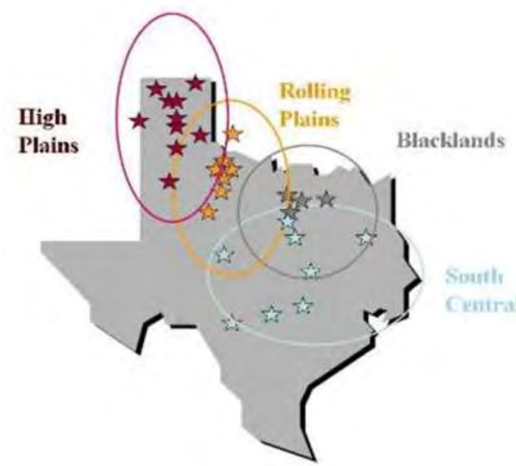
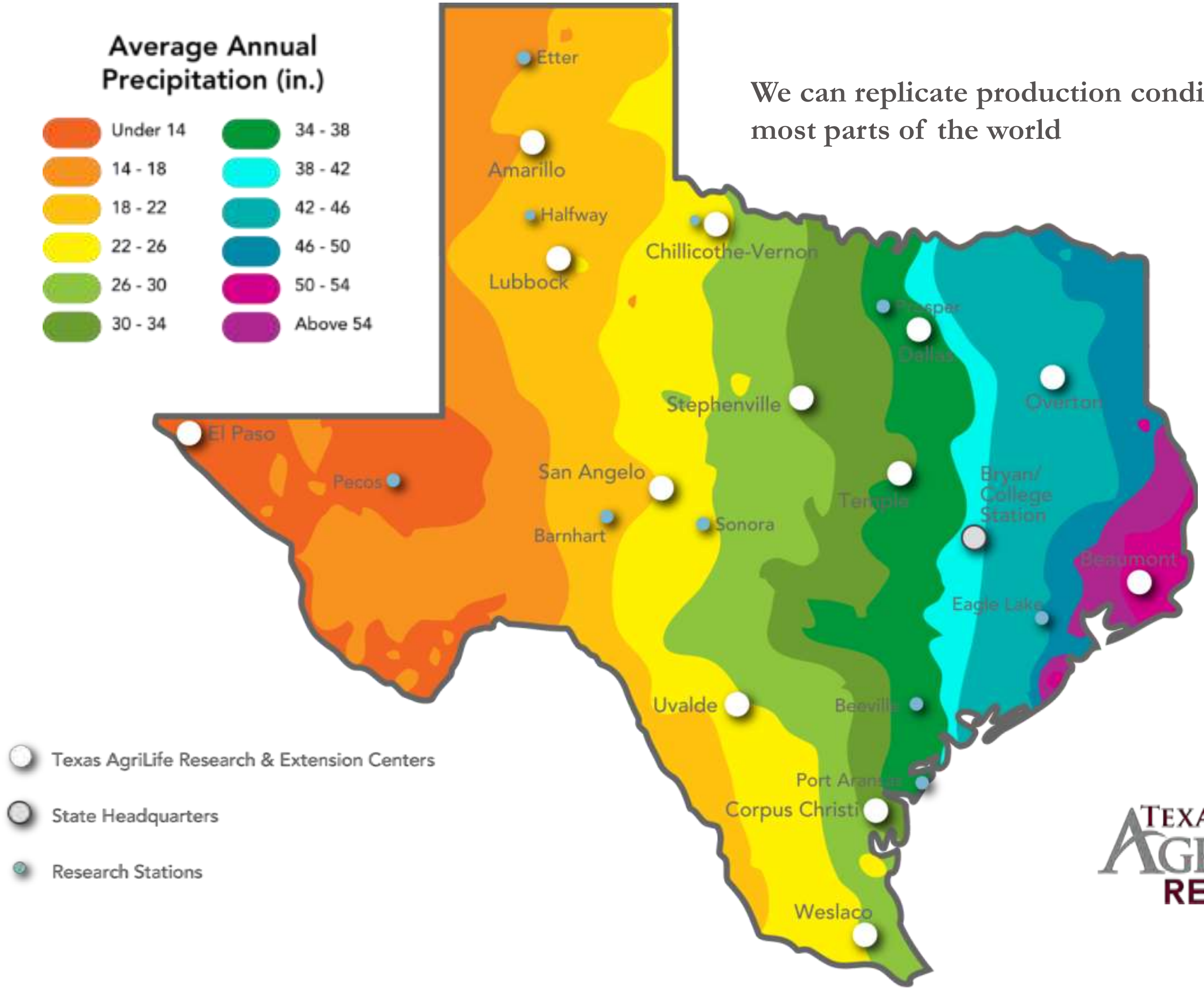
Essential components for wheat breeding

- Multi-environment testing
  - Diverse environments (20+)
  - Multiple stresses
- Germplasm
  - Diverse germplasm: Synthetics/Parent building/Hybrid wheat
  - Doubled-haploid production
  - Genome selection
  - Novel traits/Gene editing
- High throughput genotyping/Phenotyping
  - Genes/QTL mapping
  - Marker assisted breeding/BC
  - High throughput phenotyping (UAS & ground-based)
- End-use quality evaluation



Precipitation Diversity



We can replicate production conditions for most parts of the world

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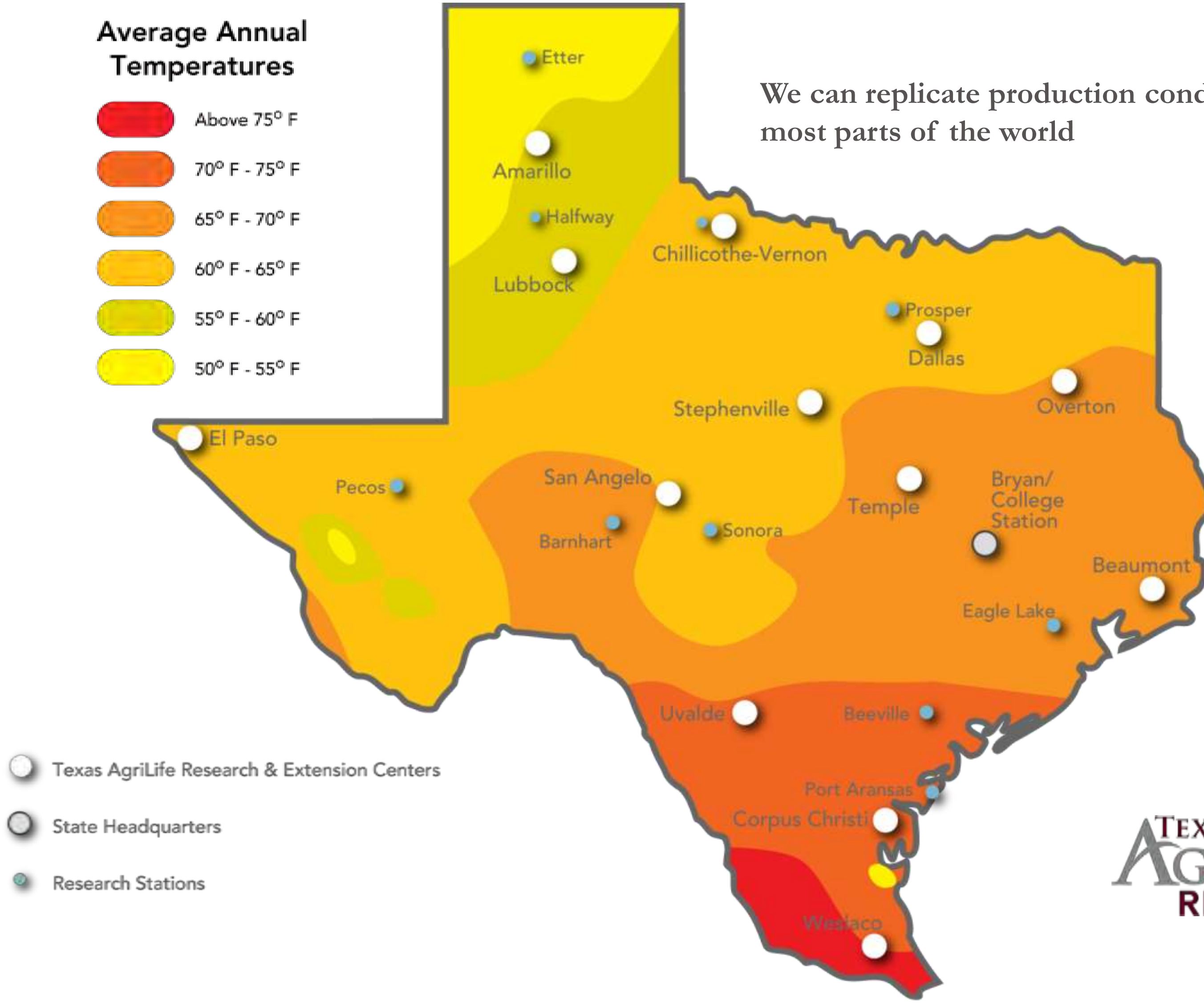
Temperature Diversity

We can replicate production conditions for most parts of the world



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Soil Diversity

We can replicate production conditions for most parts of the world

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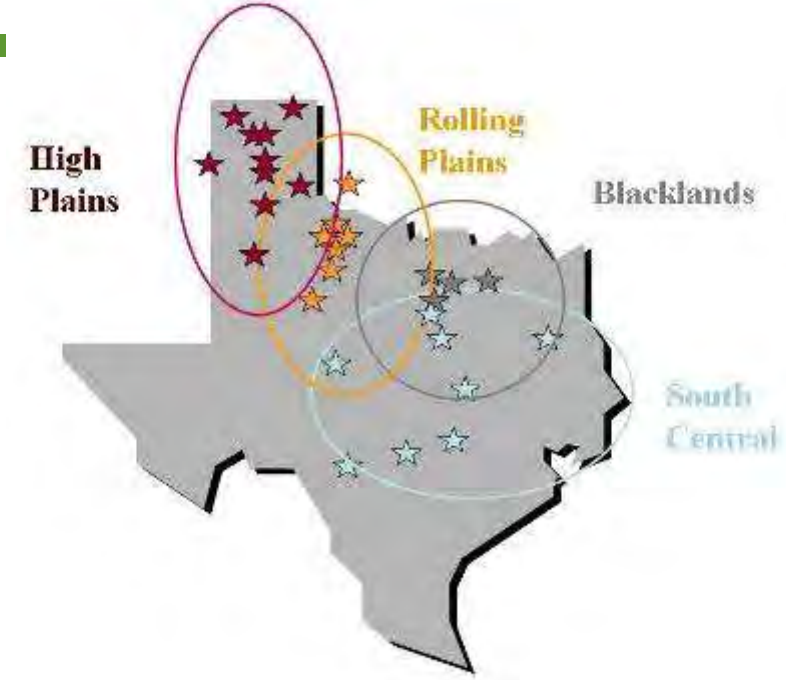

High Plains
- Drought tolerance
- Water-use efficiency
- WSMV resistance
- Russian wheat aphid

Rolling Plains
- Drought / high temp
- Hessian fly

Blackland
- Septoria diseases
- Powdery mildew
- Hessian fly

STATE WIDE
- Grain yield
- End-use quality
- Forage production
- Rust resistance
- Greenbug resistance

Central / South Texas
- High temp tolerance
- Low/intermediate vernalization
- Hessian fly

Diseases

- Leaf, stripe, and stem rusts
- Septoria
- Tan spot
- PMD
- BYDV
- WSMV
- Karnal bunt

Ug99

- Major gene resistance easily defeated (*Sr*24, *Sr*31, and *Sr*36)
- US programs will release newer novel genes introgressed into adapted backgrounds
- Combine *Sr*2, *Sr*22, *Sr*24, *Sr*25, *Sr*32, *Sr*35, *Sr*39, *Sr*40, 1RS.1AL, etc, with minor gene resistance ('King Bird')

***Ug*99 is wrecking havoc In E. Africa. Are we ready in the US?**

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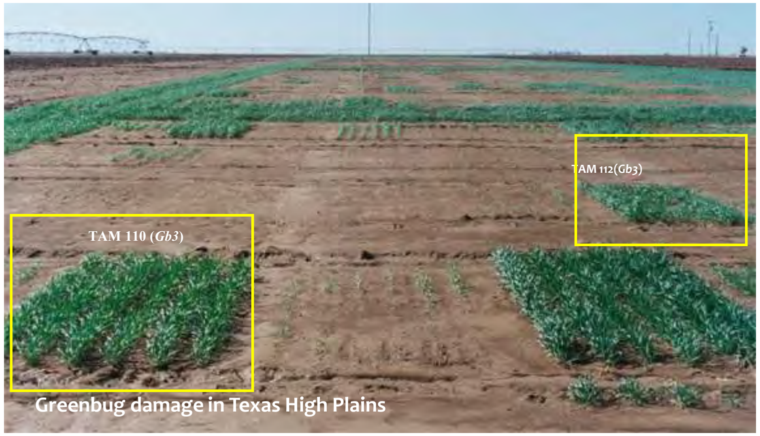
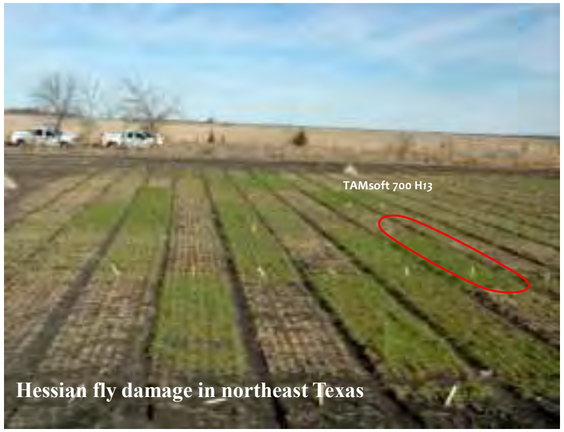
Arthropod pests

- Greenbug
- Wheat curl mite
- Hessian fly



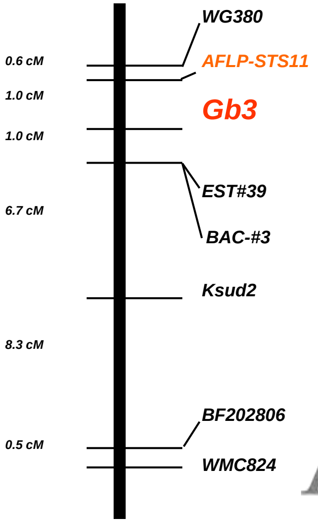


Greenbug resistance

A severe outbreak of greenbug in 2002 devastated this breeding nursery in the Texas Panhandle and only resistant wheat survived.

Gb3, the greenbug resistance gene in **TAM 112** has been mapped and diagnostic markers have been designed.

