



INTERNATIONAL CROPS RESEARCH  
INSTITUTE FOR THE SEMI-ARID TROPICS



# Drought tolerance in sorghum: Staygreen QTL validation

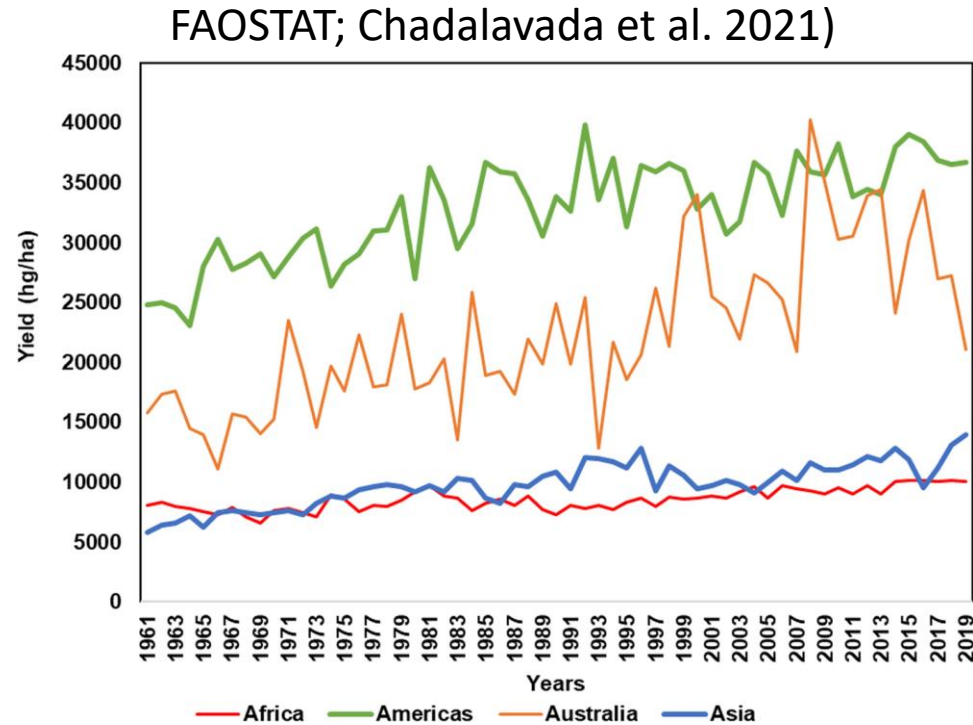
**Damaris Achieng Odeny**

Cluster Leader

Genomics, Pre-breeding and Bioinformatics

# Sorghum

- Sorghum is diploid ( $2n=2x=20$ )
- Native to Africa, 5<sup>th</sup> most important cereal
- Used as food, feed, industrial purpose
- Genome sequenced – 730 Mb



## Low yields, especially in Africa and Asia

- Average global yield significantly lower than potential
  - 1.49 MT ha<sup>-1</sup> vs 5 MT ha<sup>-1</sup>
- Low-income countries, e.g.
  - Africa average yield of <1 MT ha<sup>-1</sup>
  - Low research investment
  - Has been largely replaced by maize as indicator of food security

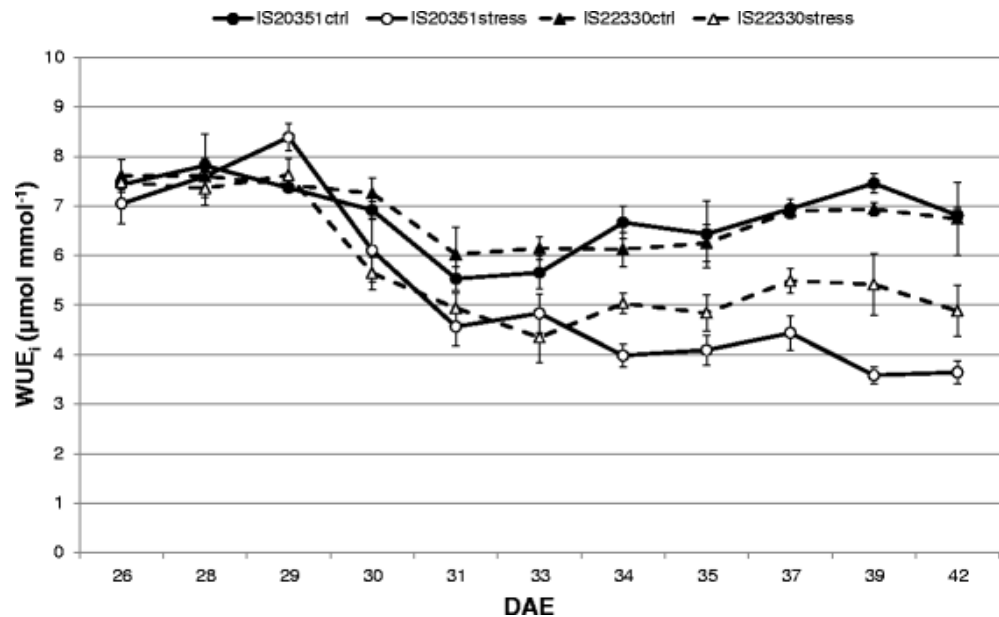
**Drought a major cause of low yields**



# Drought tolerance in sorghum

## Pre-flowering

Better Photosynthetic rates  
Lower Transpiration rates  
Better WUE  
Stomatal conductance



Fracasso et al. (2016)

