Crops to End Hunger: Case Studies in Africa and Beyond

Introgression of QTLs for Drought Tolerance into Farmers’ Preferred Sorghum Varieties

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GLOBAL
SORGHUM

Global Sorghum Association (GSA)

- **Officialy launched at the 2023 Global Sorghum Conference - Montpellier - France**
- **Mission:**
  - A Global Sorghum Conference of the Highest Level
  - Host support and long-term sustainability

Global Sorghum Association (GSA)

- **Motivation:**
  - Building and strengthening the Global Sorghum Community
    - Why?
  - *“Resilience and Sustainability in the Face of Climate Change”*
  - Synergy and Sorghum

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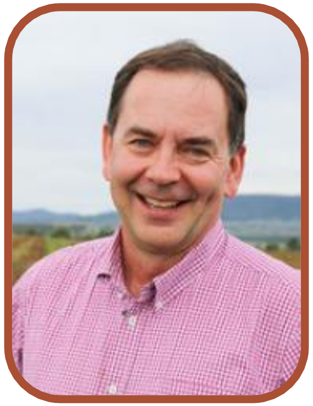

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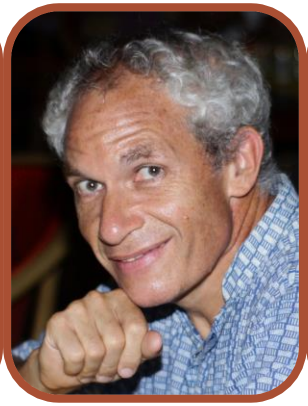

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SORGHUM CHECKOFF

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GSA incorporation and legal aspects

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INTROGRESSION OF QTLS FOR DROUGHT TOLERANCE INTO FARMERS' PREFERRED SORGHUM VARIETIES USING KASP SNPS MARKERS

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Presentation outline

- ✓ Introduction
- ✓ Objectives
- ✓ Materials and methods
- ✓ Results and discussion
- ✓ Conclusion and
- ✓ Recommendations

Introduction

- Globally, sorghum ranks as the fifth staple food crops after maize, rice, wheat, and barley (FAOSTAT, 2020)

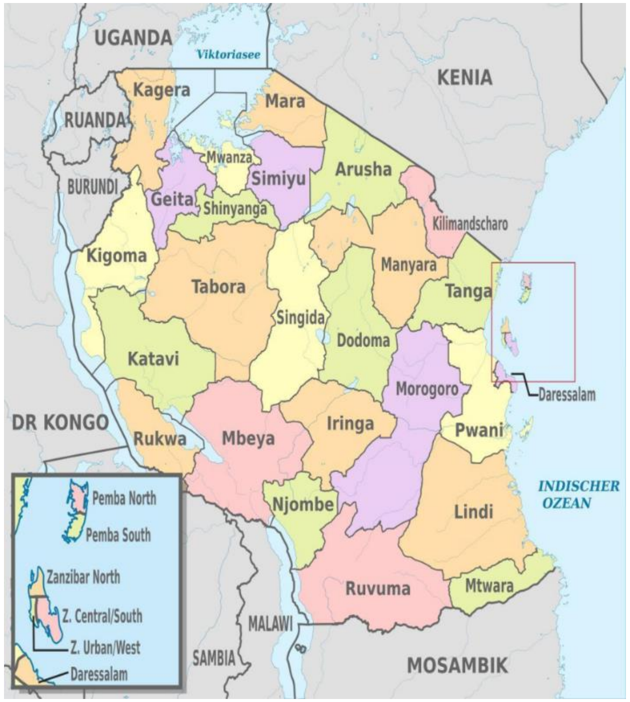
- The global production is estimated to be 59.34 million tons (USDA, 2019)

- U.S.A and Nigeria are the major producers countries in the world

(FAOSTAT, 2020)

- Over 100 million people in Eastern Africa depend on sorghum as the source food security (Gudu *et al*., 2013)

- Tanzania produces an estimate of 800,000 mt annually in more than 10 sorghum producing regions



Uses of sorghum

- Prevents diabetes
- Prevents cancer
- Improves bone health
- Improves digestion
- Iron reduces anaemia

Introduction (cont')