Chromosome 7DL



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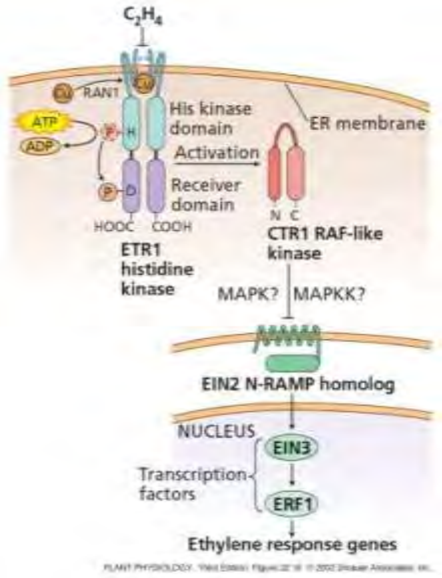
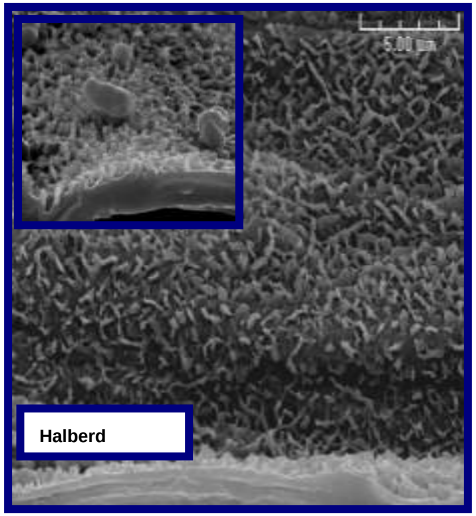
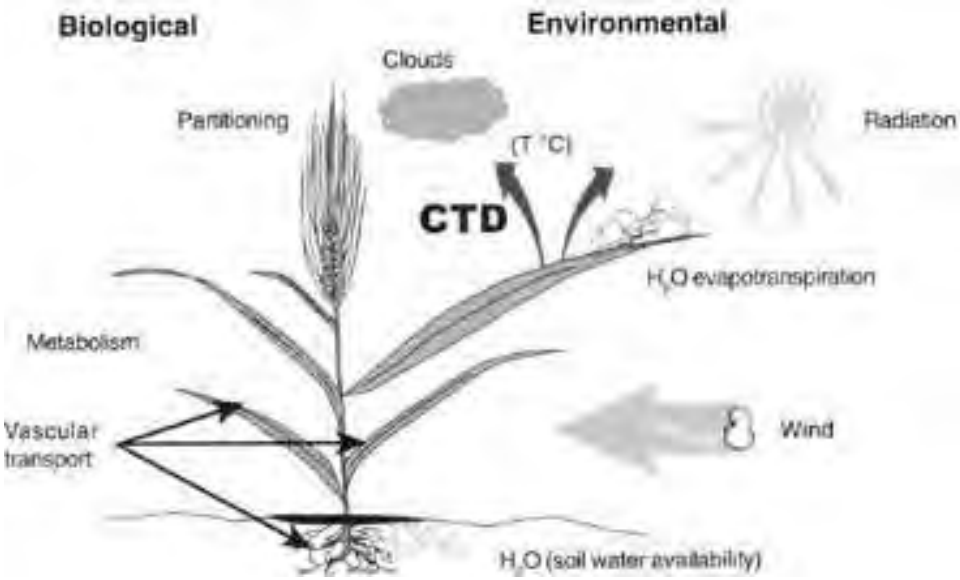
Heat and Drought Stresses







Reynolds et al., 2001

Flow diagram of TAM Wheat Breeding

Year	Where / trial name	Generation	Entries	Reps	Locations
Year 1	Greenhouse Crossing Block		2000		
Year 2	Greenhouse rows	F_1	2000		
Year 3	Field plots	F_2	2000		
Year 4	Field plots	F_3	1600	1	2
Year 5	Field plots	F_4	1200	1	2
Year 6	Head-rows	$F_{4:5}$	90,000	1	2
Year 7	Preliminary yield trials	$F_{4:6}$	3000	1	2
Year 8	Year 1: Advanced yield trials	Advanced	700	2	5
Year 9	Year 2: Advanced yield trials	Advanced	200	2	7
Year 10	TXE		15	3	18
Year 11	SRPN, TXE, and increase		6	3	42
Year 12	SRPN, TXE, UVT, increase, and WQC		2	3	65
Year 13	Release		1		

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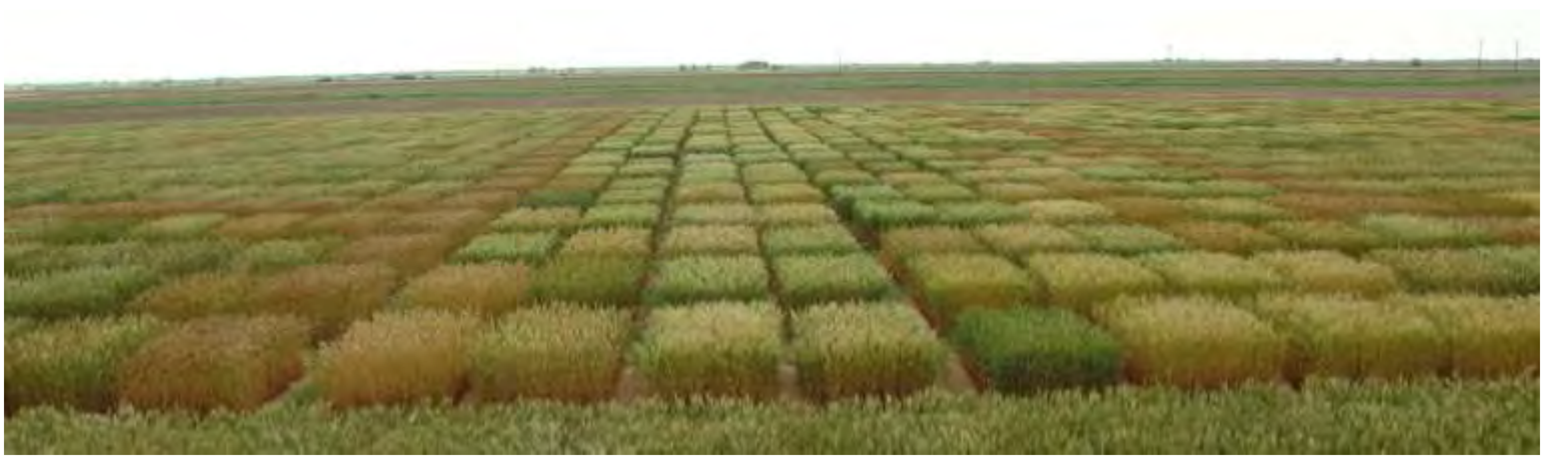
Wheat Variety Survey 2014

Texas	Kansas	Colorado	Nebraska*
TAM 111 – 16.7%	Everest – 14.3%	Hatcher – 25.2%	Settler CL – 9.5%
TAM 112 – 5.3%	TAM 111 – 11.6%	Byrd – 14.8%	TAM 111 – 6.0%
Duster – 4.0%	T158 – 5.0%	TAM 111 – 5.4%	SY Wolf – 5.6%
Fannin – 3.2%	TAM 112 – 4.6%	Snowmass – 5.1%	Brawl CL Plus – 5.3%
WM 135 – 3.0%	Armour – 4.2%	Ripper – 4.4%	Overland – 4.2%



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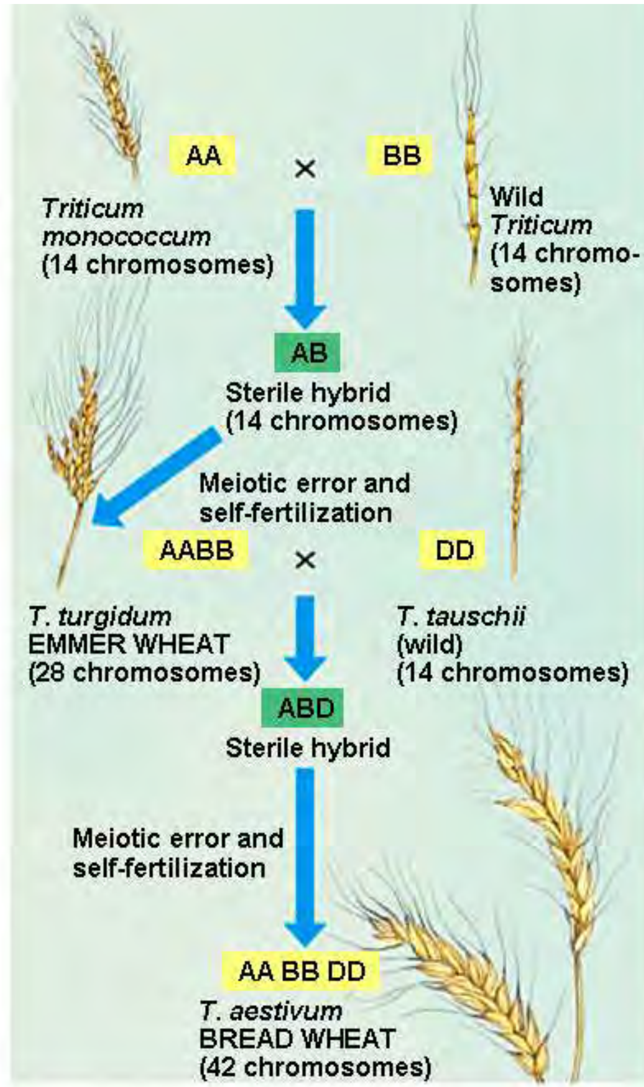
* Nebraska variety survey is based on 2015 planting

Synthetic wheat

- Synthetics contribute to higher yields through increasing seed size and weight
- Yield of improved synthetics mostly influenced by head number and seed per head
- Improving yield could result from selections of **seed per head** or **head number** in improved synthetic lines



Contributions of synthetics to wheat improvement

- Increased the genetic diversity of wheat
- Increased yield under drought and optimum conditions
- Contributed to insects, nematodes and fungal disease resistance
  - Leaf, stem and yellow rusts
  - Nematodes
  - Septoria leaf blotch
  - Greenbug
- Salinity tolerance

Synthetic wheat

All synthetics combined well for SEED WEIGHT

Synthetic that combined well for HEAD NUMBER

(S3,S5,S8,S9)

x

RP that combined well for SEED PER HEAD

(TAM111)

↓

Highest Yielding progeny

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Synthetic wheat

- Seed yield is positively correlated (*P* < 0.01) with head number, seeds per head, and single kernel weight
- Preliminary studies show that there is wide range of diversity in the association mapping panel for abiotic and biotic stress tolerance
- Work in progress:
  - Identify SNP markers associated with drought tolerance
  - Determine genetic gain achievable for yield via indirect selection for tiller number and seeds per head
  - Identify new genotypes with higher yield and better tolerance to abiotic and biotic stresses

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Hybrid wheat: Reasons for renewed interest

- Availability of new next generation sequencing technology
- Performance potential
  - Higher biomass and yield
  - Drought tolerance
  - Consistent performance
  - Good and consistent quality
  - Good disease resistance and agronomic adaptation
  - Vigorous root system
  - Increase production in marginal lands and low fertility levels
- Higher prices of wheat

Source: http://www.cropco.co.uk/cropco-services/hybrid-wheat/

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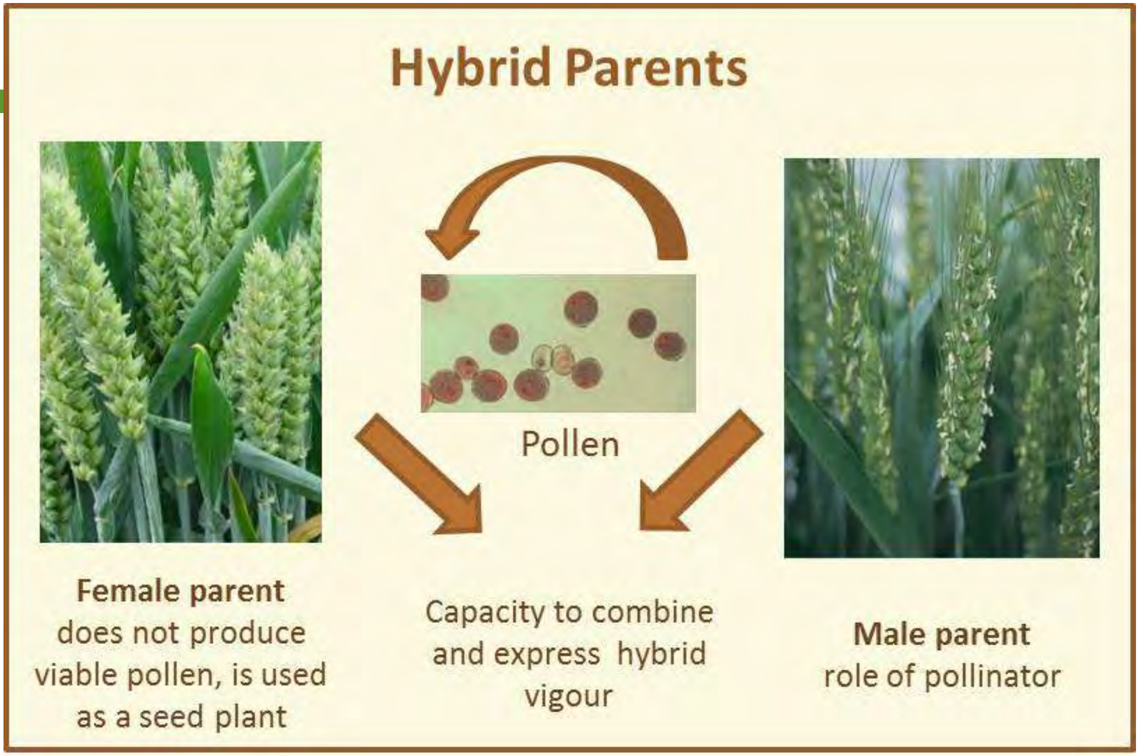
Hybrid wheat - Challenges

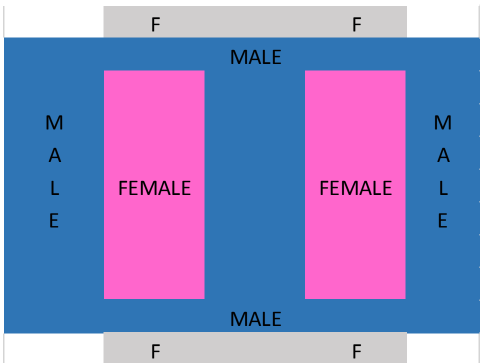
- Poor pollen dispersal and viability
- Floral morphology
- Large cost of seed increase



Source: http://www.hybridwheat.net/Presentation-616.aspx

Gapping CMS female wheat plant with the lemma and palea separated and stigma exposed to receive pollen

Crossing Block



- Elite lines screened for floral traits and yield
- Three crossing blocks; one each in Lincoln, Bushland and Prosper
- Number of female plots surrounded by single male parent
- Female plots sprayed with CHA (CROISOR®) from Saaten-Union
- Few female plants covered with bags to check sterility
- Female plots harvested upon maturity