## Post-flowering – most critical

Better root architecture

Longer seed filling duration

Faster seed filling rate



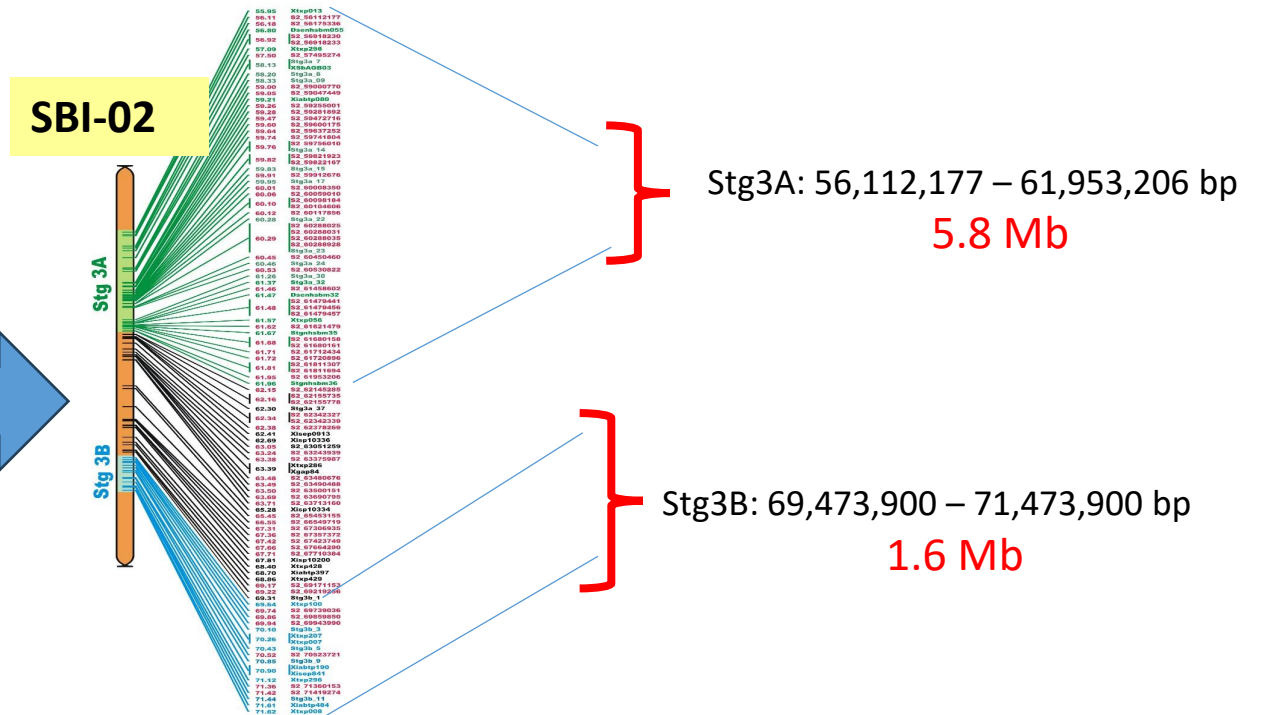
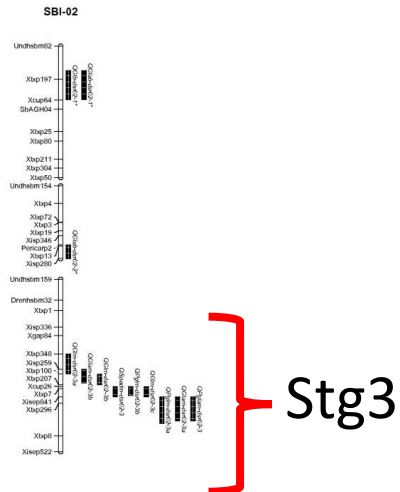
## Staygreen trait

# The Staygreen trait

- Delayed senescence – better yield
- Four major QTLs identified: *Stg1*, *Stg2*, *Stg3* and *Stg4*.
- *Stg3A* and *Stg3B* further studied, markers developed for introgression

**Table 1. Molecular Markers and Stay-green QTLs for Drought Tolerance in Sorghum.**

Molecular Marker	QTL	Position in Chromosome
Xtxp114, Xtxp38, xiabxp 3 7 8, SSR markers	<i>Stg1</i>	SB1-03
XnhsbSFCILP67, Xtxp120, Xtxs584, and Xtxp31, SSR markers	<i>Stg2</i>	SB1-03
Xtxs1307, Xtxs1111, Xtxp1, Xtxp56, Xtxp286, SSRs markers	<i>Stg3</i>	SB1-02
Xtxs713, Xtxs387, Xtxp225, Xtxp15, SSR markers	<i>Stg4</i>	SB1-05

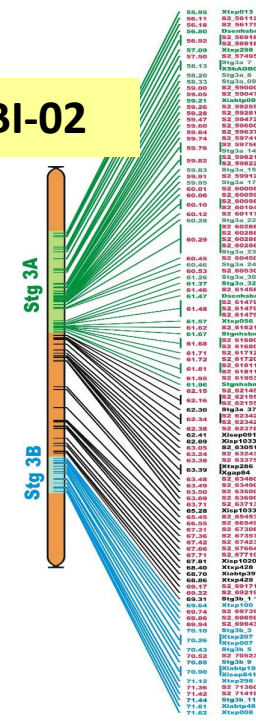


# KASP Assay marker development

- Provided to Intertek by Santosh Deshpande

	SNP	Locus	Position on V3.1.1
1	snpSB0035	Stg3A	56112177
2	snpSB0040	Stg3A	59000770
3	snpSB0049	Stg3A	59821923
4	snpSB0054	Stg3A	60098184
5	snpSB0072	Stg3A	61811307
6	snpSB0091	Stg3B	67306935
7	snpSB0095	Stg3B	67710384
8	snpSB0098	Stg3B	69739036
9	snpSB0101	Stg3B	70523721
10	snpSB0103	Stg3B	71419274