- Sorghum is a C4 crop which is characterized by drought tolerance
- Drought is among the major constraints of sorghum production globally
- It causes 50-60% yield loss that leads to food insecurity in sub-Saharan Africa and Asia
- In Tanzania, drought is common in central zone where, the productivity is 1 tons/ha
- In Tanzania, breweries have increased the demand of sorghum grain (Ministry of Agriculture, 2020)

Tamiru, 2016

Introduction (cont')

- Improved sorghum varieties have been developed, but have since lost uniformity and often succumb to drought stress
- Breeding for drought tolerance sorghum can be achieved by different approaches; Genome wide association, conventional screening, marker assisted back crossing (**Kamal et al., 2017)**
- However MABC does efficiently for introgression of trait of interest from the donor parent to the preferred recurrent parents
- For instance, *Stg1*, *Stg2*, *Stg3*, and *Stg4* stay-green QTLs have been mapped in B35 sorghum line using SSR Markers (Kamal et al., 2017) and recently *Stg 3A, Stg 3B stay green QTLs using SNPs markers*
- These QTLs have been introgreessed to non-stay green sorghum varieties for enhancing rentation of stay green during post flowering drought (Azzouz-Olden et al., 2020; Edema et al., 2015; Kamal et al., 2018)
- Therefore, these QTLs can be used to improve sorghum for post flowering drought in Tanzania

General objective

The general objective of this study was to develop drought tolerant and high grain yield of sorghum genotypes through maker-assisted backcrossing.

Specific objectives

The objectives of this study were to:

i. introgress STG QTLs from donor parents B35 and S35 to the recurrent parents

ii. identify STG QTLs transferred to the recurrent parents using KASP SNPs markers

Materials and methods

Study area

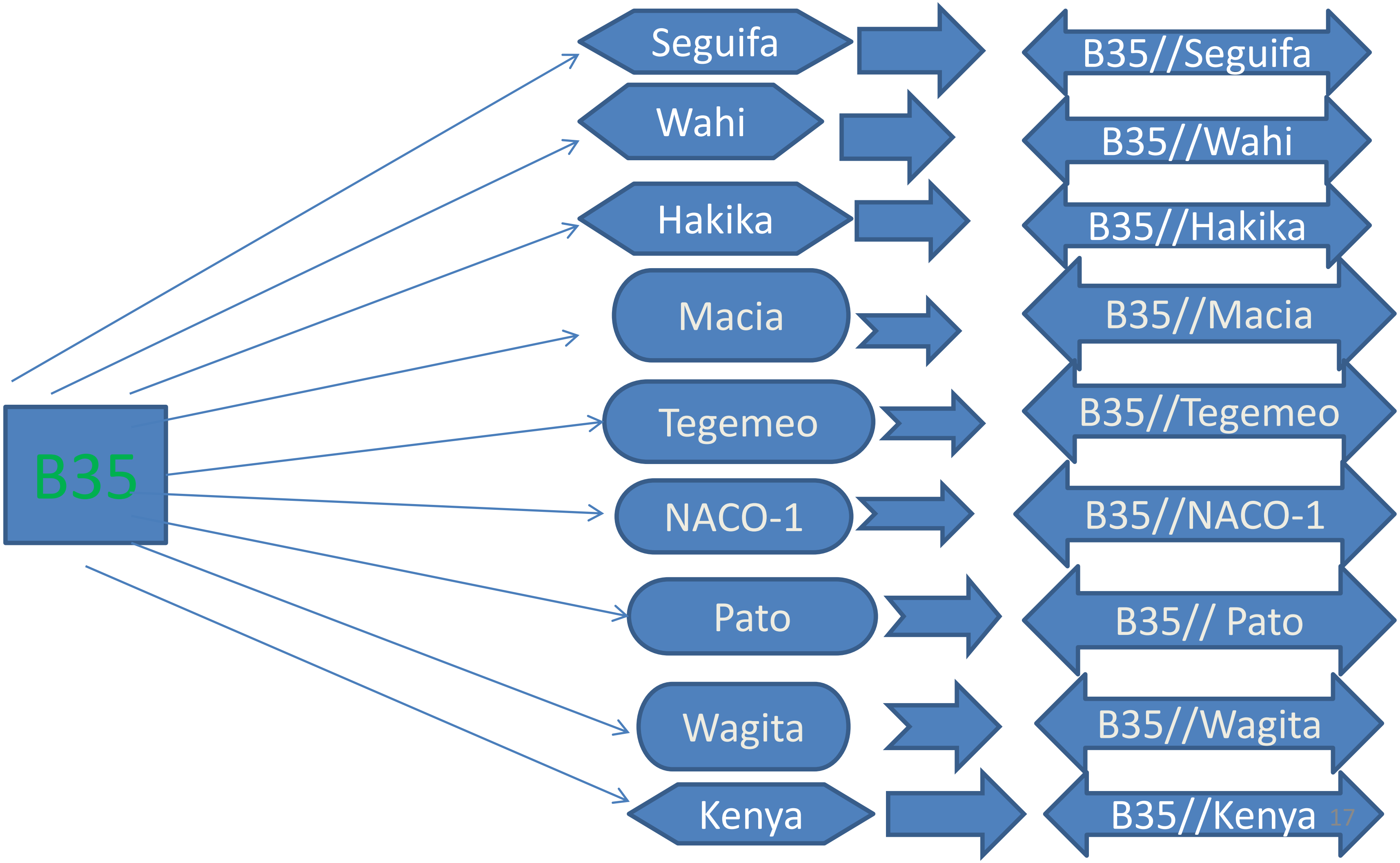
- TARI- Makutupora in Dodoma region (Longitude: 35º, 46.093'E and Latitude: 05º, 58.669'S) (Altitude: 1080 m).