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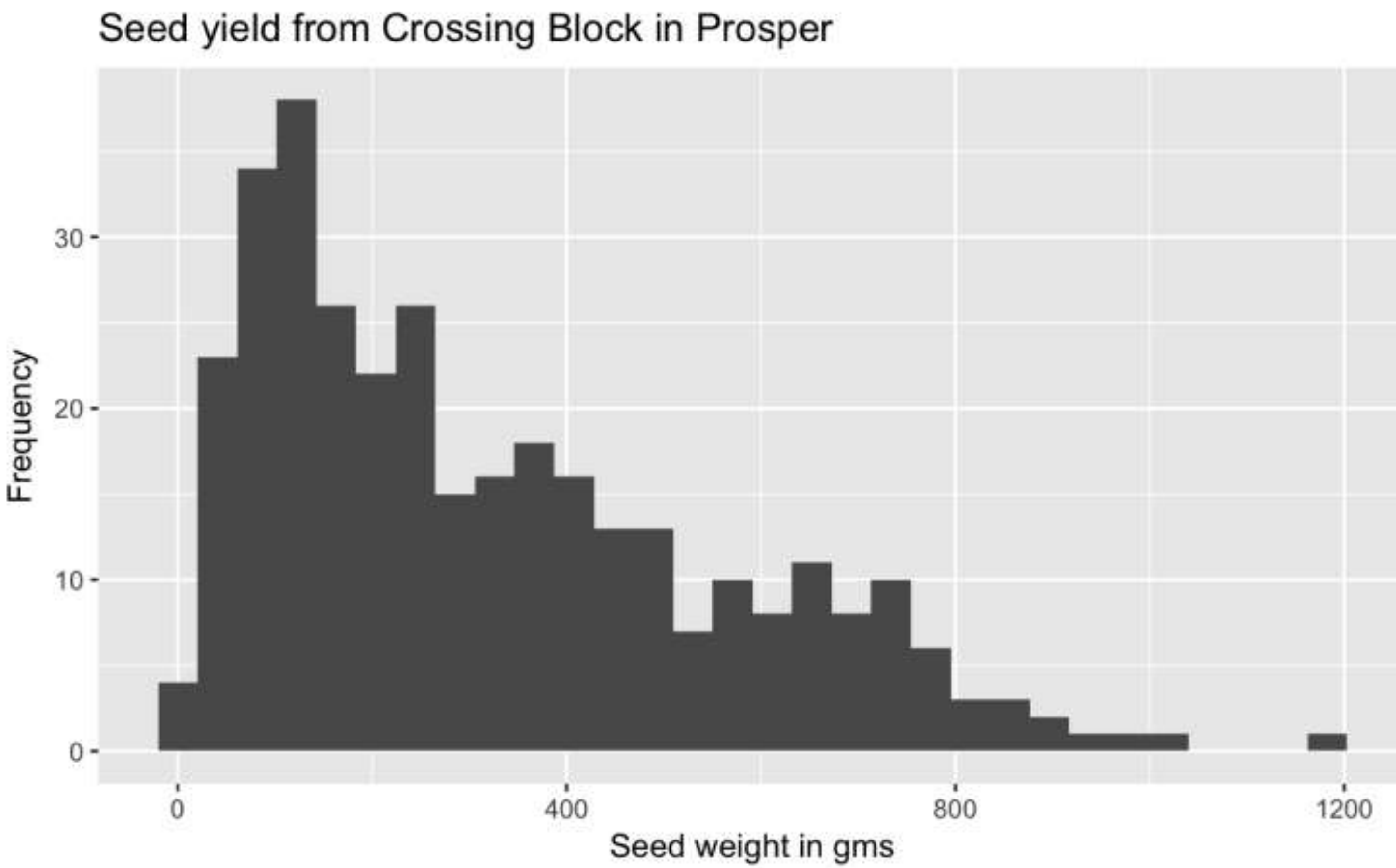
Seed yield from 2017 crossing blocks



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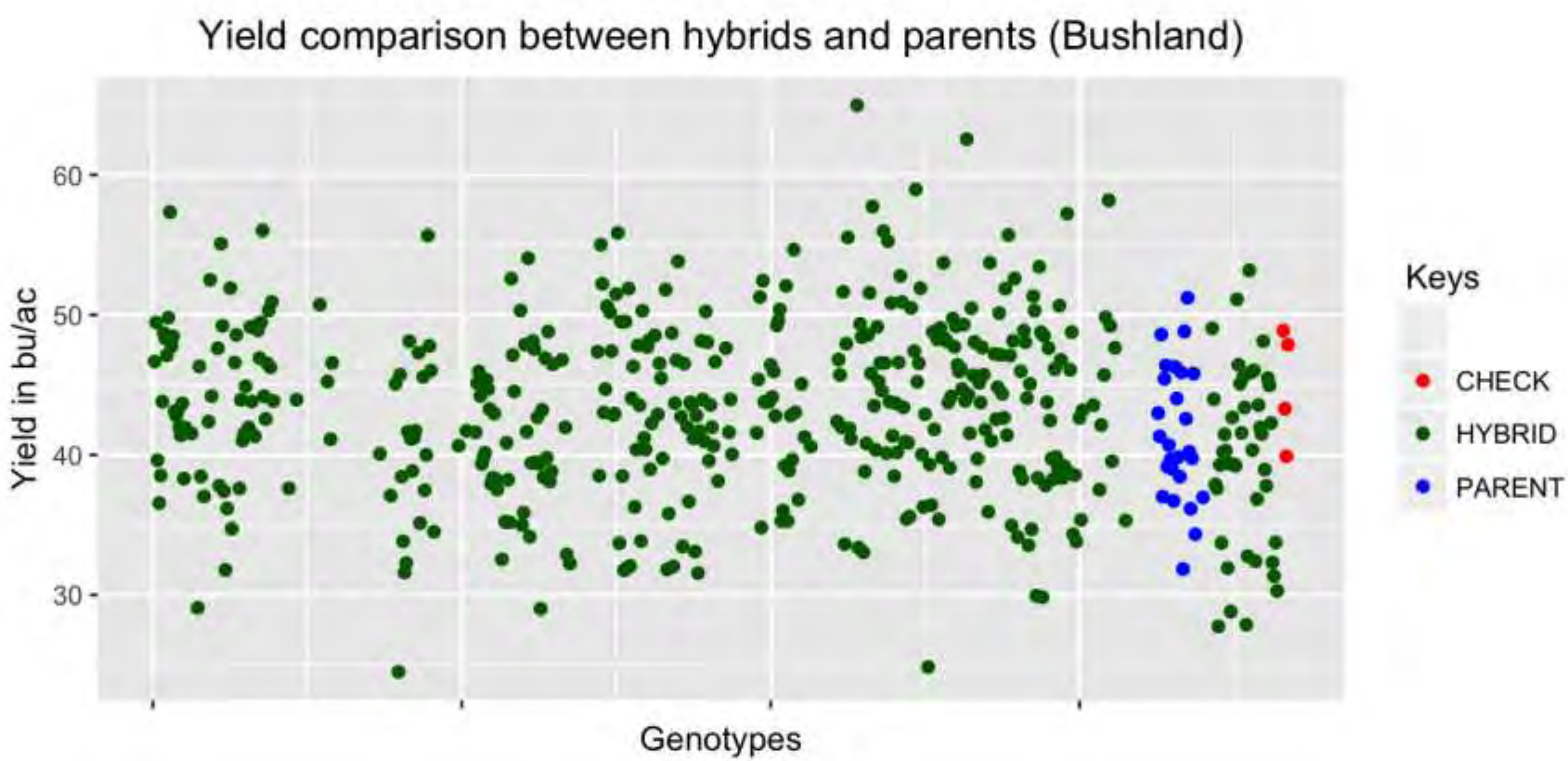
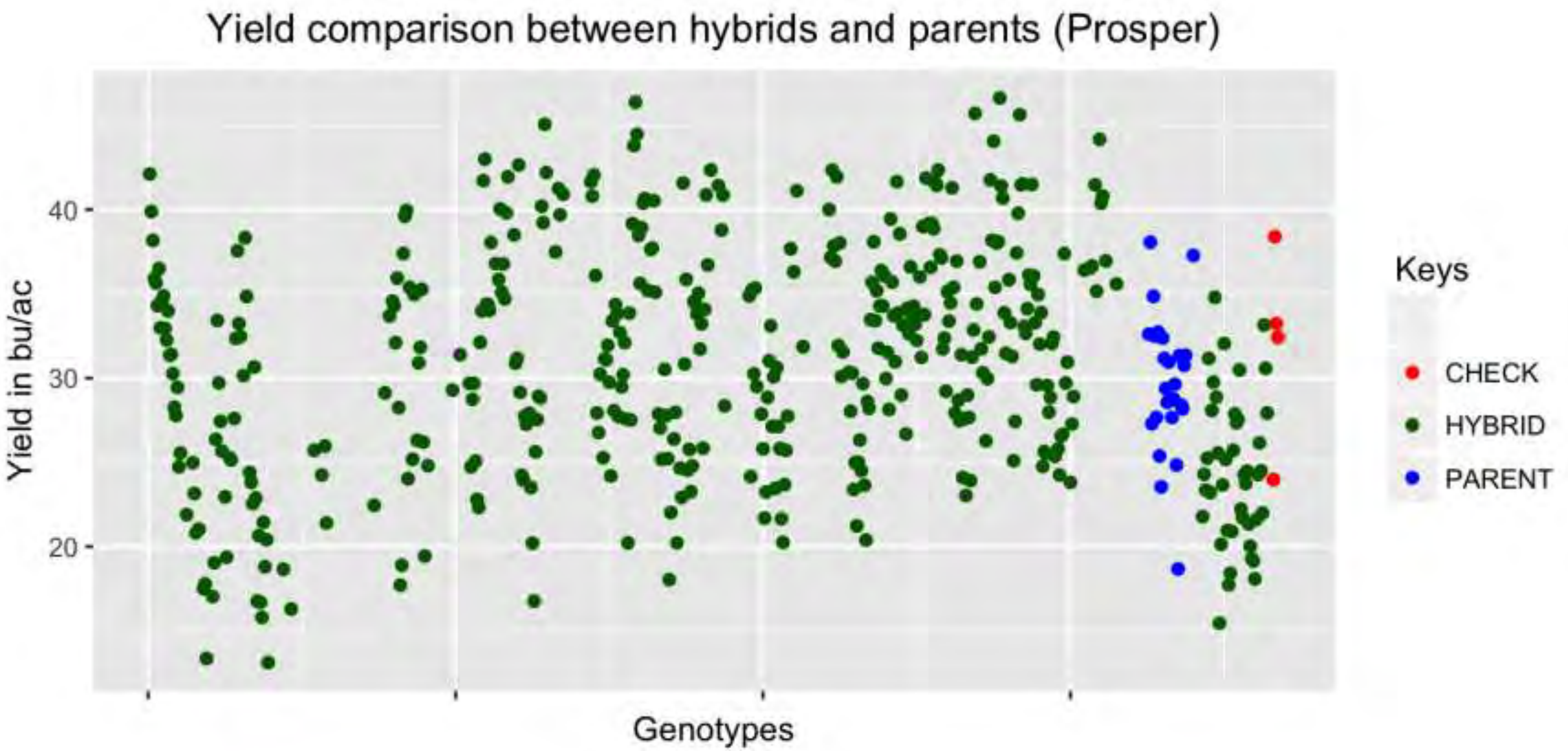
Hybrid F1 performance in Bushland, TX - 2017



- 79 lines performed better than the best check “RUTH”
- 105 lines performed better than 2nd best check ”TAM111”
- 40 lines performed better than the best parent

Hybrid F1 performance in Prosper, TX - 2017



- **67 lines performed better than the best check "Freeman"**
- **198 lines performed better than 3rd best check "TAM111"**
- **72 lines performed better than the best parent**

Wheat heterosis in Texas - 2017

Description	Bushland			Prosper		
	Heterotic lines	Highest (%)	Mean (%)	Heterotic lines	Highest (%)	Mean (%)
Mid-parent Heterosis	277	57.0	14.5	266	118	22.6
High-parent Heterosis	208	52.5	11.2	212	103	19.6
Commercial Heterosis vs "Freeman"	236	50.2	11.2	67	21.4	7.6
Commercial Heterosis vs "TAM111"	109	35.7	6.8	198	43.8	14.9
Commercial Heterosis vs "Ruth"	78	32.9	6.9	400	95	36.5
Commercial Heterosis vs "Wesley"	331	62.8	15.9	181	43.2	13.4

Best check in Greenville: Freeman (38.4 bu/ac), TAM 111 3rd place (32.8 bu/ac)
Best check in Bushland: Ruth (48.9 bu/ac), TAM 111 2nd place (47.9 bu/ac)

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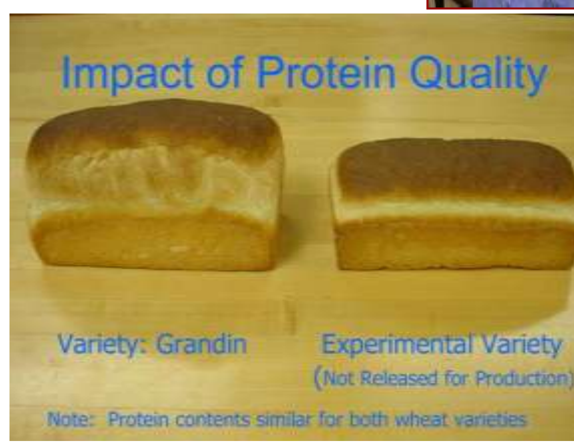
Milling and Baking Quality

- Milling (TW, hardness, weight, size, ash, flour yield)
- Mixing properties (time to peak, tolerance, absorption, Farinograph development time, mixing stability, mixing tolerance index, stability, extensibility)
- Baking (bake mix time, crumb grain, crumb texture, crumb color, loaf volume)
- Tortilla (diameter, opacity, rollability)
- Other traits (viscosity, PPO, falling number)

Mixing properties

SKCS



Tortilla

Wheat quality highlights

- Tortilla and other flatbread research
  - Small scale tortilla quality testing system
  - Clean ingredient tortilla quality
  - Predictive models for tortilla and flat bread quality
- Genotype-by-environment stability for milling and baking quality
- Computerized and digitized milling and dough quality parameters
- End-use quality screening is an integral component of the wheat breeding program
- Use of markers in end-use quality evaluation

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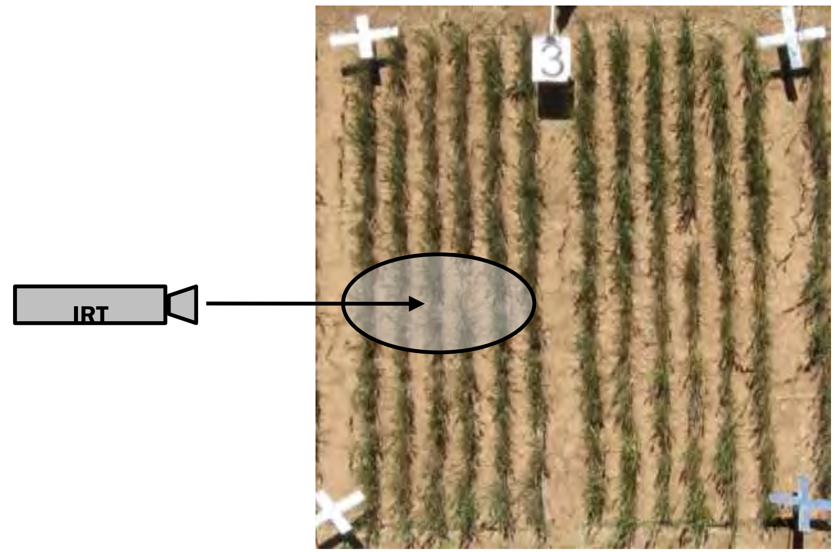
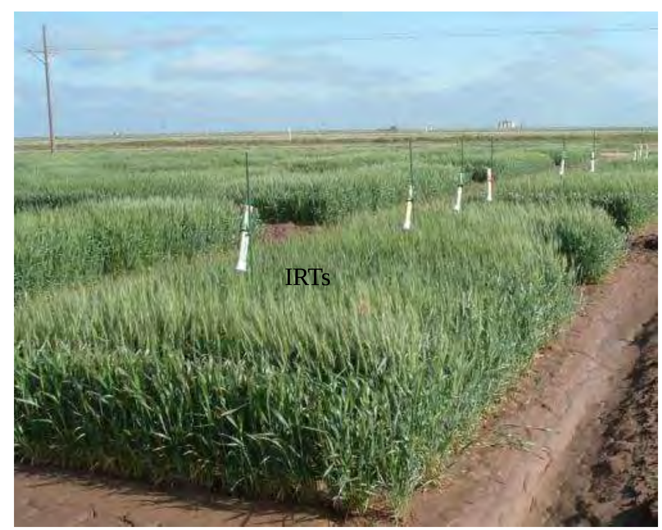
Evolution of our phenotyping: Thermal sensing

- Advantages
  - Easy
  - Inexpensive
- Disadvantages
  - Measure a small spot
  - Soil interference
  - High maintenance
  - Cannot do many lines