**SBI-02**



Stg3A: 1 marker every 1.2 Mb

Stg3A: 56,112,177 – 61,953,206 bp  
**5.8 Mb**

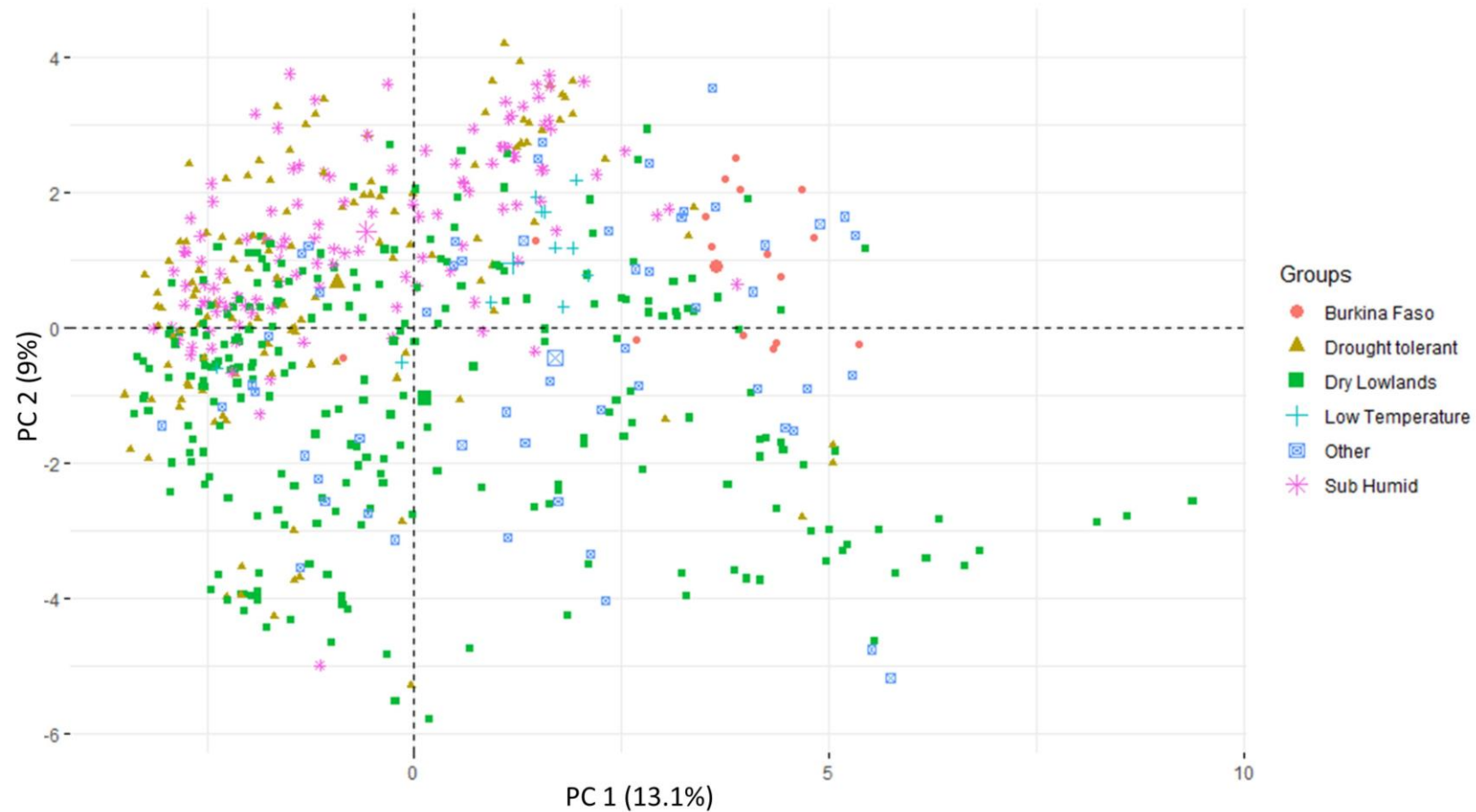
Stg3B: 69,473,900 – 71,473,900 bp

**1.6 Mb**

Stg3B: 1 marker every 0.3 Mb

# KASP Assay marker validation

- 752 breeding lines from ICRISAT – ESA program: Provided by Eric Manyasa  
161 had historical data – recorded as drought tolerant



# Performance of the Stg KASP Assays across all genotypes

- Out of the 10 markers, 9 amplified across 718 – 745 genotypes

	SNP	Locus	# Homozygous Favorable allele	# Alternate allele	Heterozygotes	Total amplified
1	snpSB0035	Stg3A	715	21	4	740
2	snpSB0040	Stg3A	649	66	13	728
3	snpSB0049	Stg3A	588	134	8	730
4	snpSB0054	Stg3A	357	335	26	718
5	snpSB0072	Stg3A	X	X	X	X
6	snpSB0091	Stg3B	712	17	1	730
7	snpSB0095	Stg3B	661	67	4	732
8	snpSB0098	Stg3B	7	728	1	736
9	snpSB0101	Stg3B	732	10	3	745
10	snpSB0103	Stg3B	13	712	1	726

# Performance of the Stg KASP Assays across drought tolerant genotypes

- 155 out of 161 amplified

	SNP	Locus	# Homozygous Favorable allele	# Alternate allele	Heterozygotes	Total amplified	% Variation explained
1	snpSB0035	Stg3A	155	0	0	155	100.0
2	snpSB0040	Stg3A	131	20	2	153	85.6
3	snpSB0049	Stg3A	134	20	0	154	87.0
4	snpSB0054	Stg3A	99	55	0	154	64.3
6	snpSB0091	Stg3B	153	0	0	153	100.0
7	snpSB0095	Stg3B	154	0	0	154	100.0
8	snpSB0098	Stg3B	0	155	0	155	0.0
9	snpSB0101	Stg3B	155	0	3	158	98.1
10	snpSB0103	Stg3B	3	151	0	154	1.9