- Temperature varies from 15-35.1°C (Tanzania Meteorological Agency- 2014). The area is characterized by mono-modal rainfall

❖ Leaf samples were collected at TARI Makutupora Centre and transported to Intertek Sweden in Sweden for genotyping in coordination with Excellence in Breeding (EiB) CIMMYT Nairobi Kenya

Materials

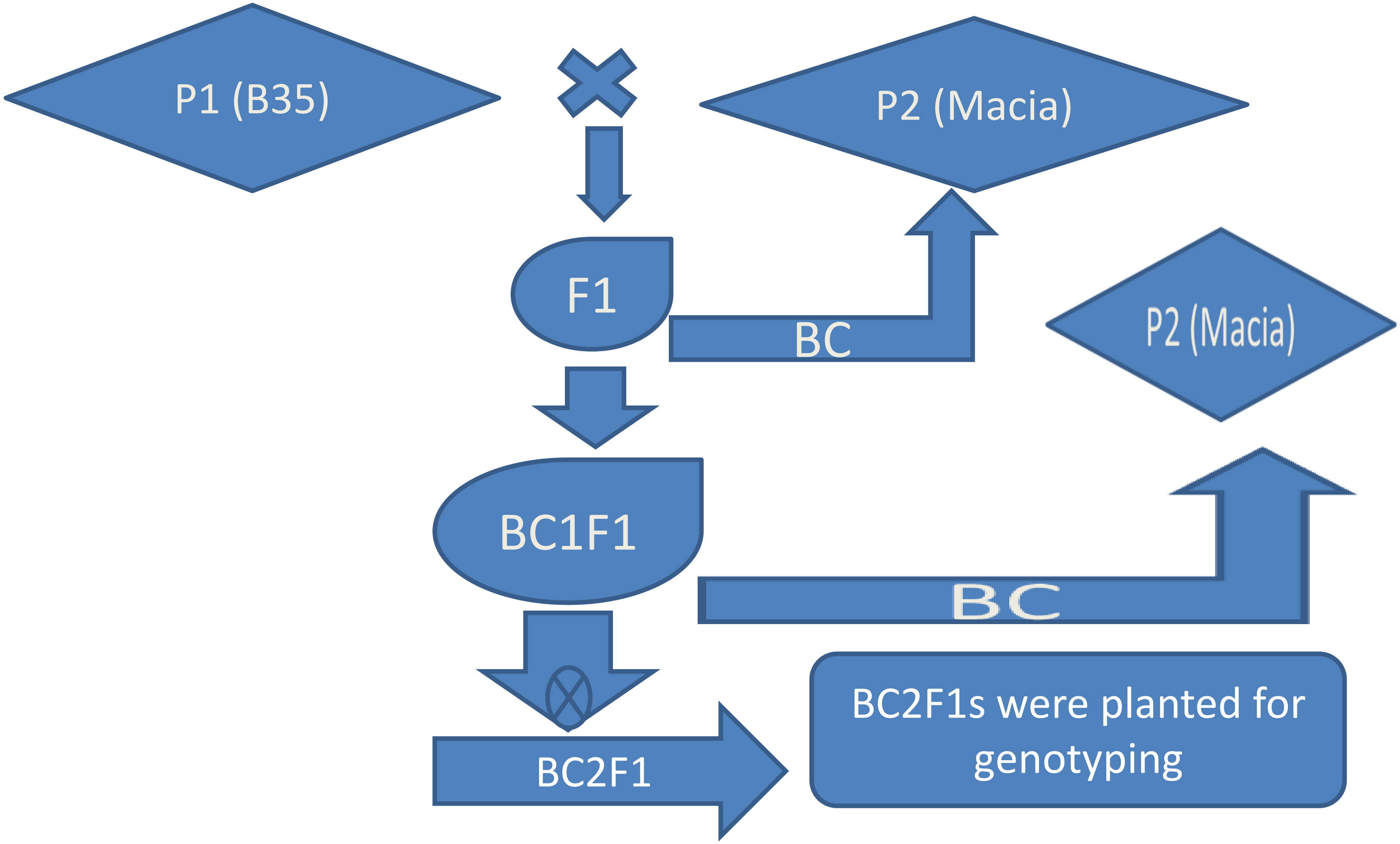
- Two donor parents and nine recurrent parents were used
- North Carolina II mating design