Evolution of our phenotyping

Special thanks to Steve Evett and his crew at USDA-ARS

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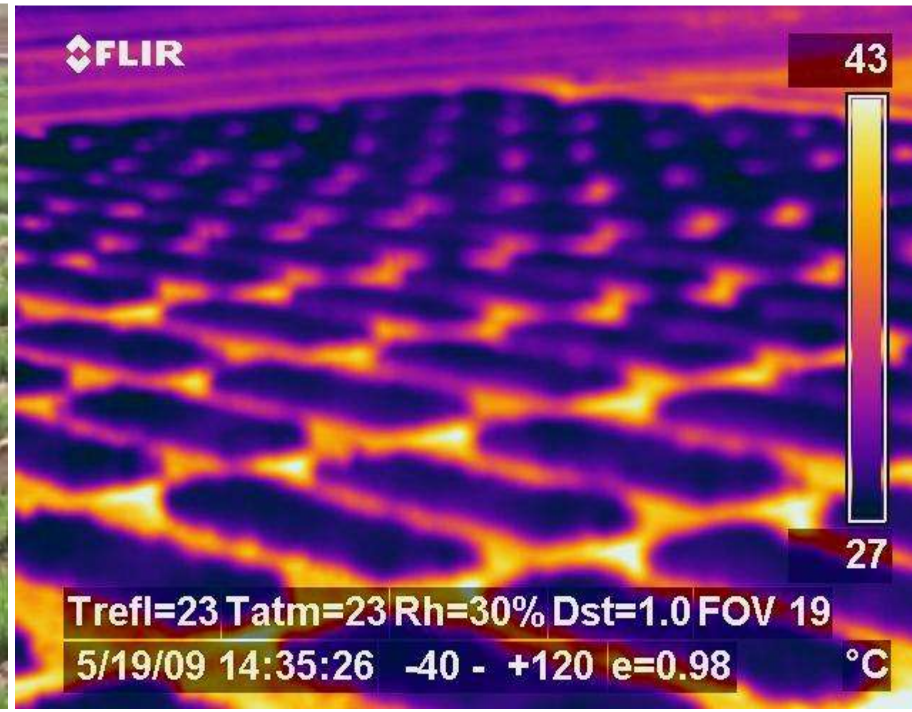
Evolution of our phenotyping: Thermal imaging



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Genotype

Environment

- Cultivar Selection
- Growth Habit
- Stress Tolerance
- Insect Tolerance
- Disease Resistance
- Earliness
- Health Status

Phenotype

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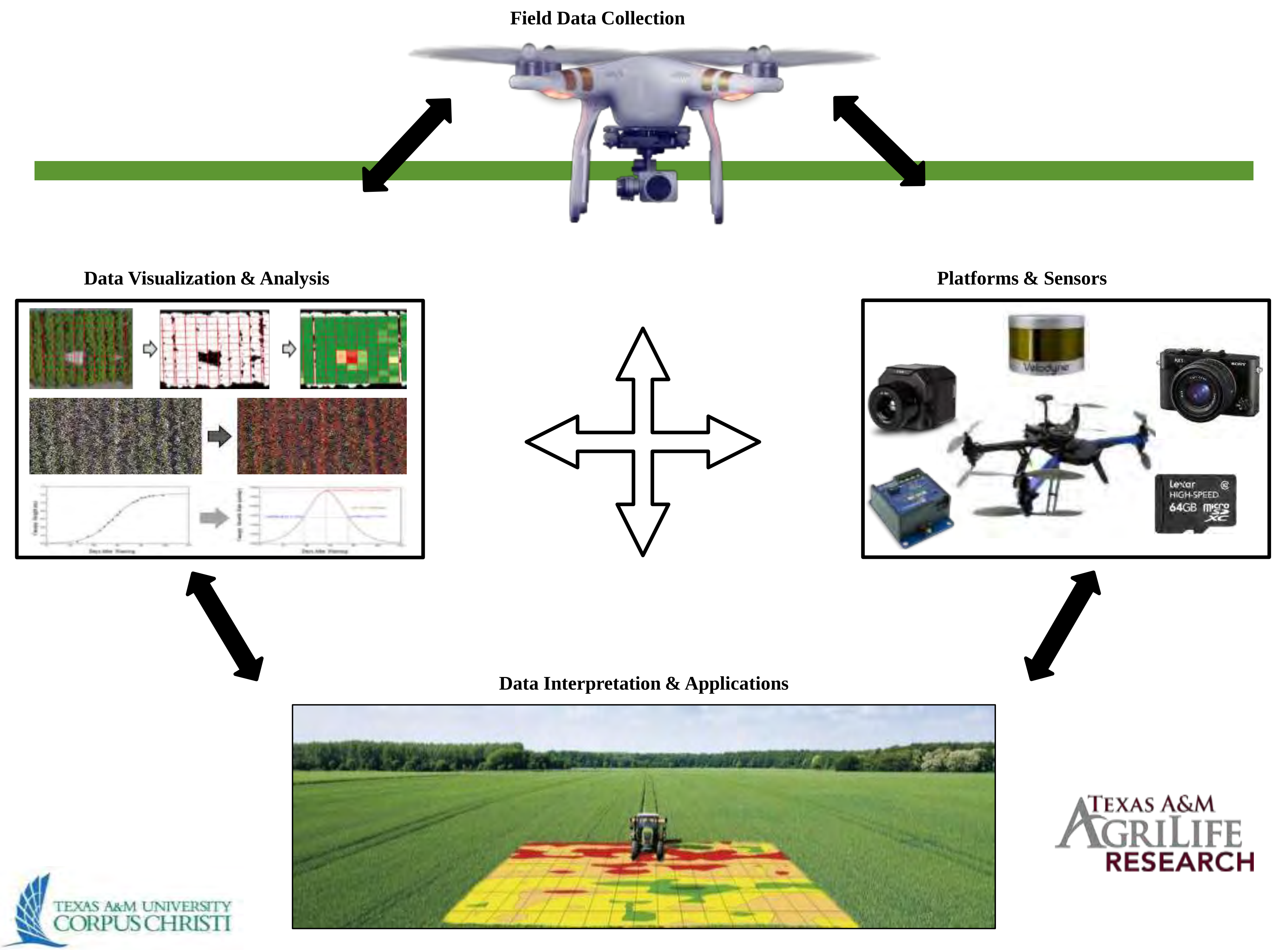
Field Data Collection
Data Visualization & Analysis
Platforms & Sensors
Velodyne
Lexar
HIGH-SPEED
64GB microSDXC
Data Interpretation & Applications
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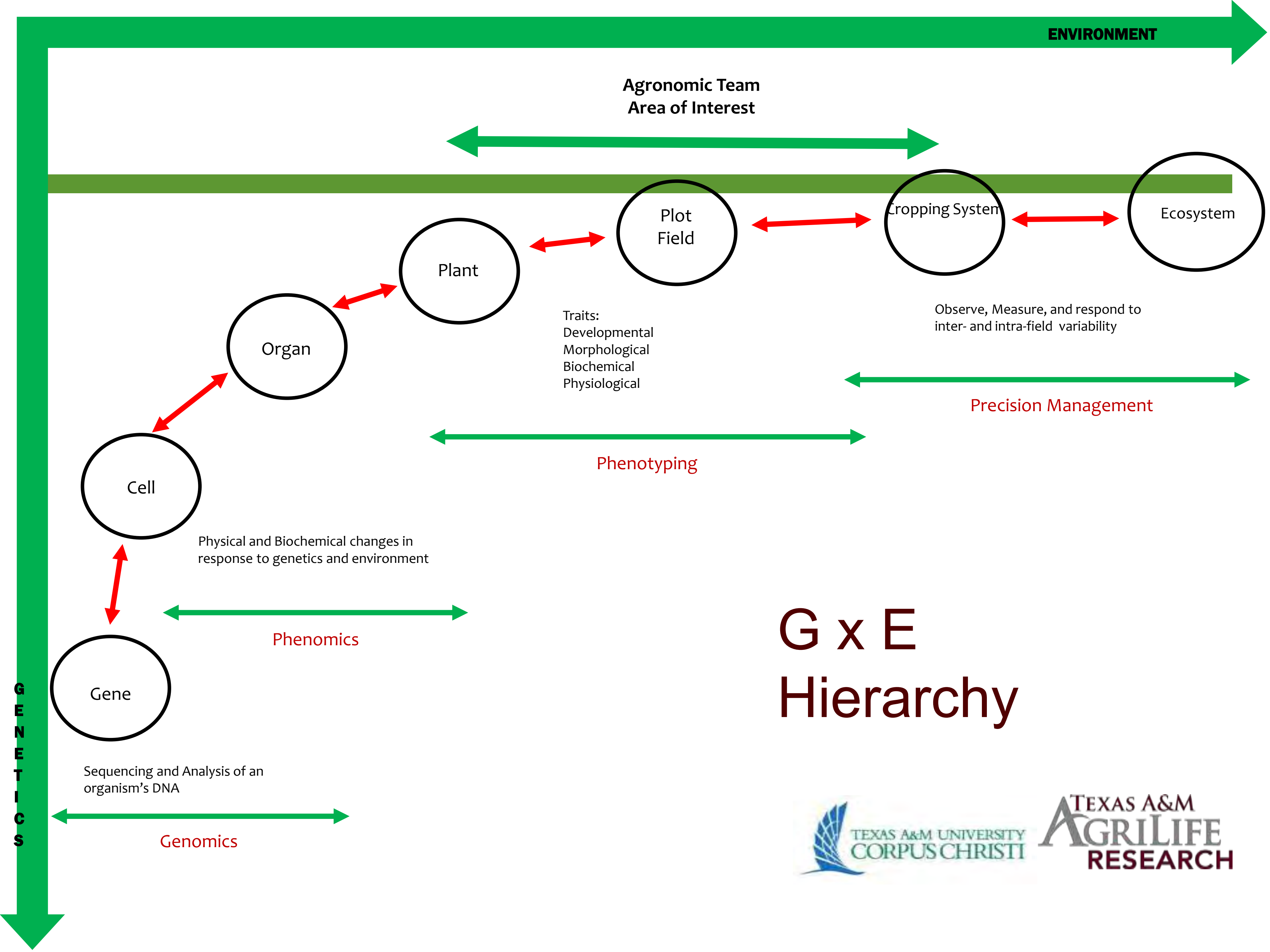
G x E Hierarchy



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Sensors & Platforms

- DJI Phantom 4
  - RGB sensor
  - 12 Mega Pixel

3DR X8+, eBee
- Tetracam ADC Snap (Multispectral sensor)
- FLIR Vue Pro R (Thermal imaging)

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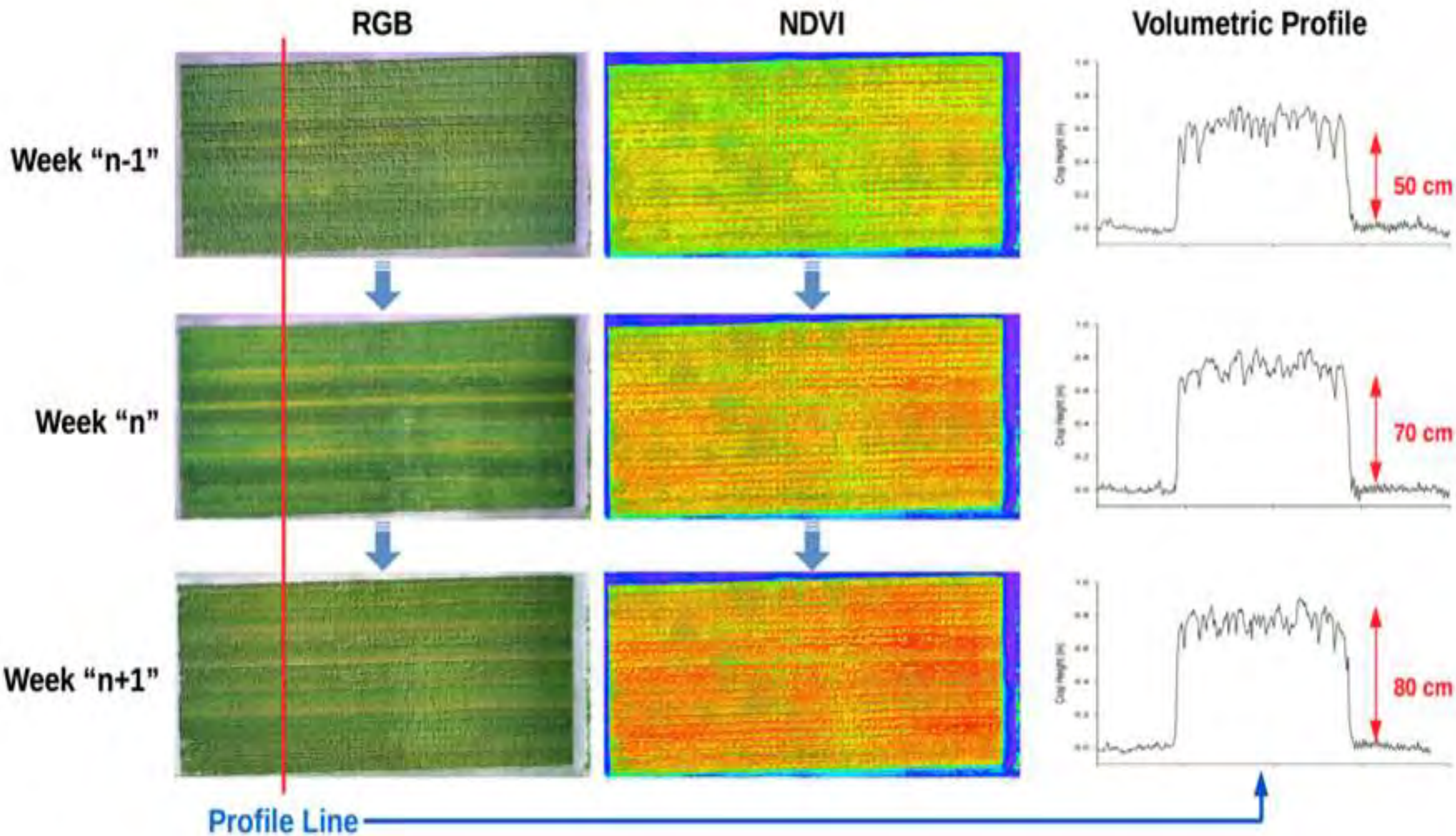
Evaluating and monitoring of growth and maturity of wheat



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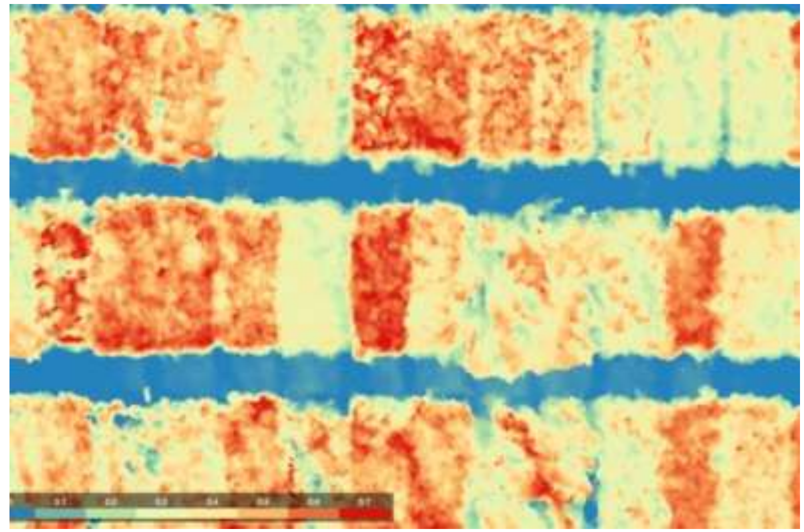
Estimating wheat canopy height