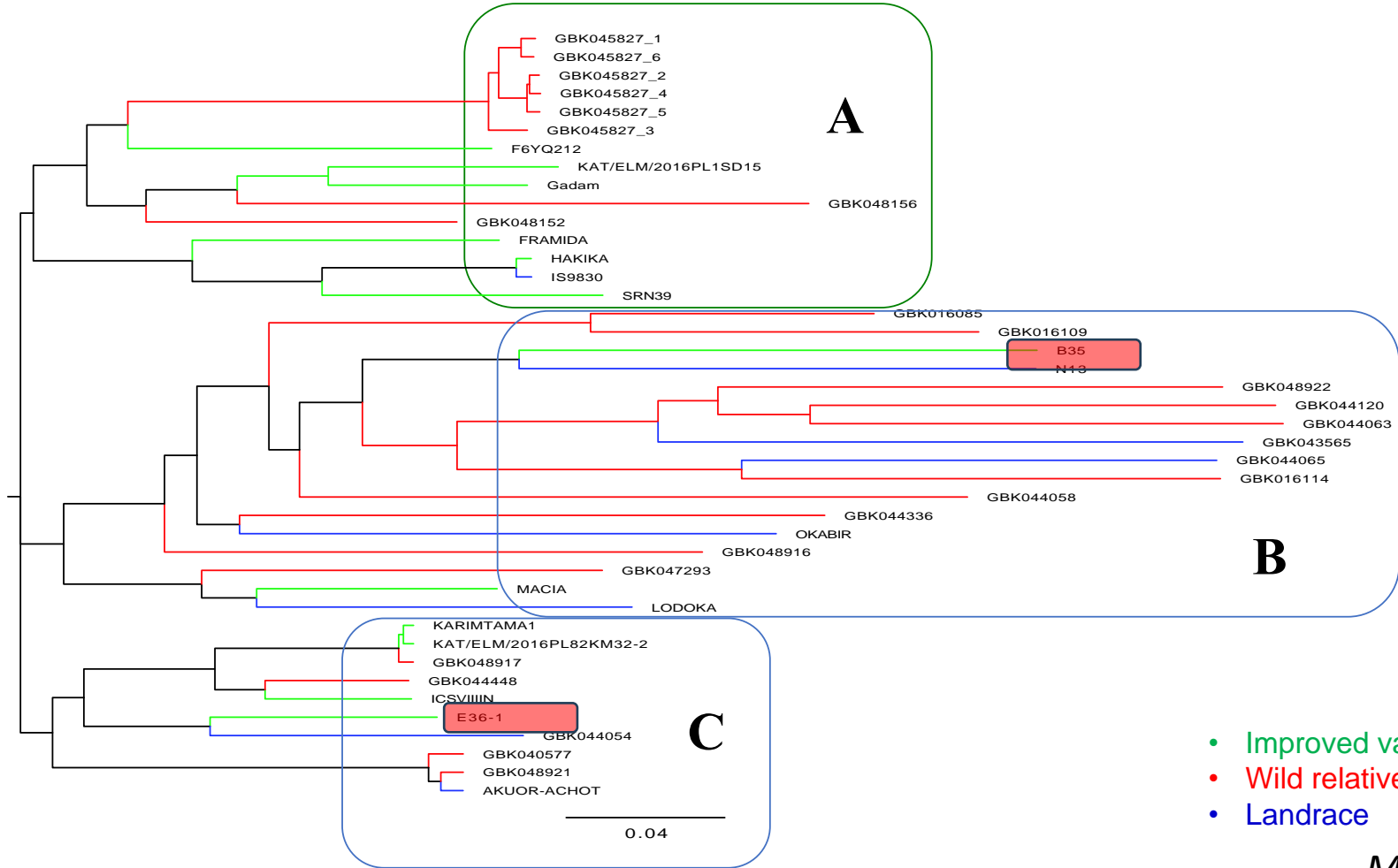
# Some Background Information

- B35 and E36-1 are the main sources of drought tolerance in ESA
- Stg3 locus common to both sources
- Can we integrate new sources to the breeding programs?

Germplasm classification	Numbers used
1. Landraces	9
2. Improved varieties	16
3. Wild accessions	17
5. B35 and E36-1	2

*Ochieng et al. (2020)*

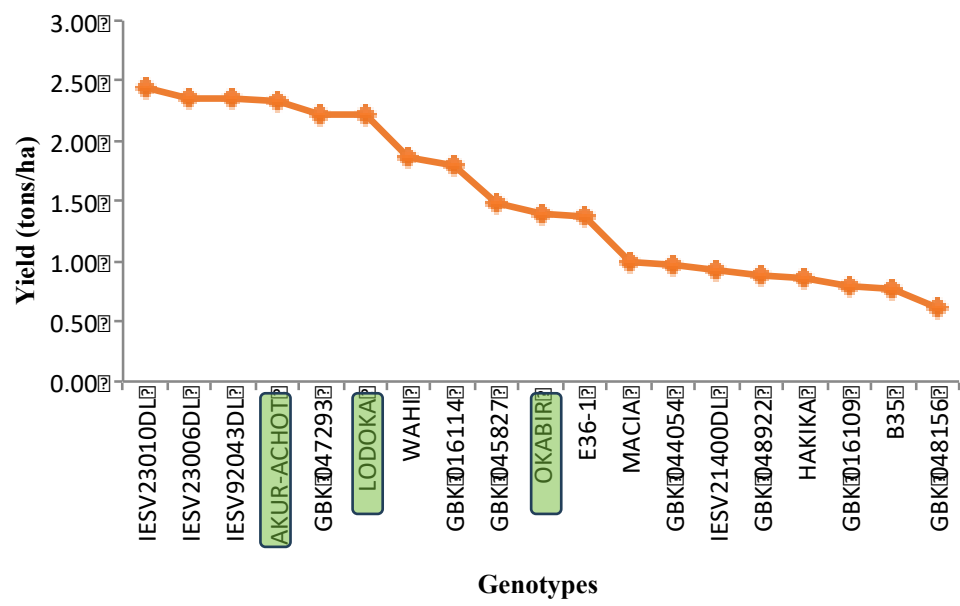
# Genetic relatedness of the germplasm



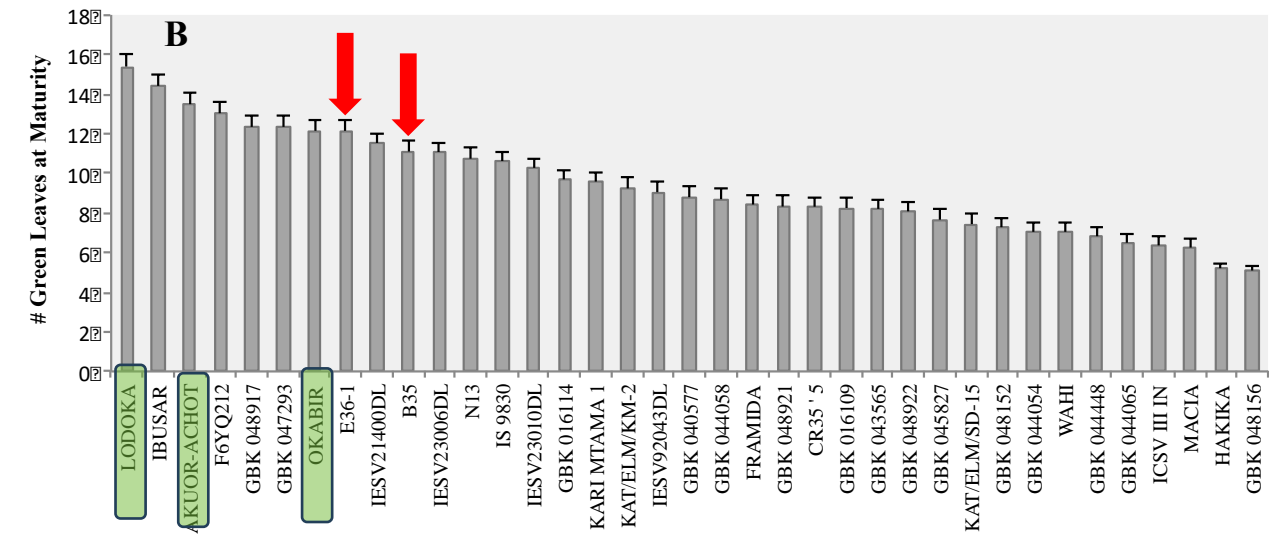
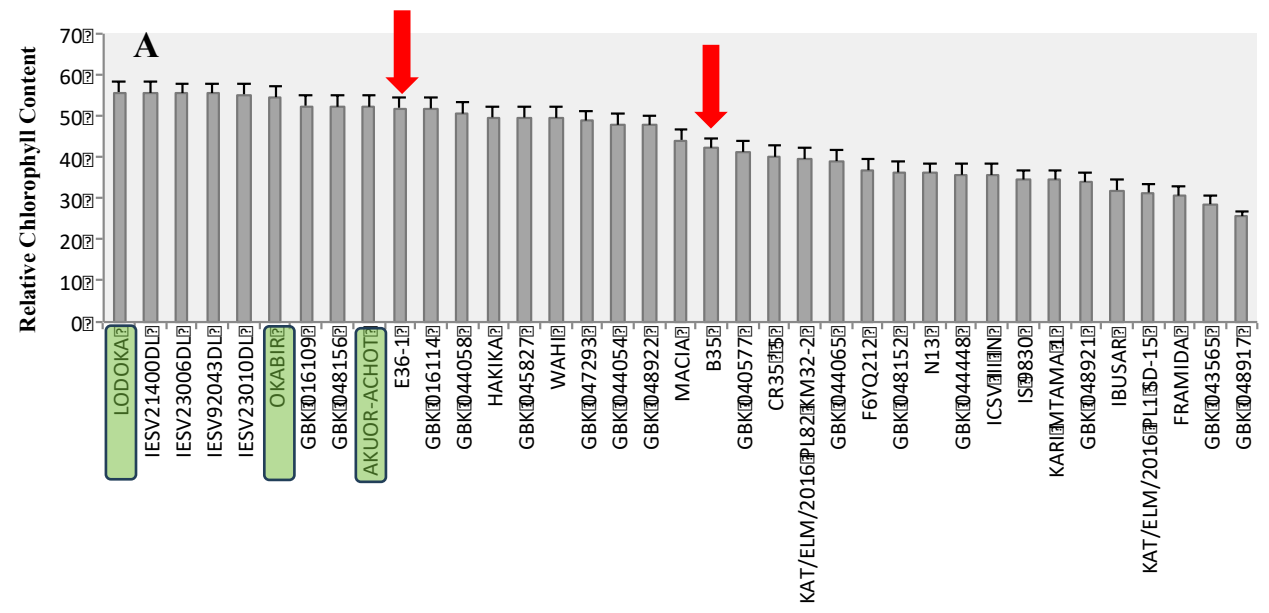
- Improved variety
- Wild relative
- Landrace