B35

Seguifa
Wahi
Hakika
Macia
Tegemeo
NACO-1
Pato
Wagita
Kenya

B35//Seguifa
B35//Wahi
B35//Hakika
B35//Macia
B35//Tegemeo
B35//NACO-1
B35// Pato
B35//Wagita
B35//Kenya



P1 (B35)

P2 (Macia)

F1

BC

P2 (Macia)

BC1F1

BC

BC2F1

BC2F1s were planted for genotyping

Genotyping BC2F1

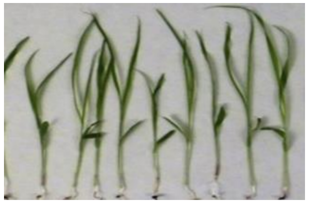
- A total of 150 leaf samples per genotype were collected 47 days from planting for genotyping using 30 SNPs

Table 1. List of SNPs markers associated with STG QTLs used for genotyping sorghum.

S.No.	Physical_Mbp	SNP Ids	Alleles	Donor Allele	Alternate Allele	Remarks/Possible
1	S2_56112177	snpSB0035	C/T	C	T	grain types
2	S2_56918230	snpSB0037	C/T	C	T	
3	S2_56918233	snpSB0038	A/T	A	T	
4	S2_57495274	snpSB0039	A/G	A	G	PCD triggering
5	S2_59000770	snpSB0040	C/T	C	T	
6	S2_59047449	snpSB0041	C/G	C	G	
7	S2_59255001	snpSB0042	T/C	T	C	
8	S2_59281892	snpSB0043	T/C	T	C	
9	S2_59472716	snpSB0044	A/T	A	T	
10	S2_59821923	snpSB0049	G/A	G	A	N2 mobilisation / SAG/SGR1
11	S2_60059010	snpSB0053	A/G	A	G	
12	S2_60098184	snpSB0054	G/A	G	A	
13	S2_61811307	snpSB0072	G/A	G	A	APETALA2 and EREBPs
14	S2_62145285	snpSB0075	A/G	A	G	Recurrent parent-specific
15	S2_62155735	snpSB0076	T/A	T	A	Heat shock domain
16	S2_62155778	snpSB0077	T/C	T	C	
17	S2_62378269	snpSB0080	C/T	C	T	Recurrent parent-specific
18	S2_63375987	snpSB0083	T/C	T	C	Chloroplast precursor/ubiquitionus
19	S2_63690795	snpSB0087	A/G	A	G	
20	S2_65453155	snpSB0089	C/A	C	A	SGR2
21	S2_67306935	snpSB0091	A/C	A	C	panicle compactness
22	S2_67357372	snpSB0092	C/G	G	C	Salt responsive
23	S2_67423749	snpSB0093	A/T	A	T	
24	S2_67664290	snpSB0094	T/C	T	C	
25	S2_67710384	snpSB0095	A/G	A	G	
26	S2_69739036	snpSB0098	C/G	G	C	Aspartic proteases through SA
27	S2_69859850	snpSB0099	C/T	C	T	
28	S2_70523721	snpSB0101	C/G	C	G	SGR3
29	S2_71360153	snpSB0102	A/G	A	G	
30	S2_71419274	snpSB0103	C/G	G	C	