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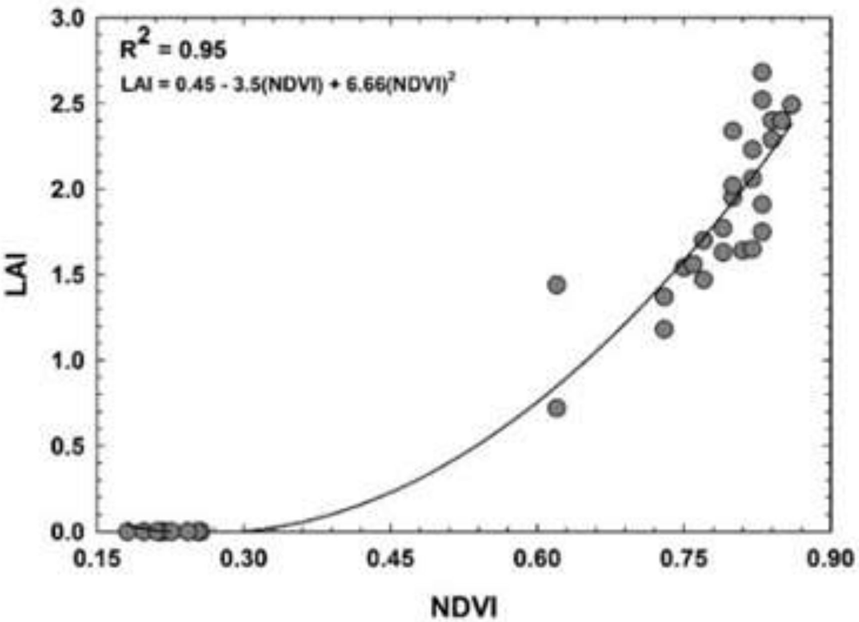
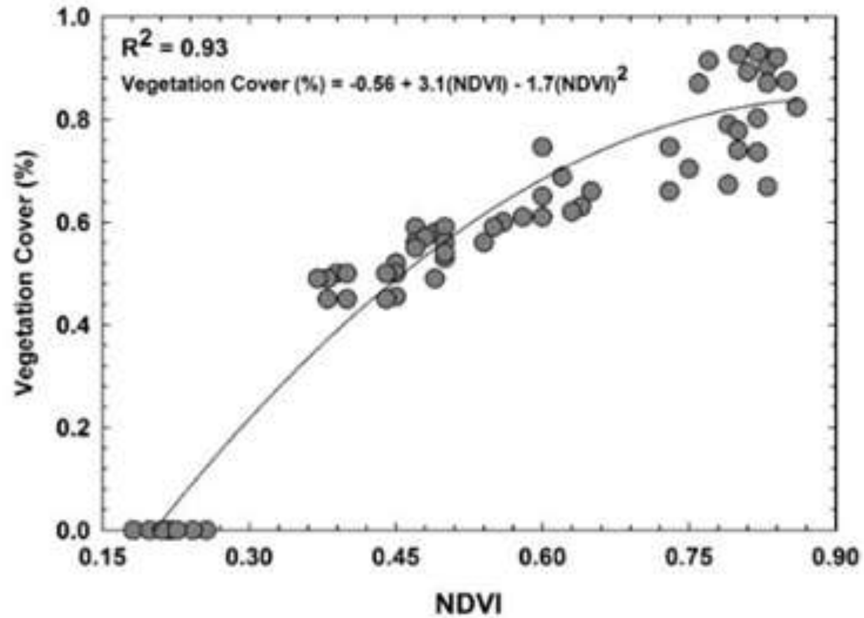
Estimating wheat biomass using UAS





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Estimating reaction to wheat fungal diseases using UAS

- Several genotypes were grown at Castroville, Texas (highly favorable environment for natural inoculation for wheat leaf rust)

- RGB (red, green, and blue) image was taken on March 31 and April 14, 2017 using a UAS platform: DJI Phantom rotary wing

- Green seeker was used to collect NDVI data

$$NDVI = \frac{(NIR - Red)}{(NIR + Red)}$$

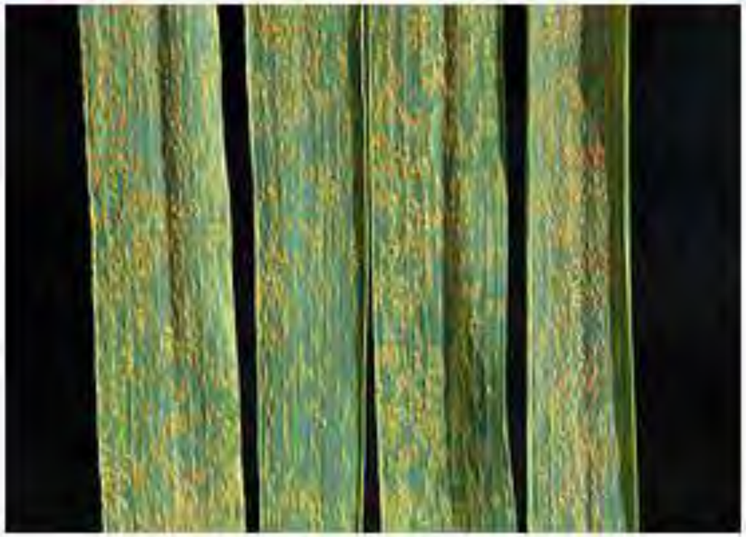
- Disease type and severity notes were taken

- Coefficient of Infection (COI) calculated from

  visually obtained data (severity and disease type)

Incidence of leaf rust

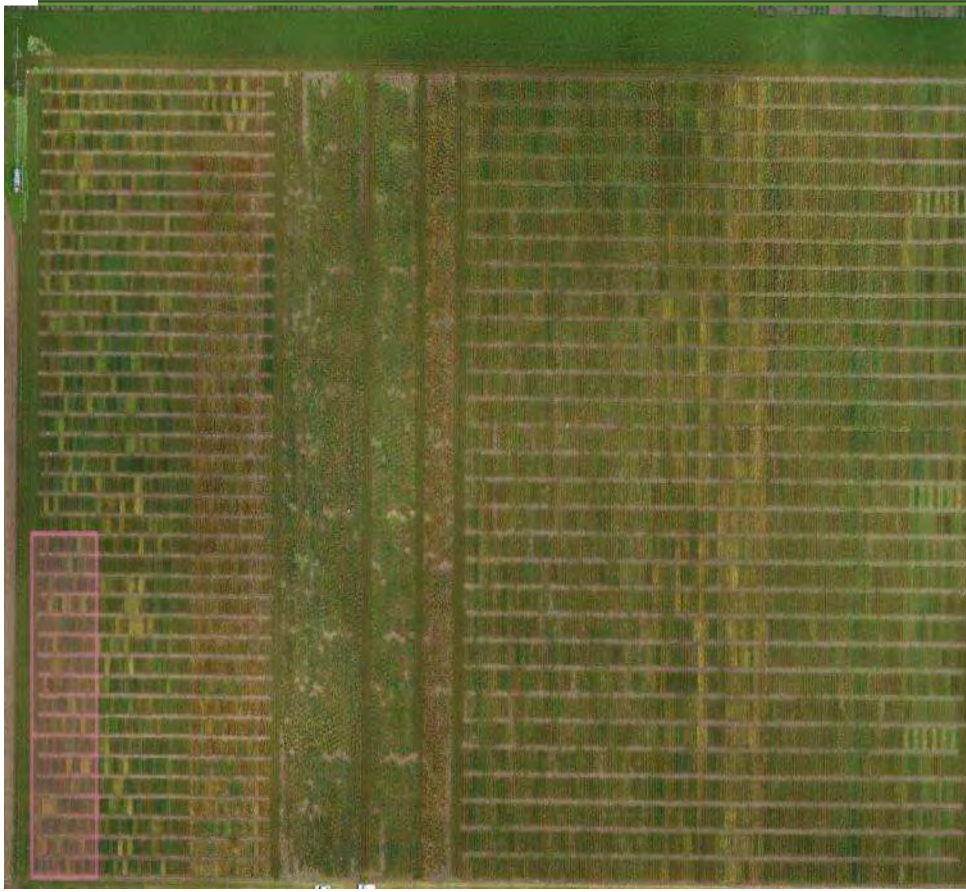
Wheat leaf rust



Symptoms of wheat leaf rust

Common names	Brown rust Leaf rust
Causal agents	Puccinia triticina
Hosts	wheat
EPPO code	PUCCRT
Distribution	Worldwide

UAS - Image processing



RGB image of the entire research area

Boundary selection of each plot for ROIs

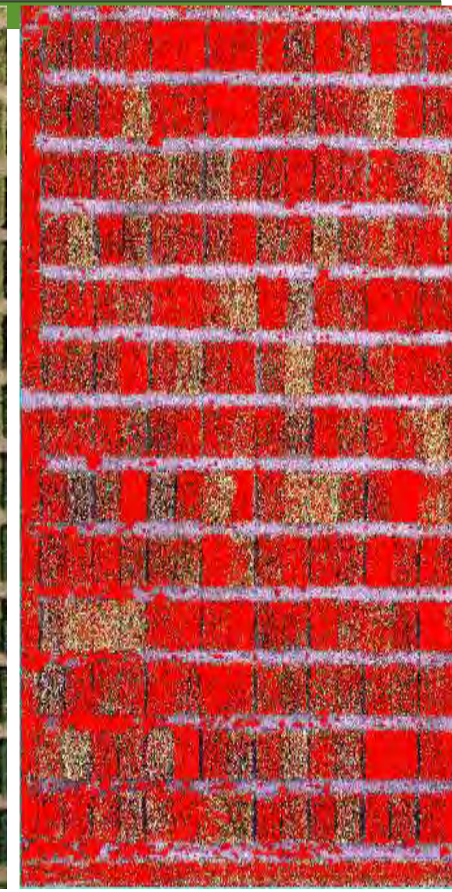


Image classification (UVT)
Bright red: Less infected

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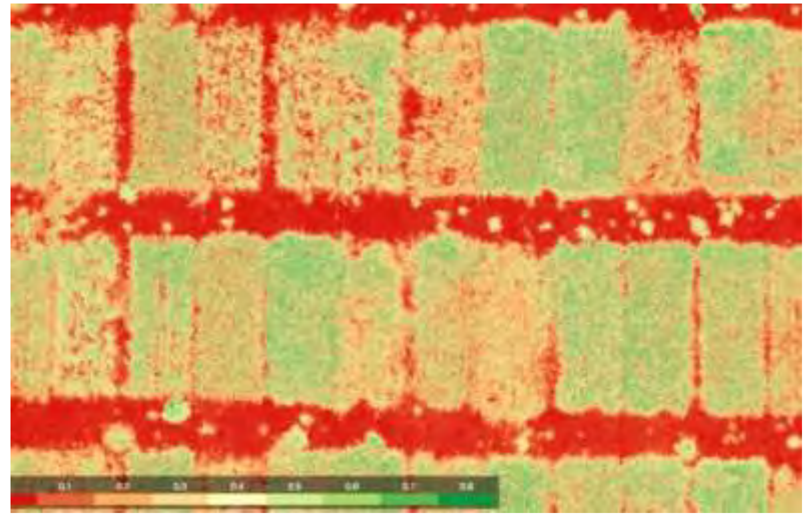
Estimating reaction to wheat fungal diseases



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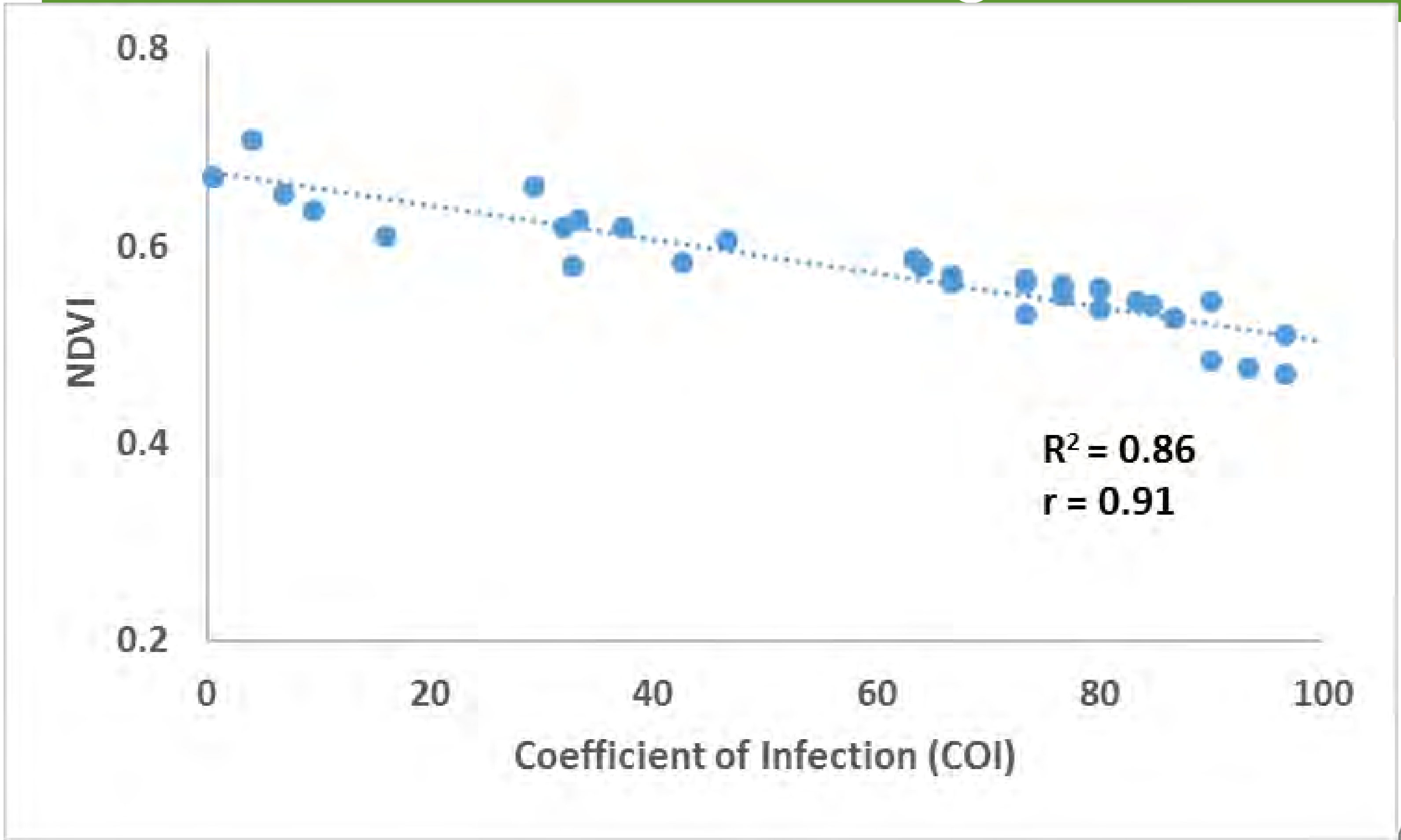
Estimating reaction to wheat fungal diseases using UAS