*Modified from Muchira et al. (2021)*

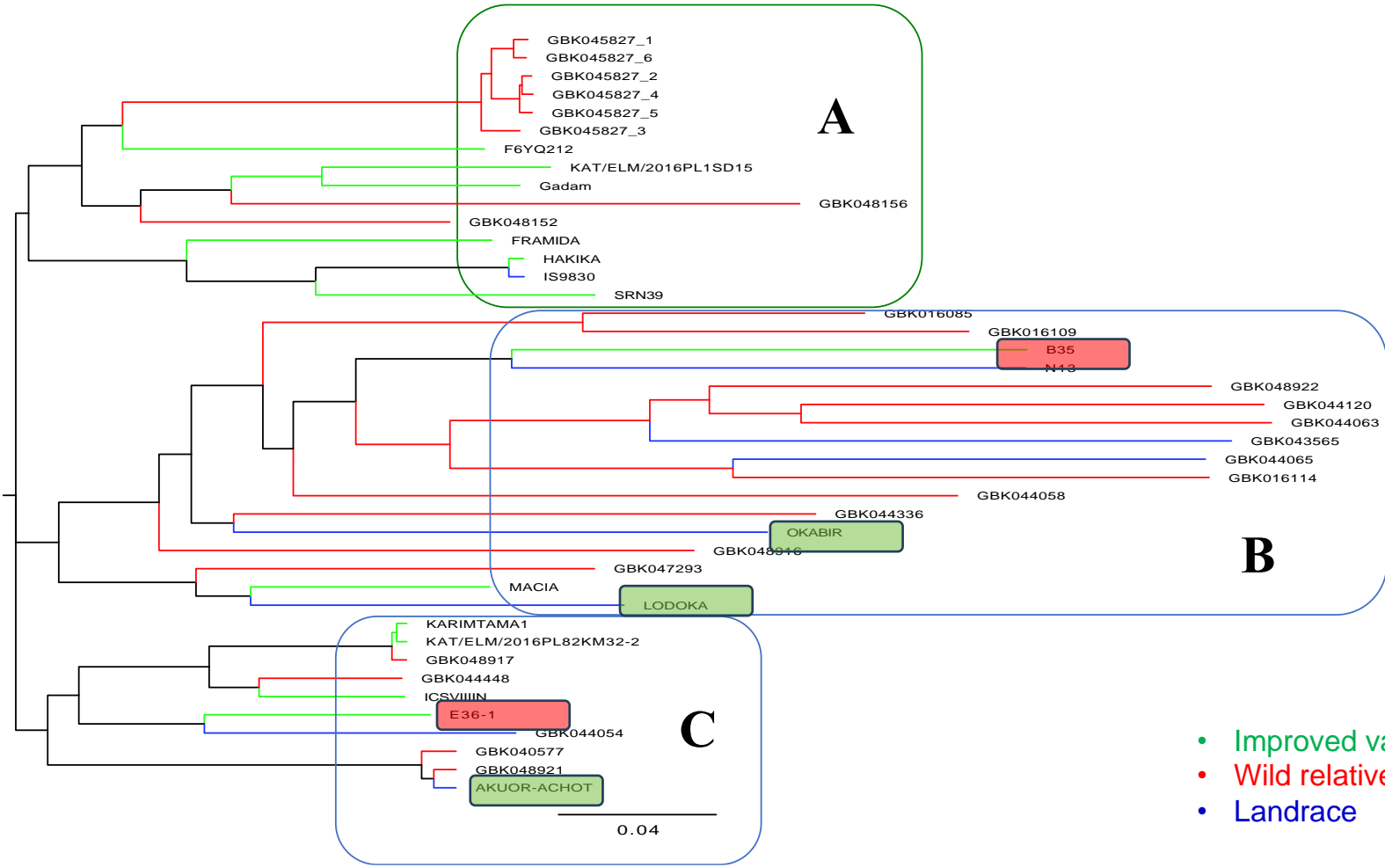
# Identifying novel sources of drought tolerance



Ochieng et al. (2020)



# Relatedness of Okabir, LODOKA and Akuor-Achot with B35 and E36-1



- Improved variety
- Wild relative
- Landrace

# Performance of Okabir, LODOKA and Akuor-Achot with KASP Assays

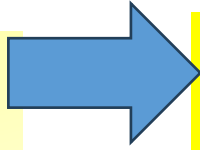
	SNP	Locus	Allele	Favorable	Akuor-Achot	Lodoka	Okabir
1	snpSB0035	Stg3A	C/T	CC	CC	CC	CC
2	snpSB0040	Stg3A	C/T	CC	CC	CC	CC
3	snpSB0049	Stg3A	G/A	GG	GG	GG	AA
4	snpSB0054	Stg3A	G/A	AA	AA	GG	AA
6	snpSB0091	Stg3B	A/C	AA	AA	AA	AA
7	snpSB0095	Stg3B	A/G	AA	AA	AA	AA
8	snpSB0098	Stg3B	C/G	GG	CC	CC	CC
9	snpSB0101	Stg3B	C/G	CC	CC	CC	CC
10	snpSB0103	Stg3B	C/G	GG	GG	CC	CC

- Is the drought mechanism different in these new sources?