BC2F1s were selfed to BC2F3 followed by genotyping with a KASP marker (LGC, UK)

150 leaf samples were collected per line



DNA extraction

DNA purity

Amplification of DNA using SNPs markers

QTLs identification for drought tolerance

QTLs selection

BC2F3 genotypes were genotyped using 10 KASP SNPs markers

- ✓ Marker data analysis was performed using SNPviewer from Biosearch Technologies
- ✓ Subsequently, genotyped materials were compared in flapjack v. 1.21.02.04

Results and discussion

❖Phenotypic data

Table 2. Five per cent of the best performance BC_2F_1 populations and their parents under field condition

Population	PH	NGLM	PL	PW	EX	GWT
B35*Wahi	117.4c	2.167ef	32.22f	7.5fg	2.5b	0.096e
S35*Hakika	135defg	2de	30.25e	7.25ef	2a	0.08d
B35*NACO	144.1gh	3g	24.92d	8.17g	3c	0.127f
B35*Macia	132.7def	1bc	23.88d	5.07cd	3c	0.027a
B35*Seguifa	141.7fgh	2.833fg	20.75c	6.58e	2.83bc	0.11e
S35*Pato	146.4h	1bc	21.75c	8.17g	2.5b	0.112e
B35 DP	88a	0.33ab	17.17ab	3.5a	3c	0.013a
Pato RP	131.3de	1.67cde	15.83a	6.67e	2.67bc	0.064c
Seguifa RP	139.7efgh	1.67cde	21.33c	5.67d	2.67bc	0.067cd
Hakika RP	131.2de	1bc	29.17e	5.5d	3c	0.028a
Macia RP	113.4bc	0.0a	21.33c	4ab	3c	0.019a
NACO RP	113.8bc	1.33cd	21.47c	5.13cd	3c	0.047b
S35 DP	104.5b	2de	18.33b	4ab	3c	0.024a
Wahi RP	128.3d	1bc	30.17e	4.5bc	3c	0.021a
SE	2.72	0.22	0.5	0.2	0.11	4.43
LSD	5.37	0.43	0.98	0.4	0.33	8.72
CV (%)	6.5	43.2	6.3	10.5	11.7	22.3

a-h are compared by Bonferroni mean test; EX—Inflorescence exertion

Results and discussion (cont’)

BC_2F_1 genotyping results

- Seventy one (10%) out of 728 BC_2F_1 sample collected for genotyping were heterozygous
- The markers snpSB00049, snpSB00077, snpSB00102 and snpSB00103 indicated heterozygous allele in 37 samples of S35*Pato background
- The rest (19) SNP markers showed homozygous allele of the BC_2F_1 samples used for genotyping
- Selection of the genotypes for advancing to BC2F3 populations was based on the best five performing plants in response to genotypic data where
- At least one sample of plant was selected per genotypes (Platten et al., 2019)

Table 3. Favourable alleles and STG QTLs identified after genotyping BC$_2$F1 populations of sorghum.

SNP Markers	FA	QTL	Physical Location	Role
snpSB00039	A	STG 3A	S2_57495274	Retains STG and triggers PCD
snpSB00040	C	STG 3A	S2_59000770	Retains STG and triggers PCD
snpSB00041	C	STG 3A	S2_59047449	Retains STG and triggers PCD
snpSB00043	T	STG 3A	S2_59281892	Retains STG and triggers PCD
snpSB00044	A	STG 3A	S2_59472716	Retains STG and triggers PCD
snpSB00049	G	STG 3A	S2_59821923	Retains STG and enhance grain yield
snpSB00053	A	STG 3A	S2_60059010	Retains STG and enhance grain yield
snpSB00076	T	STG 3A	S2_62155735	Retains STG and regulate heat shock domain
snpSB00077	T	STG 3A	S2_62155778	Retains STG and regulate heat shock domain
snpSB00083	T	Interval	S2_63375987	Retains STG and regulates chloroplast precursors
snpSB00087	A	Interval	S2_63690795	Retains STG and regulates chloroplast precursors
snpSB00089	C	STG 3B	S2_65453155	Retains STG and enhances grain yield
snpSB00093	A	STG 3B	S2_67423749	Retains STG and regulates salt
snpSB00094	T	STG 3B	S2_67664290	Retains STG and regulates salt
snpSB00095	A	STG 3B	S2_67710384	Retains STG and regulates salt
snpSB000101	C	STG 3B	S2_70523721	Retains STG and enhances grain yield
snpSB00102	A	STG 3B	S2_71360153	Retains STG and enhances grain yield
snpSB00103	G	STG 3B	S2_71419274	Retains STG and enhances grain yield

SNP—Single nucleotide polymorphism; FA—Favourable alleles; QTL—Quantitative trait loci; STG—Stay-green; PCD—programmed cell death.