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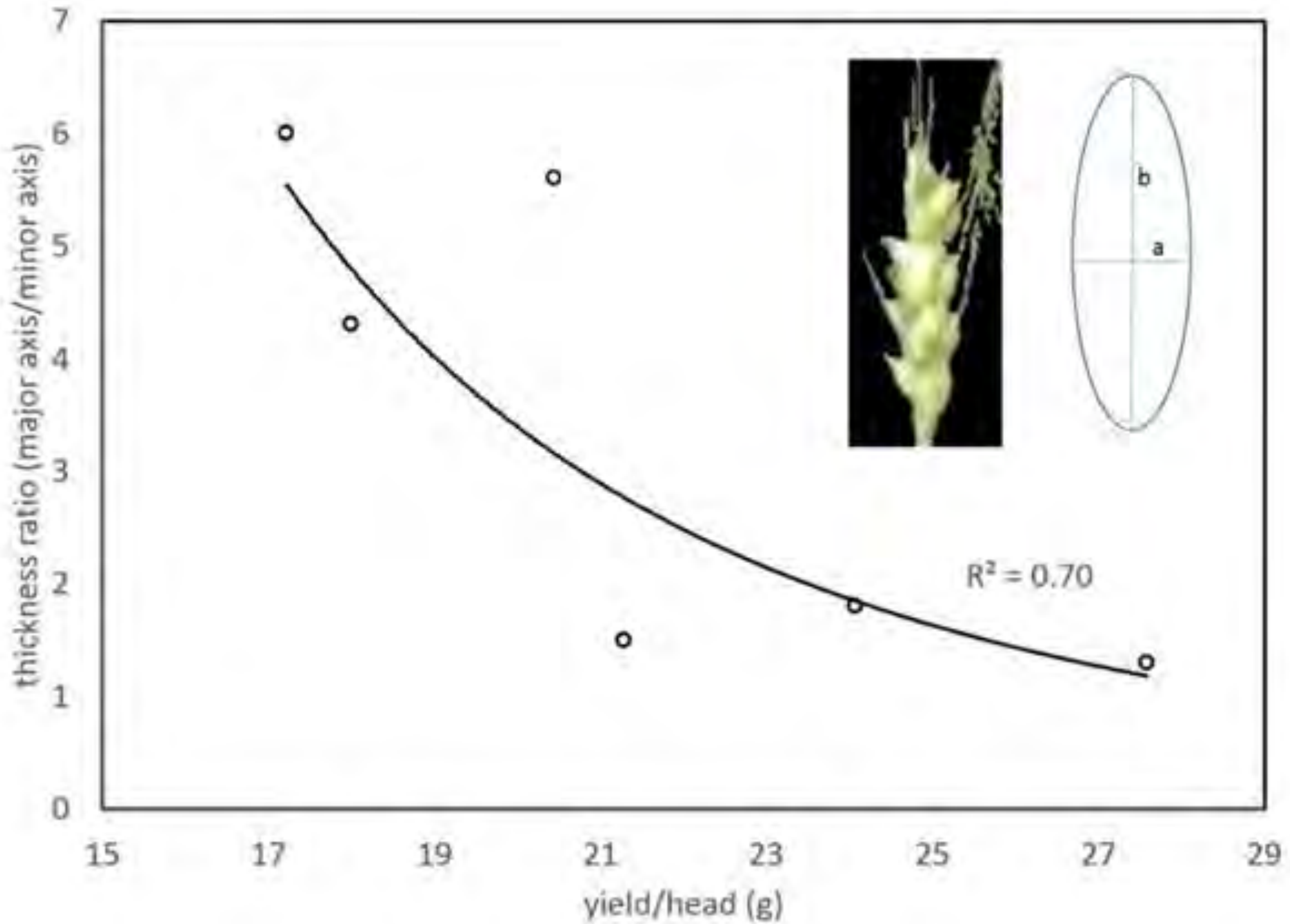
Estimating wheat yield from images



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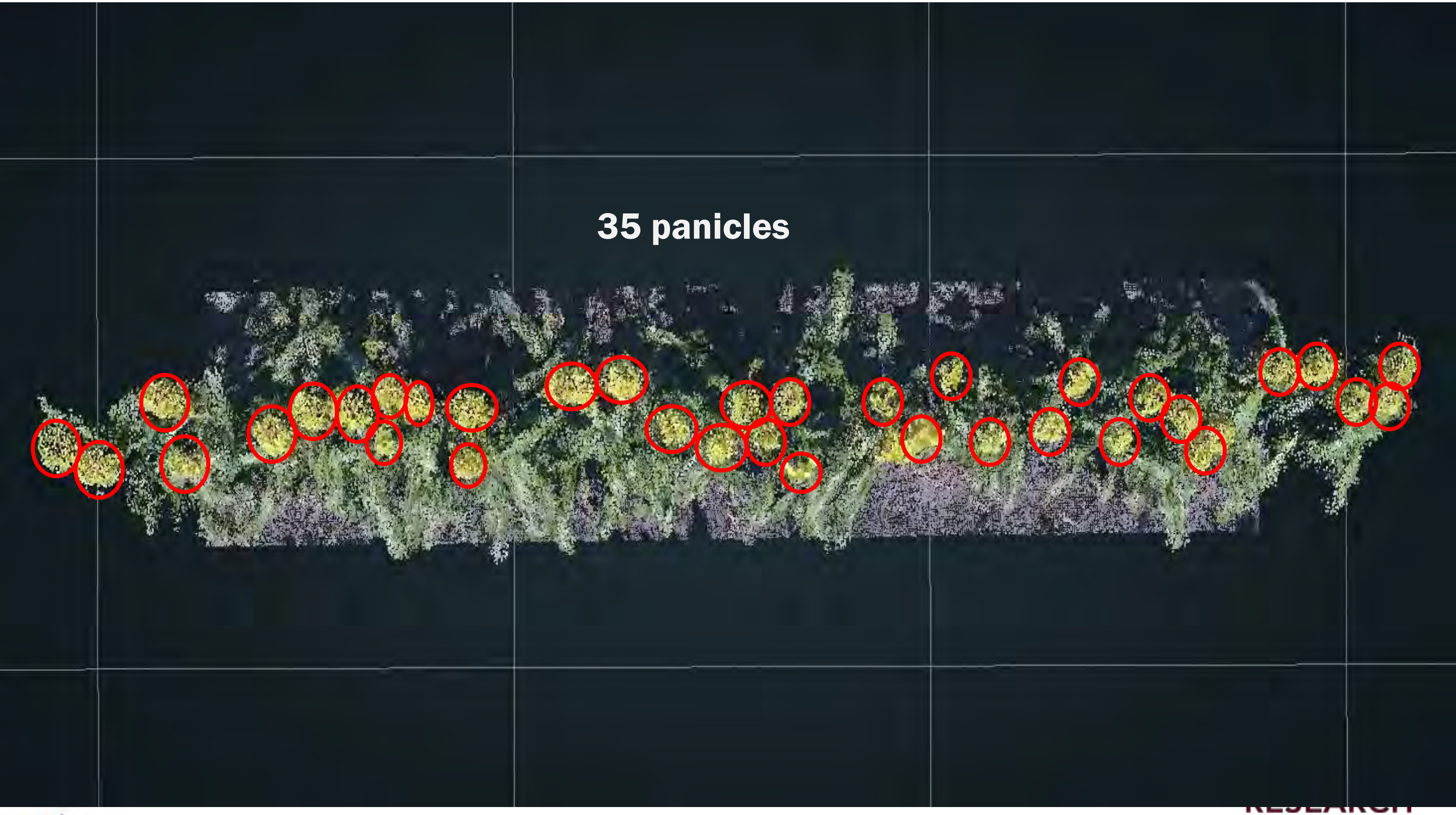
Tiller count in wheat will be done similar to panicle count in sorghum & maize



Highlights of physiology/phenotyping

- Biomass at anthesis is important for maintaining high yield under drought in the Southern High Plains
- Newer TAM cultivars use soil water more efficiently. All cultivars are drought-tolerant but have different mechanisms to respond to drought
- Cooler canopy, conferred by varying mechanisms and traits, contribute to higher yield in new drought-tolerant cultivars

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Highlights of physiology/phenotyping

- UAS/Ground vehicle phenotyping
  - High Throughput Phenotyping for grain and forage
  - UAS data is highly associated with ground-based data
  - Spectral vegetation indices associated with biotic and abiotic stress tolerance can be phenotyped quickly and efficiently
- Ground penetrating radar
- Terrestrial laser scanning
- Spectral reflectance/screening of epicuticular wax
- Canopy temperature

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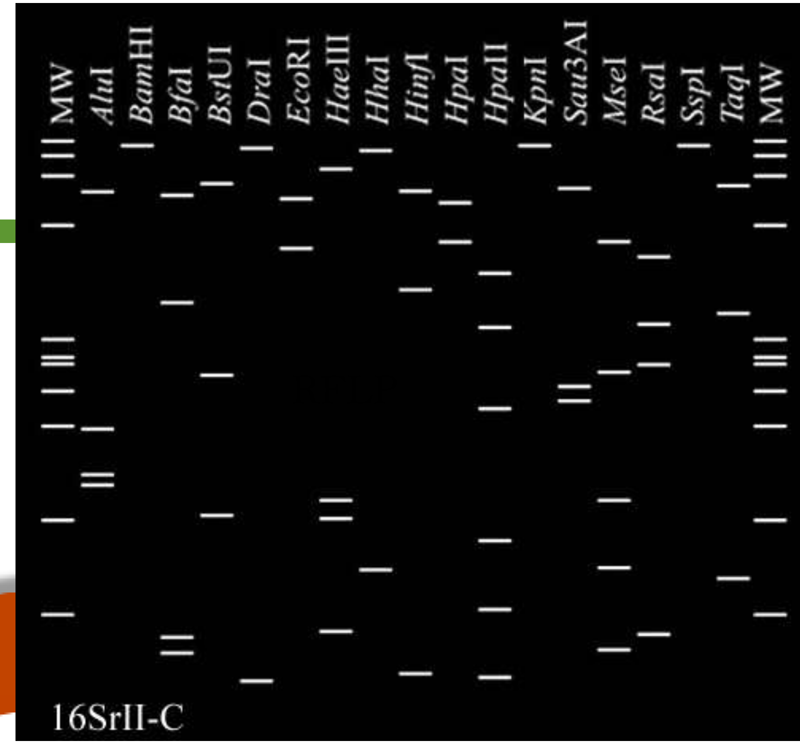
Genomics & Bioinformatics Unit

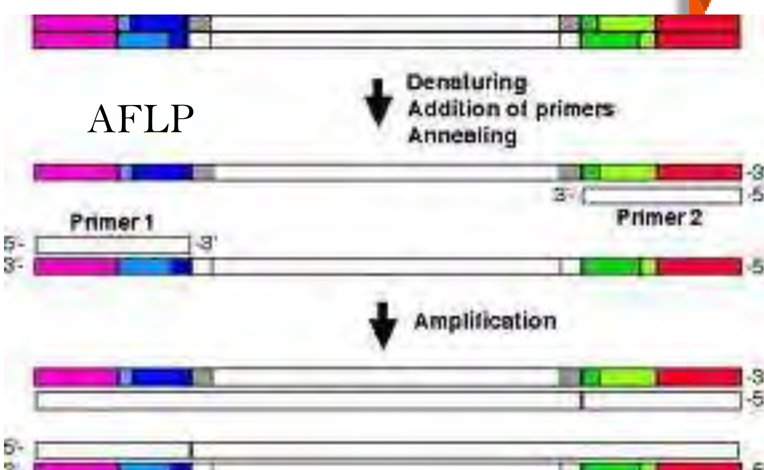
Genotyping By Sequencing



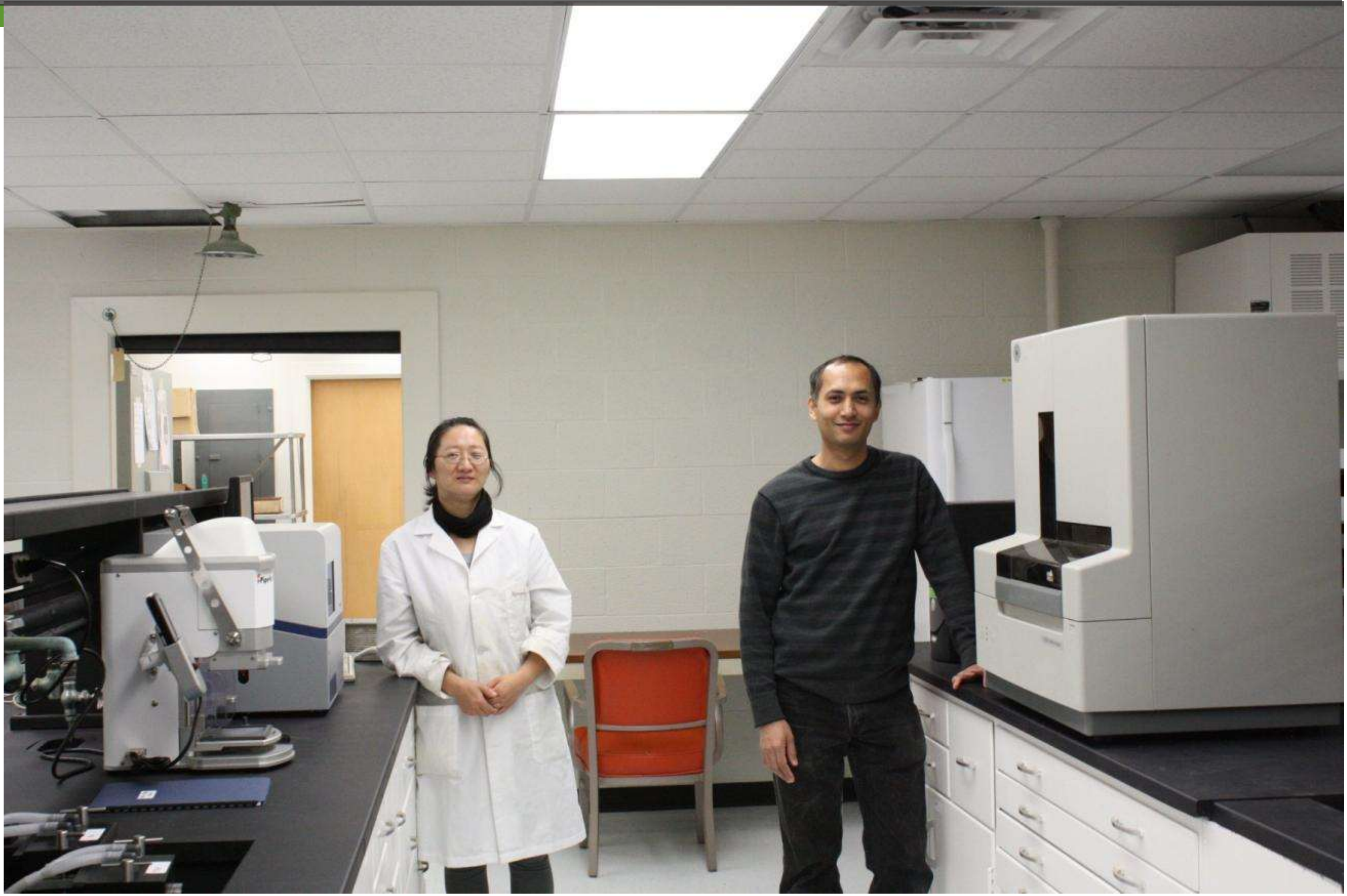
1. Genome Size and complexity
2. Lack of reference

Key factors

1. Reproducible
2. Sensitivity, specificity
3. Cost effective



AgriGenomics Laboratory



Genotyping and marker assisted breeding

- SNP genotyping (LGC Genomics, KASP)

High-throughput SNP genotyping service

1. Labconco FreeZone (freeze-drier)
2. TALBOYS homogenizer
3. Fastprep 24 homogenizer
4. Eppendorf Centrifuge
5. QIAxtractor
6. BMG PHERAstar Plus
7. Hydrocycler (KBiosciences)
8. Eppendorf Thermocycler