# Drought tolerance in sorghum

## Pre-flowering

Better Photosynthetic rates  
Lower Transpiration rates  
Better WUE  
Stomatal conductance



## Post-flowering – most critical

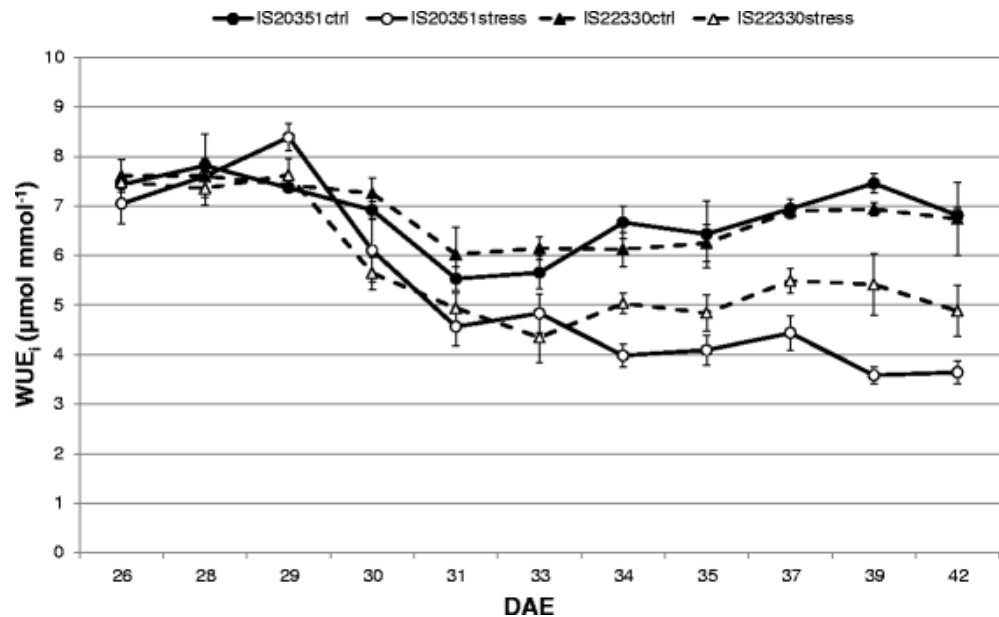
Better root architecture

Longer seed filling duration

Faster seed filling rate



Staygreen trait



Fracasso et al. (2016)

# Staygreen and root architecture

Focused on Chromosome 2

Stg3A: 69,473,900 – 71,473,900 bp

1.6 Mb

Stg3B: 56,112,177 – 61,953,206 bp

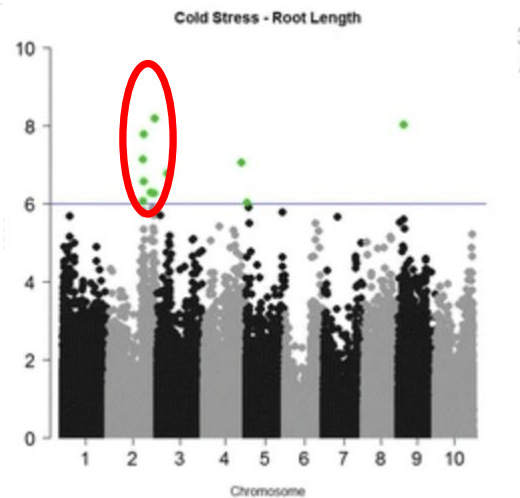
5.8 Mb

QTL ID	Publication	Population	Trait description	LG Start - end	Genes under QTL
<b>QRTWD2.2 – 2.11</b>	Parra-Londono et al. 2018	Diversity set	Root width	72,106,555 – 72,500,648	55
<b>QRTL2.8</b>	Chopra et al. 2017	Sorghum Association Panel (SAP)	Root length	72,546,626-72,935,339	55
<b>QRTL2.9</b>	Chopra et al. 2017	Sorghum Association Panel (SAP)	Root length	72,749,666 – 73,414,069	99
<b>QRTL2.3</b>	Chopra et al. 2017	Sorghum Association Panel (SAP)	Root length	73,466,507-74,272,206	118
<b>QRTDW2.4</b>	Mace et al. 2012	B923296/SC170-6-8	Root dry weight	74,735,754-76,031,254	174

Sorghum QTL Atlas: <https://aussorgm.org.au/sorghum-qtl-atlas/search/>

# Co-localization with root length under cold stress

Version	Gene Name	LG	bp_start	bp_end	Annotation	Selection Signature	Browse gene at JGI
3.0	Sobic.002G373600	2	73153554	73160861	von Willebrand factor type A domain containing protein, expressed		<a href="https://phytozome.jgi.doe.gov/pz/portal.html#!gene?search=1&amp;detail=1&amp;method=5228&amp;searchText=locusName">https://phytozome.jgi.doe.gov/pz/portal.html#!gene?search=1&amp;detail=1&amp;method=5228&amp;searchText=locusName</a>
3.0	Sobic.002G373700	2	73163583	73166165	expressed protein	<b>LAZY 2</b>	<a href="https://phytozome.jgi.doe.gov/pz/portal.html#!gene?search=1&amp;detail=1&amp;method=5228&amp;searchText=locusName">https://phytozome.jgi.doe.gov/pz/portal.html#!gene?search=1&amp;detail=1&amp;method=5228&amp;searchText=locusName</a>
3.0	Sobic.002G373800	2	73167726	73170579	elongation factor protein, putative, expressed		<a href="https://phytozome.jgi.doe.gov/pz/portal.html#!gene?search=1&amp;detail=1&amp;method=5228&amp;searchText=locusName">https://phytozome.jgi.doe.gov/pz/portal.html#!gene?search=1&amp;detail=1&amp;method=5228&amp;searchText=locusName</a>



Root length under cold stress

Root length under drought stress????