Results and discussion (cont’)

Table 4. BC$_2$F$_1$ sorghum populations scored with heterozygous alleles

NS	SNP Markers	H. Allele	DP Alleles	RP Alleles	NS	SNP Markers	H. Allele	DP Alleles	RP Alleles	
BC$_2$F$_1$ (B35*Wahi)					BC$_2$F$_1$ (B35*Seguifa)					
82	snpSB0102	G/A	AA	GA	567	snpSB0103	G/C	CC	CC	
90	snpSB0102	G/A	AA	GA	569	snpSB0103	G/C	CC	CC	
94	snpSB0102,	G/A,	AA,	GA,	581	snpSB0103	G/C	CC	CC	
BC$_2$F$_1$ (S35*Hakika)					BC$_2$F$_1$ (S35*Pato)					
127	snpSB0103	G/C	CC	CC	595	snpSB0049,	G/A,	GG,	AA,	
131	snpSB0102,	G/A,	AA,	GA,	601	snpSB0103	G/C	CC	CC	
138	snpSB0103	G/C	CC	CC	610	snpSB0049,	G/A,	GG,	CC	
BC$_2$F$_1$ (NACO Mtama 1)					624	snpSB0102	G/A	AA	AA	
213	snpSB0103	G/C	CC	CC		snpSB0103	G/C	GG	CC	
276	snpSB0103	G/C	CC	CC	645	snpSB0102	G/A	AA	AA	
304	snpSB0102	G/A	AA	AA	656	snpSB0103	G/C	CC	CC	
307	snpSB0102,	G/A,	AA,	AA,	659	snpSB0102	G/A	AA	AA	
319	snpSB0103	G/C	CC	CC		snpSB0103	G/C	CC	CC	
320	snpSB0103	G/C	CC	CC	663	snpSB0103	G/C	CC	CC	
BC$_2$F$_1$ (B35* Macia)					668	snpSB0103	C/T,G/C	CC	CC	
347	snpSB0103	G/C	CC	CC	671	snpSB0103	G/C	CC	CC	
348	snpSB0102,	G/A,	AA,	AA,	679	snpSB0103	G/C	CC	CC	
352	snpSB0103	G/C	CC	CC	685	snpSB0102,	G/A,	AA,	AA,	
391	snpSB0103	G/C	CC	CC		snpSB0103	G/C	CC	CC	
395	snpSB0103	G/C	CC	CC	703	snpSB0102	G/A	AA	AA	

H—Heterozygous; DP—Donor parent; NS—Number of samples; RP—Recurrent parent.

Results and discussion (cont’)

BC_2F_3 phenotyping results

- The mean grain weight per panicle across the environments was the highest (0.068 kg) in the genotype NA316C