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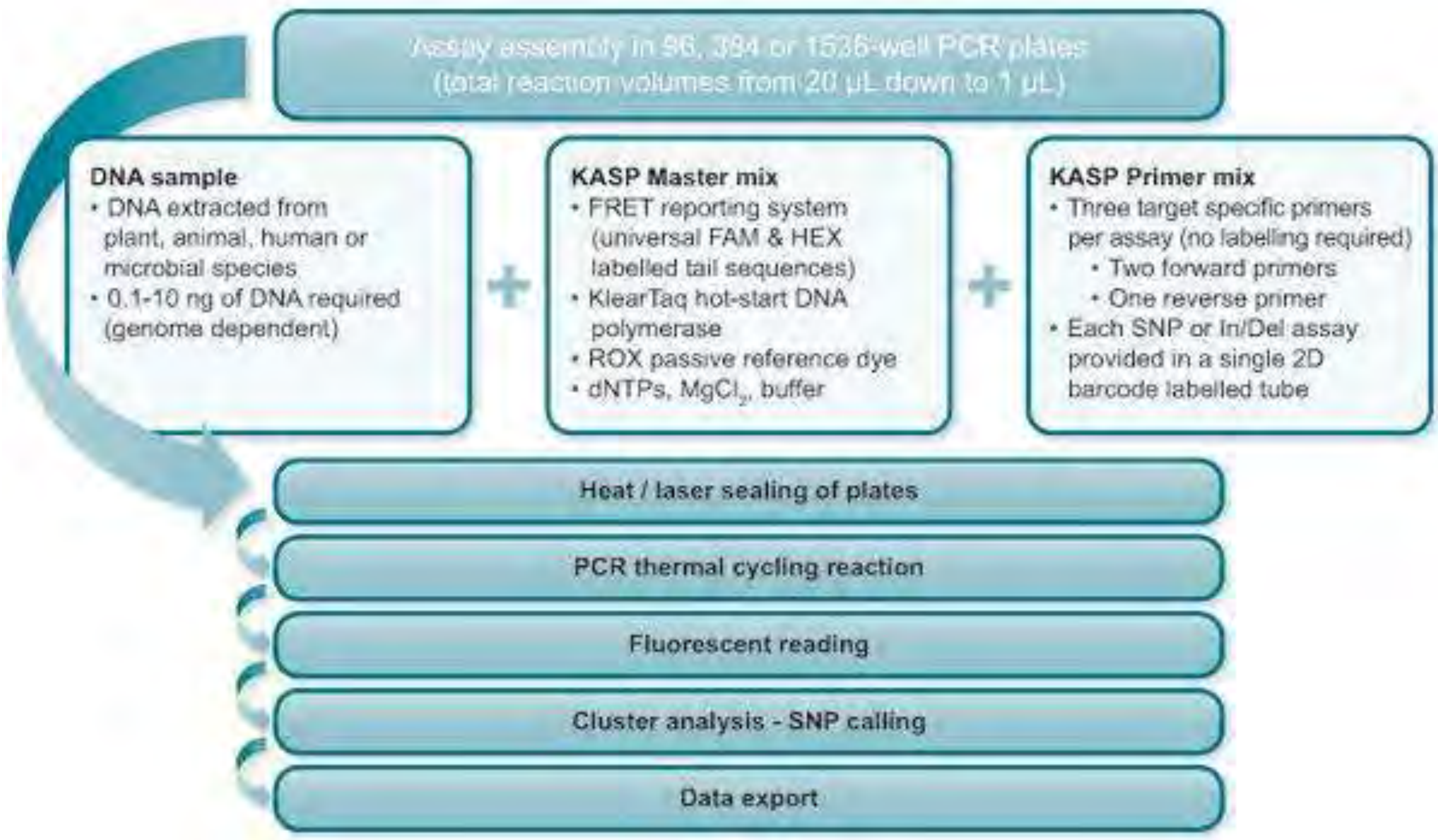
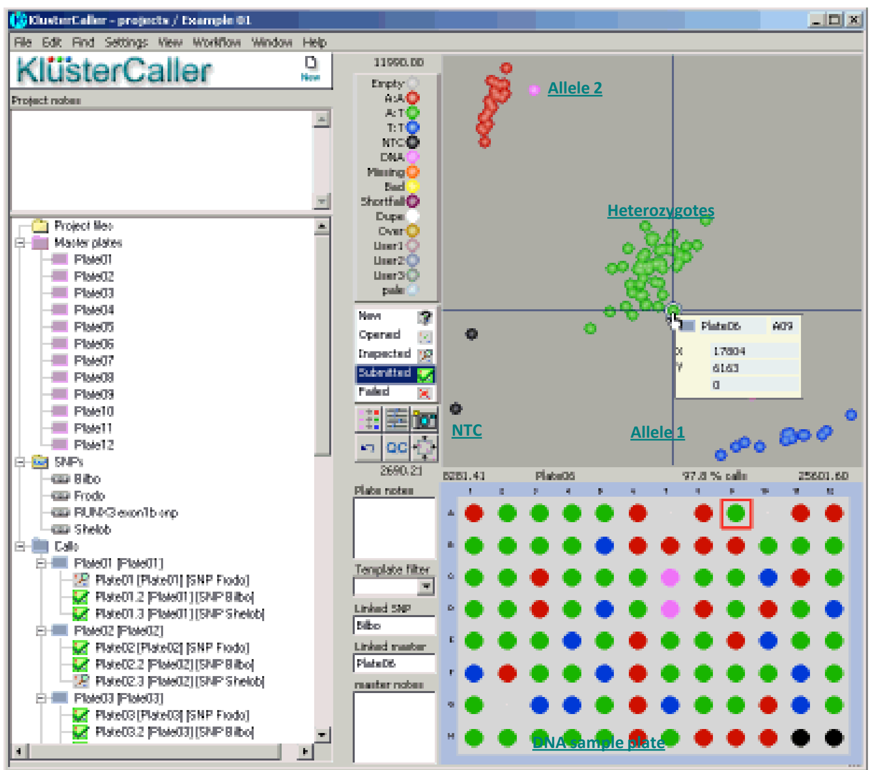
KASP Workflow



Cluster analysis and reporting

- KlusterCaller™ software (KBiosciences)
  - Scatterplots- FAM and HEX data plotted on x-axes and y-axes
  - ROX data- normalization



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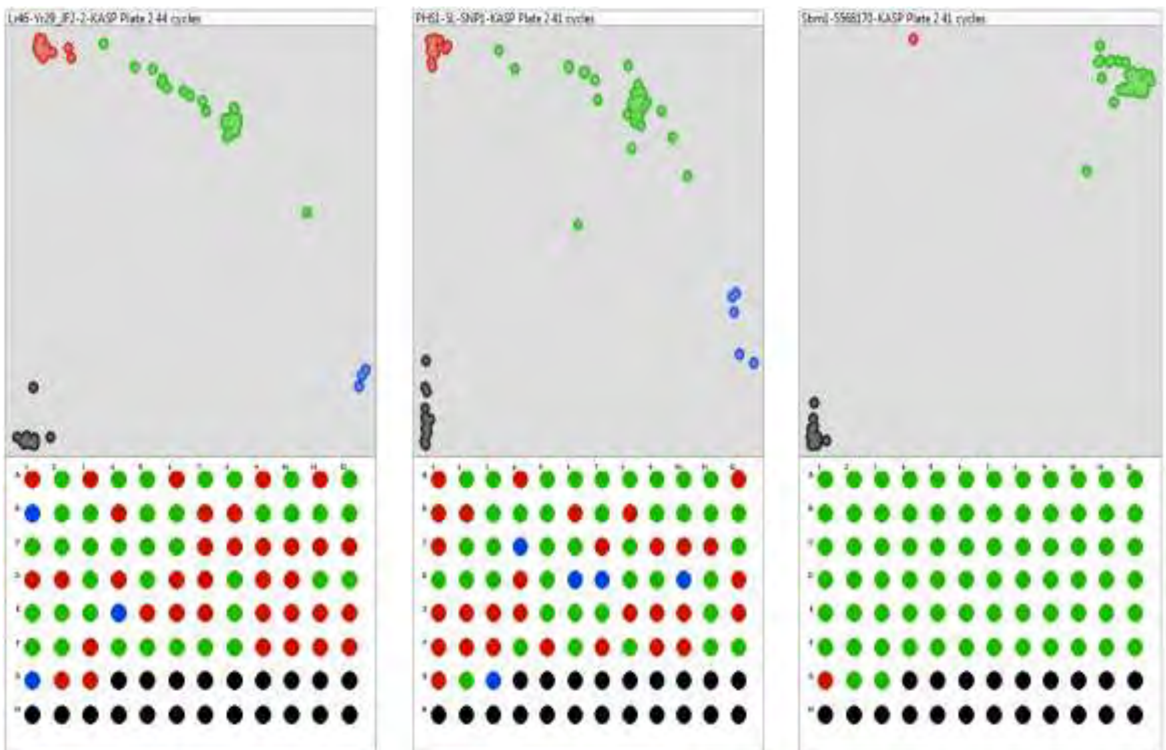
SNP's fully utilized at AGL

30 SNP markers were screened on 48 parents and 159 populations: 16 out of 30 SNPs can be used for genotyping



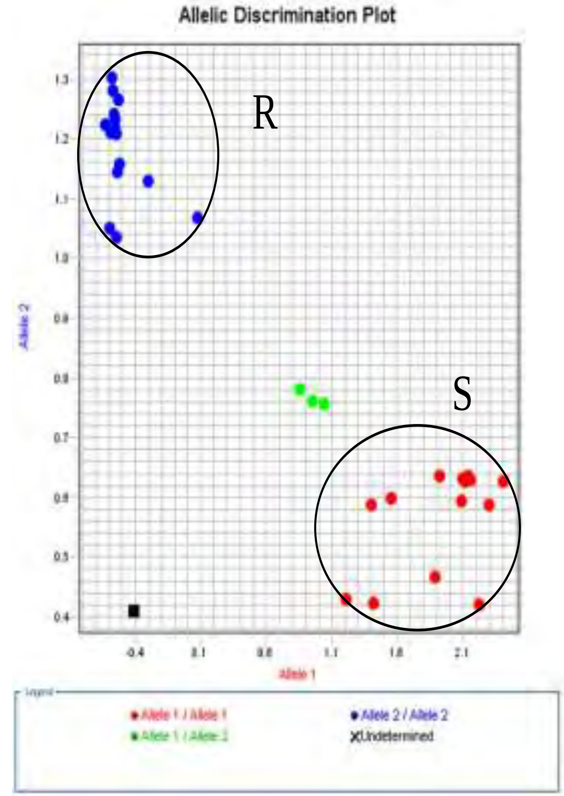
SNP markers available at the AGL

SNP		Primer		Trait
Fhb1_SNP319-KASP		Fhb1_SNP319-AL1-FAM-WT		Fusarium head blight resistance
Fhb1B_umc_3837-KASP		Fhb1B_3837_AL1		Fusarium head blight resistance
FHB3BS-SNP8-KASP		snp3BS-8_AL1		Fusarium head blight resistance
Glu-D1-DX-KASP		Glu-D1d_SNP_AL1		Gluten strength
Lr34Inron4_A-KASP		Lr34-int4_A_AL1		Leaf rust resistance
Lr34JagExon22-KASP		Lr34jagger_AL1		Leaf rust resistance
LR42-113325-01-KASP		LR42-113325_01_AL1		Leaf rust resistance
LR46-Yr29-JF2-2-KASP		Lr46_JF2-2A_AL1		Leaf rust resistance
Lr9a-KASP		Lr9a_AL1		Leaf rust resistance
Lr42-TC425250_08-KASP		Lr42-TC425250_08_AL1		Leaf rust resistance
PHS1-SL-SNP1-KASP		PHS1-MFT_1587_AL1		Pre-harvest sprouting
RhtB1_cim-KASP		RhtB1_AL1		Height
RhtD1-KASP		RhtD1_AL1		Height
Sr36-Pm6_8085-KASP		Sr36_8085_AL1		Stem rust resistance
Sr40-Seg2-SNP2-KASP		Sr40-Seg2-SNP2-_AL1		Stem rust resistance
1A1R_8035-KASP		1A1R_8035_AL1		Stem rust, PMD

Genotyping genes/QTL Highlights

- Kompetitive allele specific PCR (KASP) SNP markers have been developed for wheat streak mosaic virus resistance, *Wsm2*, greenbug resistance, *Gb3*, and wheat curl mite resistance, CMC_{TAM112}.
- Apply marker-assisted breeding to develop germplasm lines with tolerance to multiple stresses

Cultivars/lines screening



Name	SNPG b3	GB testing
PI268210	A	R
Largo	A	R
TAM110	A	R
TAM112	A	R
TAM204	A	R
TX09V7352	A	R
TX10A001099	A	R
TX10A001537	A	R
TX11A001295	A	R
TX11A001440	A	R
TX11A001549	A	R
TX11A001643	A	R
TX97V5300	A	R
TXGBE273A	A	R
TAM111	B	S
TAM113	B	S
TAM400	B	S
TAM401	B	S
TX01M5008	B	S
TX01M5009	B	S
TX01M5009-28	B	S
TX10D2063	B	S
TX11A001112	B	S
TX11A001137	B	S
[illegible]	[illegible]	[illegible]

Genotyping genes/QTL highlights

- Map genes/quantitative trait loci associated with higher yield under dryland, resistances to diseases, arthropods, and their transmitted viral diseases
- Validate and develop high throughput single nucleotide polymorphic markers
- Provided a saturated map for *Wsm2* based on 90K SNP markers
- Tightly linked markers linked to *Wsm2* have a potential to improve genetic gain in wheat
- Putative QTL for yield and yield components are localized on chromosomes 2B,1A and 3B
- Markers linked to drought tolerance QTL are being converted to KASP and tested

Stripe rust **Drought**

Greenbug **Curl mite** **WSMV**

Genotyping genes/QTL highlights

- Wild relative exploitation
  - *Triticum turgidum* for heat tolerance
  - *Aegilops tauschii* for insect and disease resistance
  - Synthetic hexaploids for biomass and grain yield
- QTL Mapping results published
  - Tiller production in wheat
  - Epicuticular wax in wheat
  - Stripe rust in wheat
  - Greenbug in wheat
  - Wheat curl mite in wheat
  - Wheat streak mosaic virus in wheat