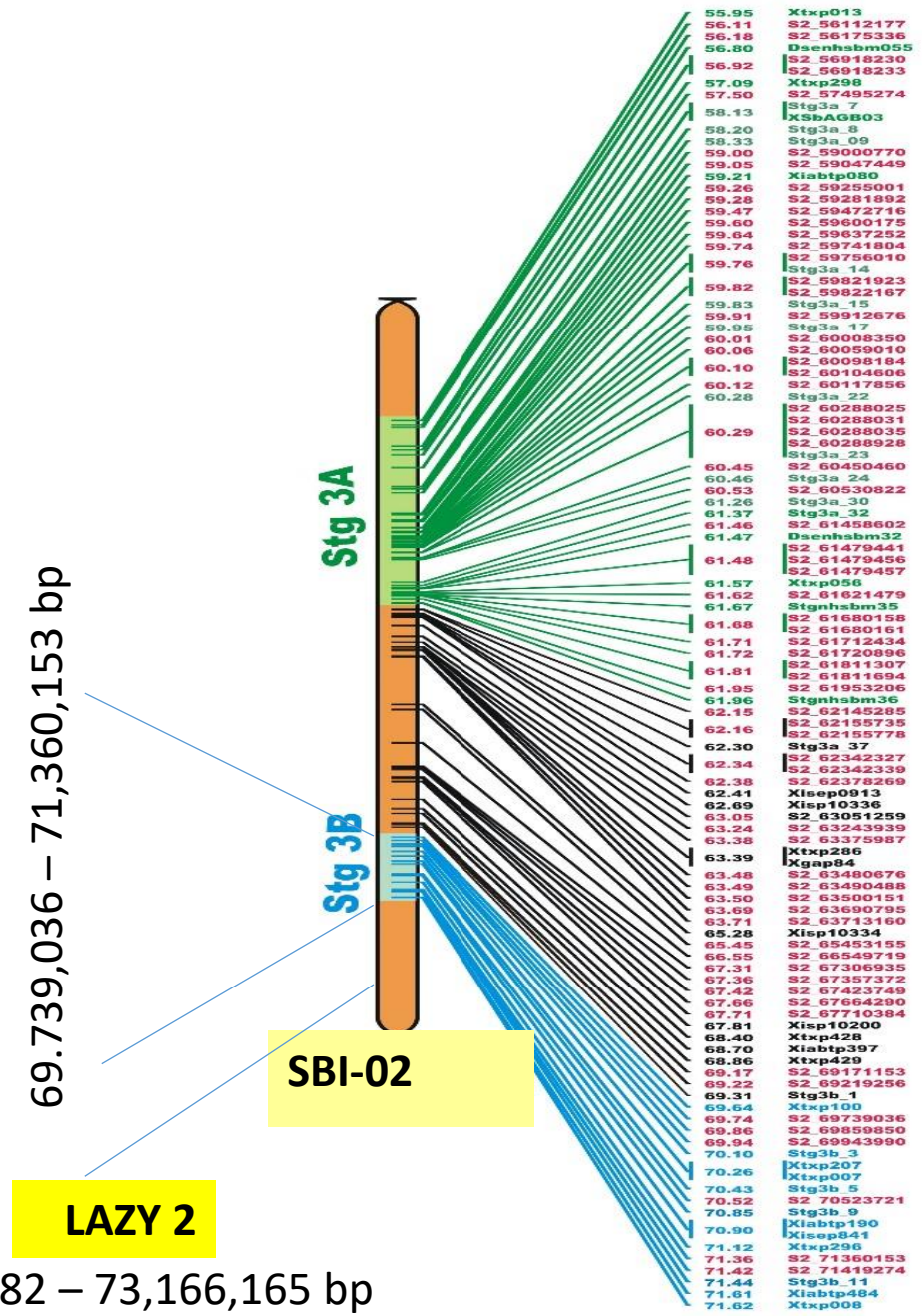
# Co-localisation of Stg3B with *LAZY2*

>SORBI\_3002G373700: OQU90252 peptide: OQU90252 pep:protein coding

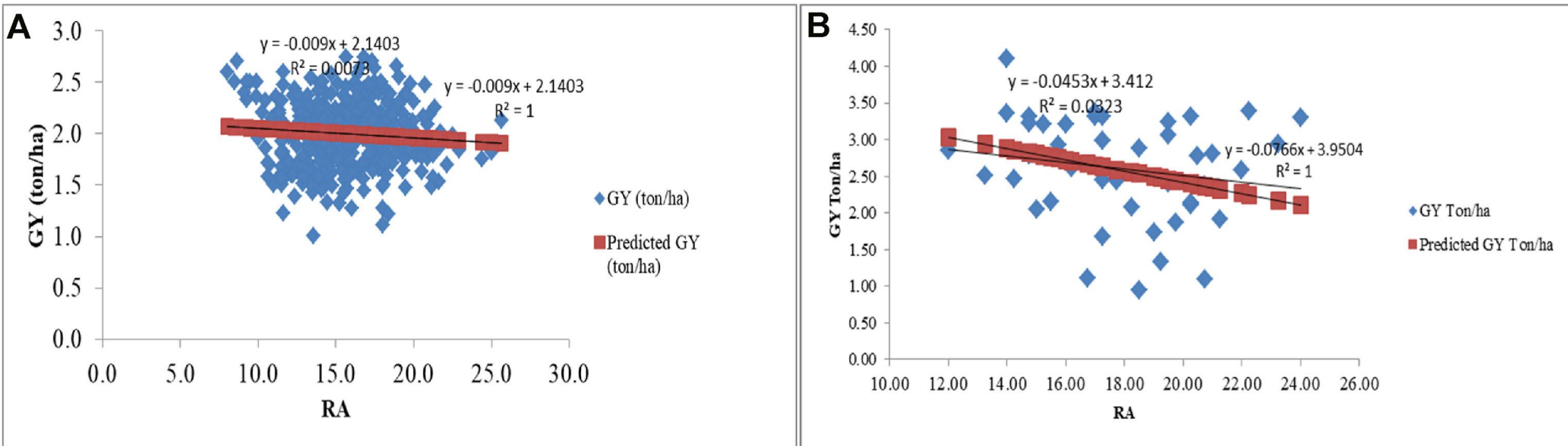
MGI INWMQNRFNKGHEKRRPEATAAAAAAFASSAHESCRQDHGREDKIPTGDWPPQGLLS  
 IGTLGDDPPPAAGDGGGGPPRASQADVLDFTIEEVKKLQDALNKLLRRAKSKSSSSSRGSG  
 ATDEDRASQLPLDRFLNCPSS **LEVDRR** ISLRHAAGDGGGENGEFSPDTQIIILSKARDLLV  
 NSNGTTIKKKSFKFLLKKMFVCHGGFAPAPSLKDPVESRIEKLFRTMLQKKMNNARPSNA  
 AVSSRKYYLEDKPSGRMMIRDGHHDEEDDEKGS DRIKWDKTD TDFIVLEI

## Two hits

1. Chromosome 2: 73163582 – 73166165 uncharacterized protein
2. Chromosome 1: 62946190 – 62948207 root specific expression



# Correlation has been shown between RA and yield in staygreen genotypes



The result showed that narrow root angle genotypes had better grain yield performance, while wider root angle genotypes had lower grain yield performance.

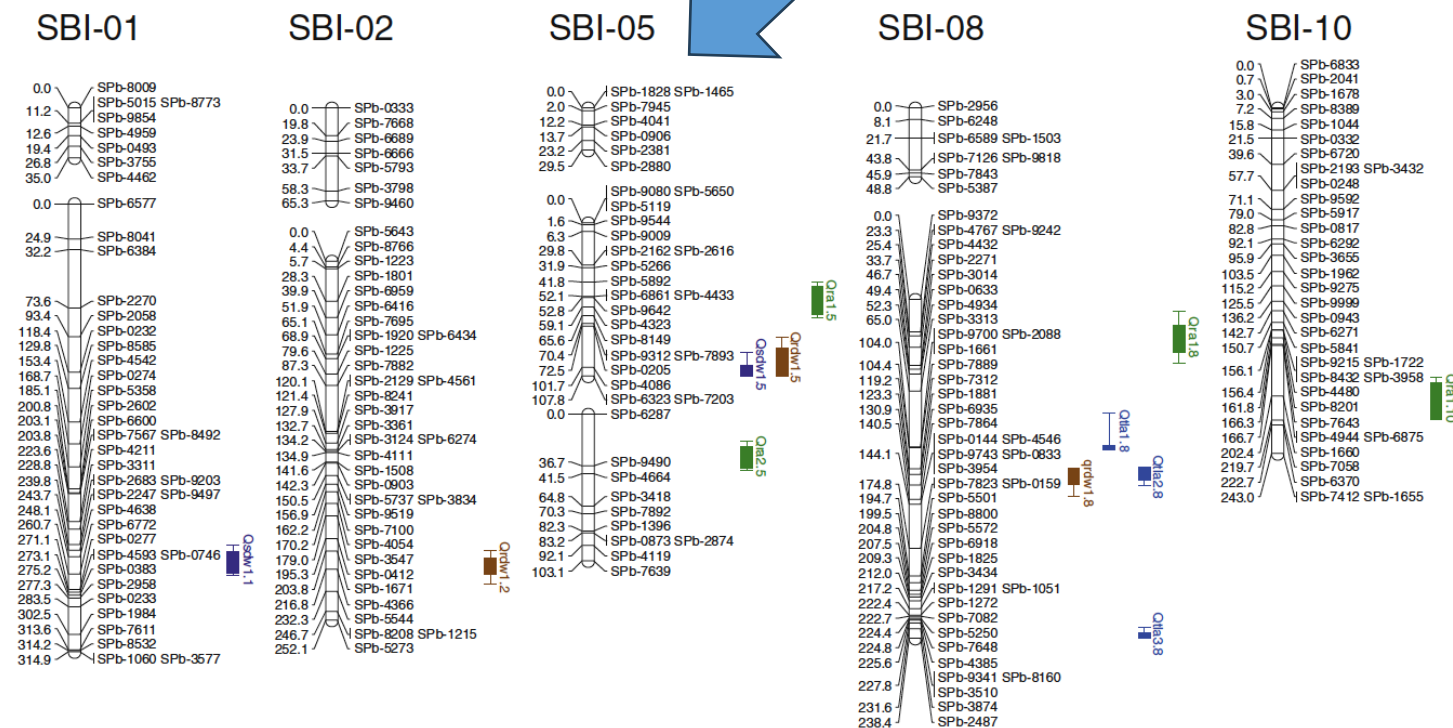
# Other QTLs for nodal root angle not necessarily on LG2

**QTL for nodal root angle in sorghum (*Sorghum bicolor* L. Moench) co-locate with QTL for traits associated with drought adaptation**

E. S. Mace · V. Singh · E. J. Van Oosterom · G. L. Hammer · C. H. Hunt · D. R. Jordan

*Stg4* located on SBI-05

$R^2$  of 30 and 10 %



**Fig. 3** Localisation of QTL for five traits measured in this study on a genetic linkage map based on the B923296/Sc170-6-8 RIL mapping population. QTL are represented by bars (1-LOD interval) and

extended lines (2-LOD interval). QTL are colour-coded according to trait; nodal root angle green; root dry weight brown; shoot dry weight dark blue; total leaf area light blue

The result showed that narrow root angle genotypes had better grain yield performance, while wider root angle genotypes had lower grain yield performance.

# So can we add a few more parameters in the phenotyping for Staygreen?

Standard protocol based on IBPGR and ICRISAT (1993)

1. Plant height
2. Flag leaf area
3. Days to 50% flowering
4. Number of green leaves at maturity
5. Relative chlorophyll content (RCC; SPAD readings)
6. Panicle weight
7. Hundred seed weight
8. Grain yield
9. Leaf rolling

10. How about root traits?

**Table 8.** Correlation coefficients of traits contributing to drought tolerance and grain yield of sorghum genotypes under well-watered and water-stressed environments.

Trait	GW	PWT	PH	NL	BM	NGLM	PW	IEX	CH	STG	PL
GW	-										
PWT	0.72 **	-									
PH	0.27 **	0.41 **	-								
NLM	0.08	0.17 **	-0.13 **	-							
BM	0.26 **	0.29 **	-0.21 **	0.04	-						
GLM	0.18 **	0.2 **	-0.2 **	0.21 **	0.39 **	-					
PW	0.53 **	0.75 **	0.5 **	0.04	0.17 **	0.06	-				
IEX	-0.17 **	-0.18 **	0.2 **	-0.07	0.01	0.1	-0.08	-			
CH	0.25 **	0.27 **	-0.26 **	-0.02	0.6	0.53 **	0.2 **	0.1	-		
LSN	-0.21 **	-0.22 **	0.09	-0.03	-0.41 **	-0.84 **	-0.11	-0.15 **	-0.56 **	-	
PL	0.35 **	0.51 **	0.18 **	1	0.29 **	0.07	0.46 **	-0.09	0.14 **	-0.1	-

\*\* Correlation coefficient is significant at  $p \leq 0.01$ , BM = root biomass, IEX—inflorescence exertion, GW = grain weight per panicle (kg), PWT = panicle weight (kg), PH = plant height (cm), CH = chlorophyll content ( $\text{g}^{-1}$ ), STG, NGLM = total number of green leaves at maturity, NLM = total number of leaves at maturity, PL—panicle length (cm), PW—panicle width (cm).

*Mwamahonje et al. (2021)*

# In summary

- Availability of the KASP markers enabled the validation and potential routine utilization of 4 markers
- We still do not understand fully the biology of Staygreen trait
- Neither do we understand the genetics
- We need to do more exhaustive phenotyping of our sources of drought tolerance to capture correctly the genetic variation
- That would also allow for more precise Genomic selection in the future

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Thank YOU