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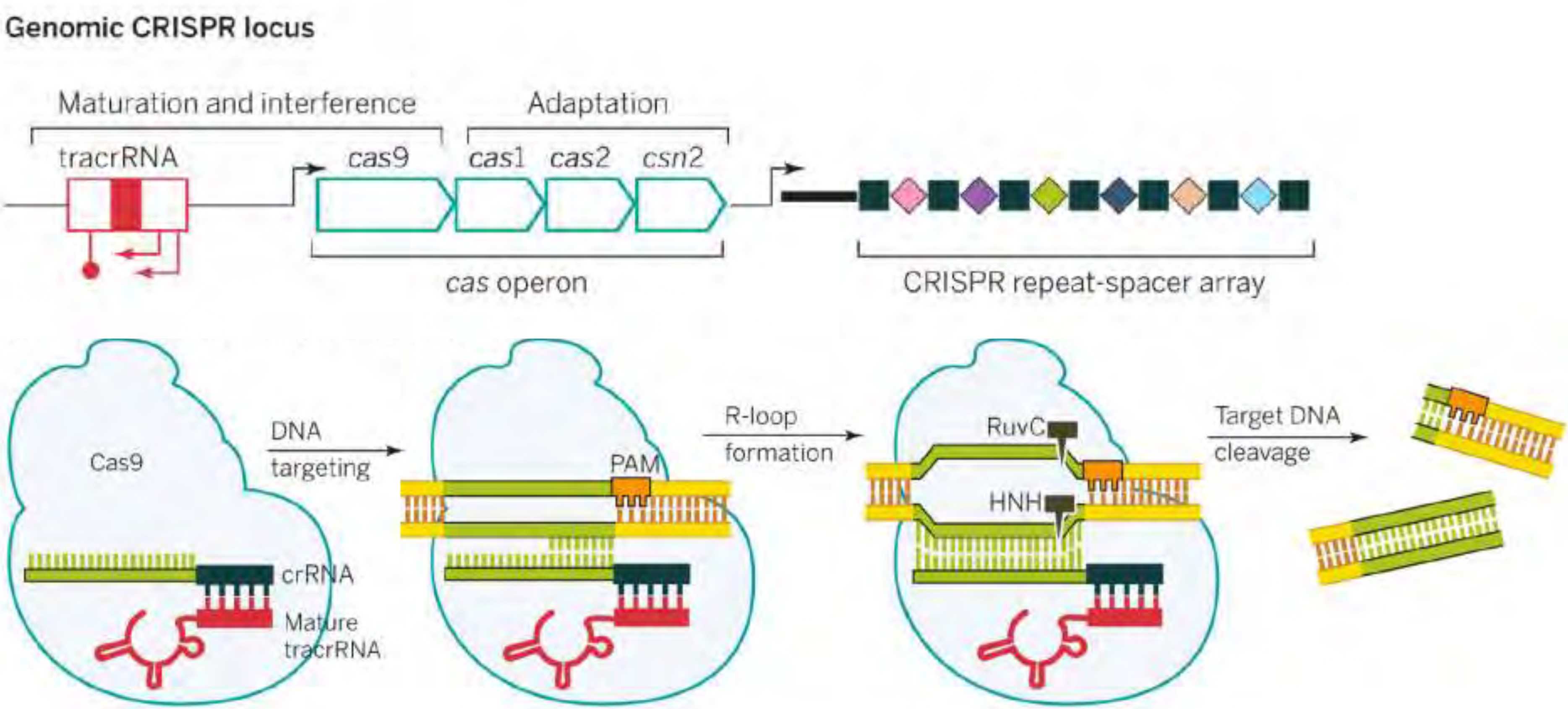
“CRISPR Everywhere”

The rise of gene editing

CRISPR/Cas9 bacterial immune system

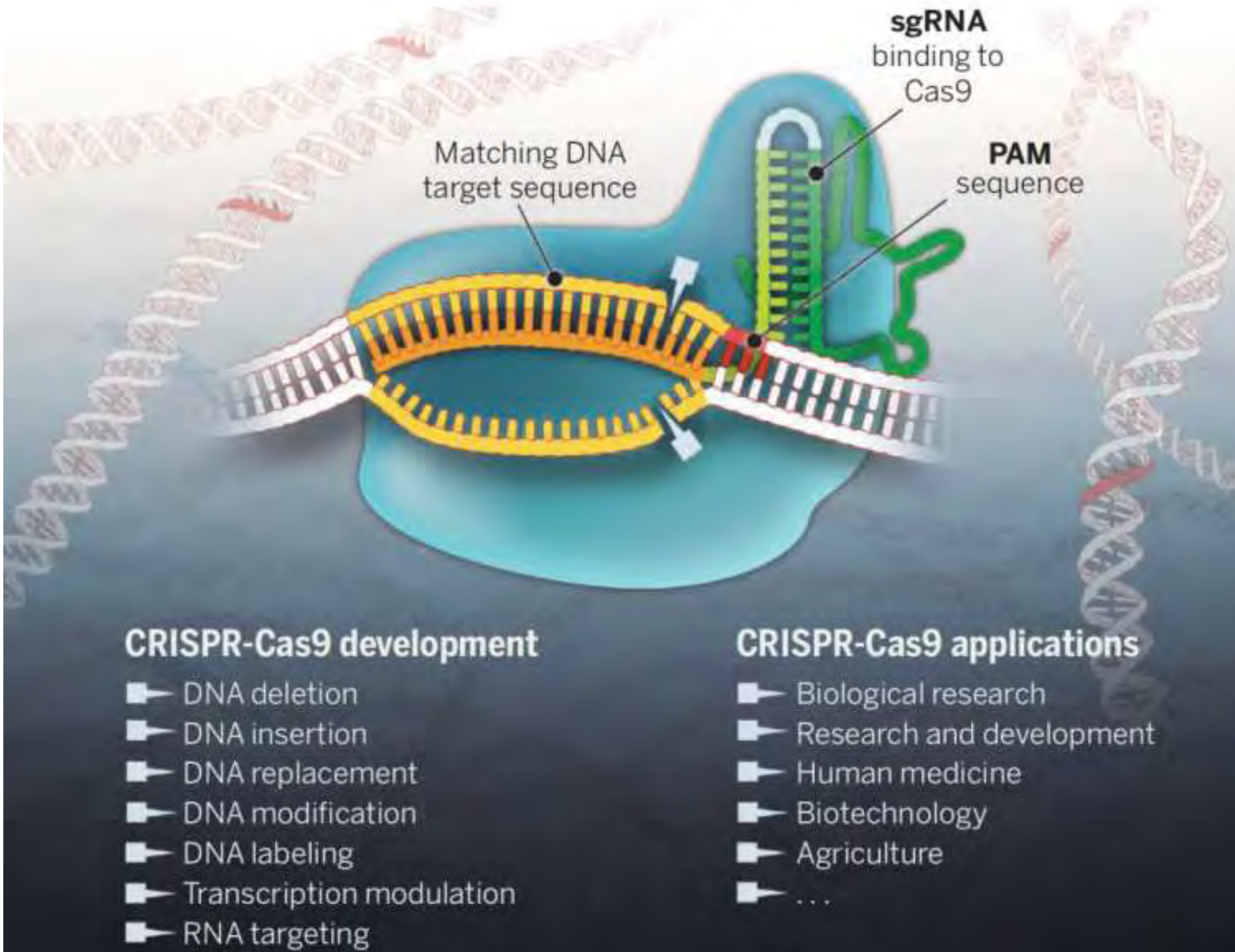
Clustered Regularly Interspaced
Short Palindromic Repeats (CRISPR)

CRISPR-associated protein 9 (Cas9)



Doudna and Charpentier 2014

CRISPR/Cas 9 technology



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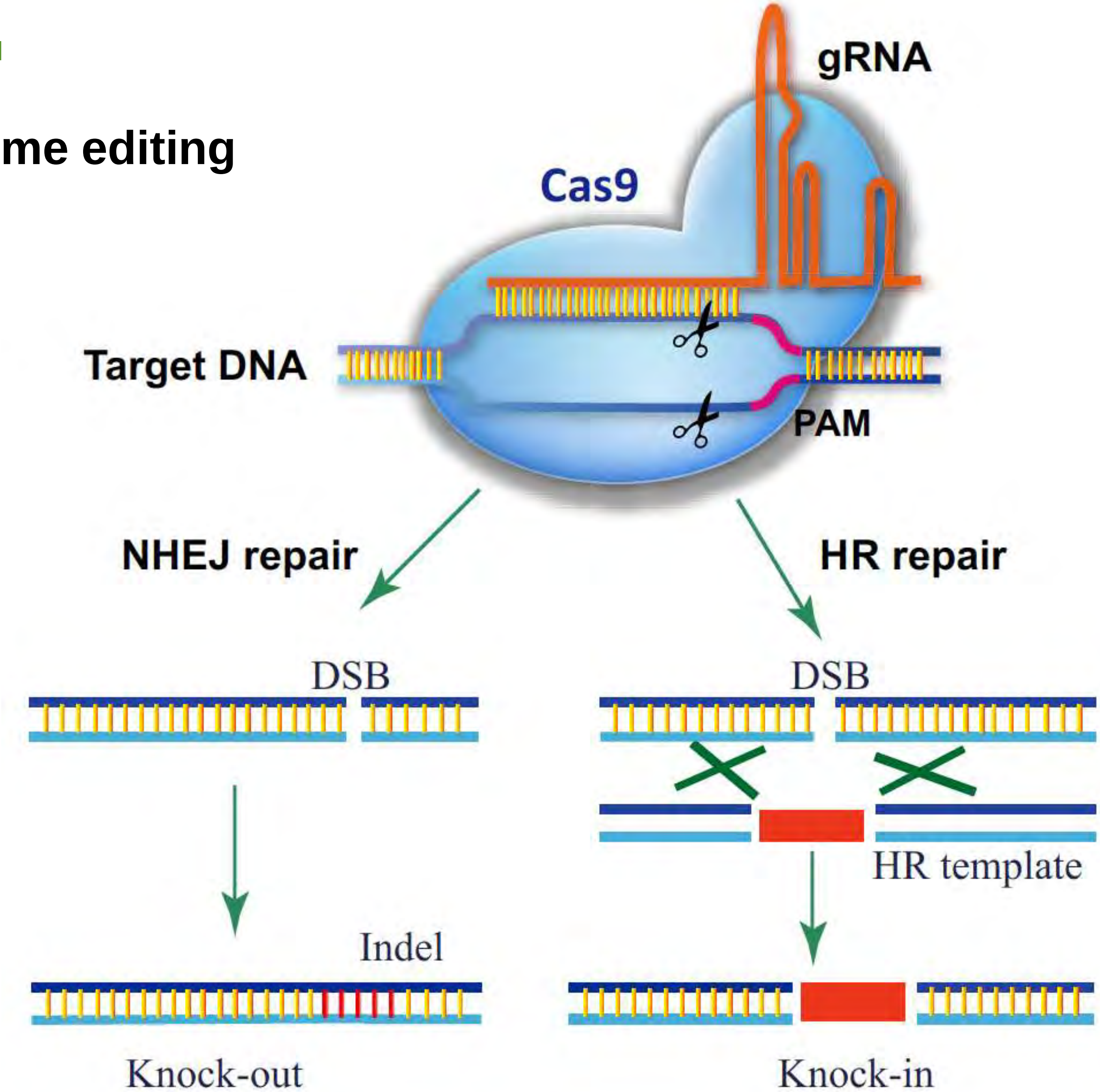
Doudna and Charpentier 2014

CRISPR-Cas9 genome editing

Introduce Cas9 and guide RNA matching target sequence

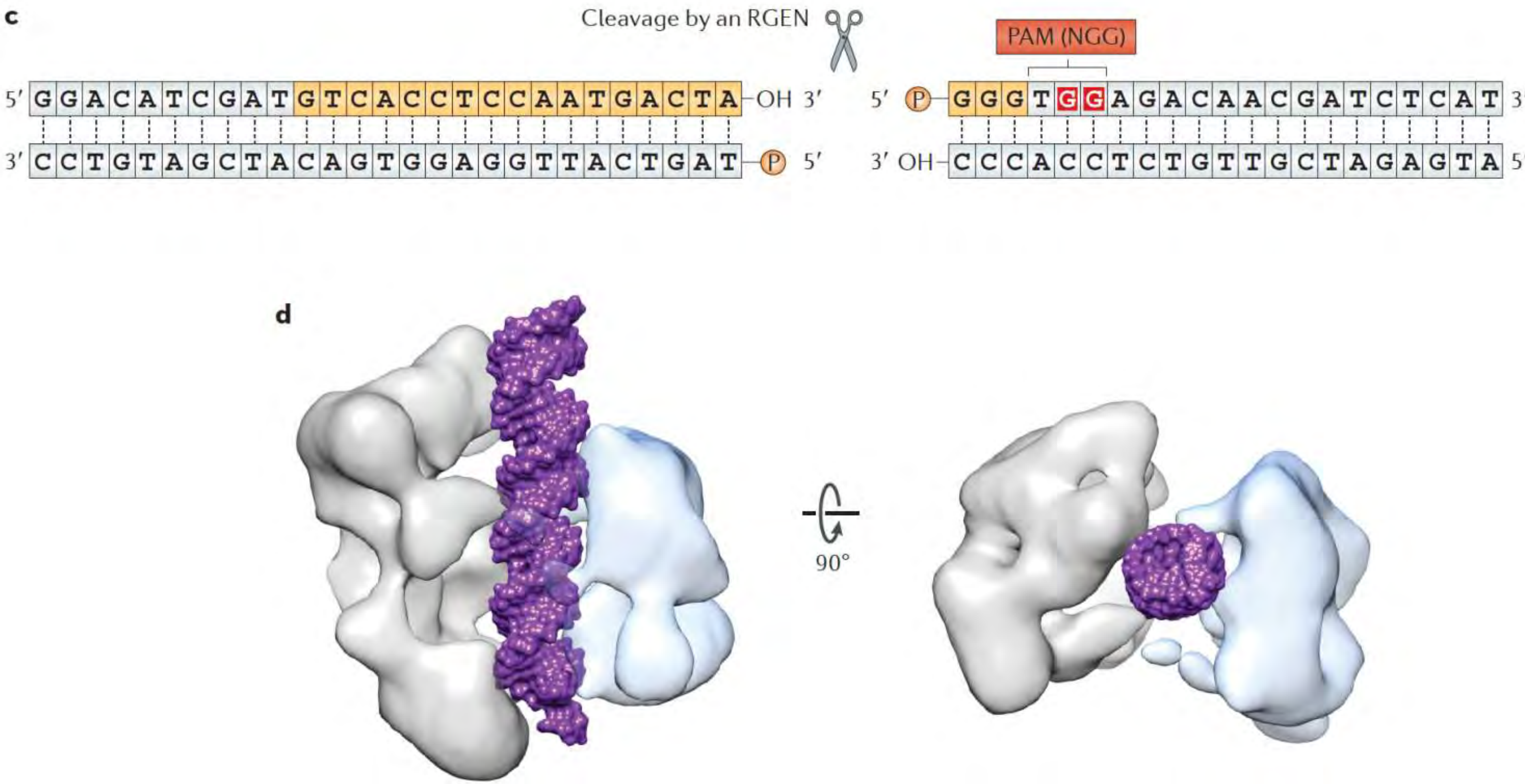
Double strand break (DSB) is created in the gene of interest

Targeted mutations (loss of function or gain of function)



Ding et al. 2016 Frontiers Plant Sci

CRISPR/Cas9 creates a double strand break at a specific target site in the genome



Kim and Kim 2014

Genome editing to rapidly combine targeted alleles for breeding

- Explore the use of genome editing for rapid pyramiding of beneficial alleles at breeding relevant genetic loci:
  - Validate genes where knock-outs provide the desired phenotypes (such *sd1*, *BadH2*)
  - Validate functional nucleotide polymorphisms by allele replacement → rapid testing of known alleles at each locus in the same background
  - Use as a replacement for marker-assisted backcrossing (MABC) to combine targeted alleles through multiplexed gene editing

M. Thomson, TAMU

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Texas A&M AgriLife Research Crop Genome Editing Lab

- New 3,500 ft^2 facility on the Texas A&M campus in College Station undergoing renovations for completion by January 2018

- Will provide research, service, and training functions to optimize protocols, set up a high throughput gene editing pipeline, and enable CRISPR/Cas9 research projects for target crops

- Starting with rice, cotton, wheat, and peanut but will rapidly expand into other crops in the future

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M. Thomson, TAMU

The Missing link: The root

Texas A&M AgriLife Research

Sean Thompson at CIMMYT in the Chihuahuan Desert, Mexico
Texas A&M/CIMMYT collaborative project

International Collaboration

- CGIAR Centers
  - International Maize and Wheat Improvement Center (CIMMYT)
  - International Center for Agricultural Research in the Dry Areas (ICARDA)
  - International Center for Tropical Agriculture (CIAT)
- Countries
  - Poland
  - Ukraine
  - Georgia
  - Turkey
  - Tunisia
- Several private sector entities

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It takes a team!!

Synergism: 1 + 1 = 3; Antagonism: 1 + 1 = -2

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