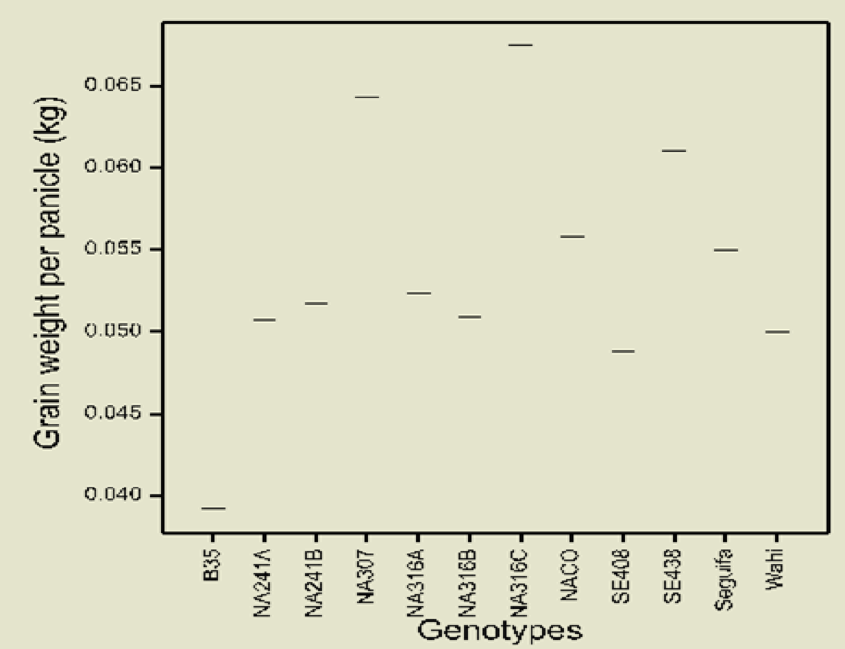


Figure 2.1. Boxplot of mean grain weight per panicle of BC_2F_3 genotypes and parents across well-watered and water-stressed environments.

- The mean STG per genotype recorded the highest (4.45) in the donor parent B35 across the environments

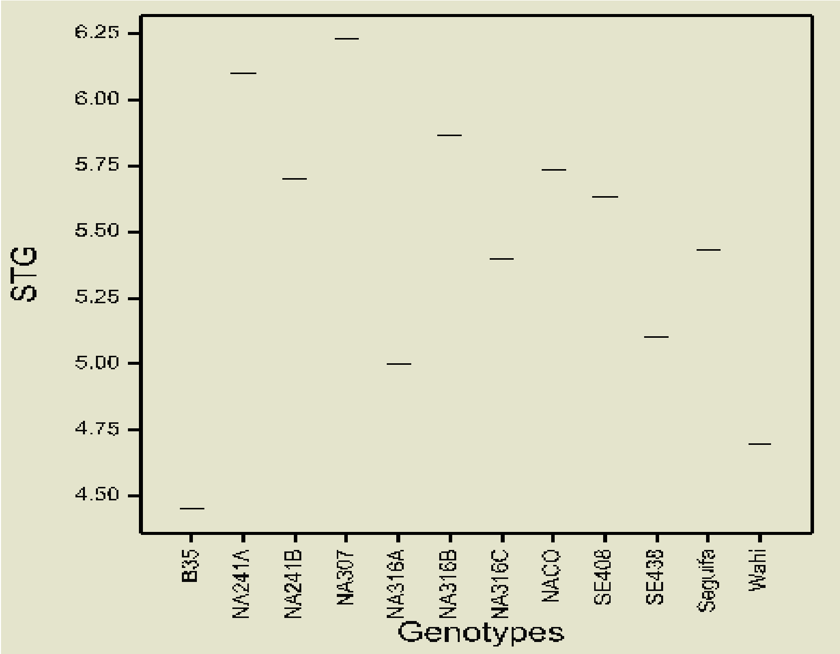


Figure 2.2. Boxplot of mean STG of BC_2F_3 genotypes and parents across well-watered and water-stressed environments (Scale: 1–9 where, 1—very low or no visible sign of susceptibility, 9—very high).

Results and discussion (cont’)

- ❖ Eight out of ten SNP markers used for genotyping of BC_2F_3 population identified favourable homozygous alleles for STGs in sorghum

Table 5. Genotypes of BC_2F_3 populations scored by favourable alleles using SNP markers

Genotype	SNP Markers	FA	No. of FA	Genotype	SNP Markers	FA	No. of FA
	BC_2F_3 (B35*NACO)				BC_2F_3 (B35*Seguifa)		
NA241A	snSB00049	GG	115	SE408	snSB00049	GG	55
	snSB00072	AA	6		snSB00089	CC	73
	snSB00089	CC	124		snSB00101	CC	73
	snSB000102	AA	123	SE438	snSB00049	GG	23
NA241B	snSB00049	GG	113		snSB00053	AA	51
	snSB00101	CC	122		snSB000102	AA	52
NA316A	snSB00049	GG	116	Seguifa (Recurrent P)	snSB00049	GG	3
	snSB00053	AA	120		snSB00053	AA	3
	snSB00101	CC	121		snSB000102	AA	3
NA316B	snSB00049	GG	70	B35 (Donor P)	snSB00042	TT	2
	snSB00053	AA	115		snSB00049	GG	2
	snSB000102	AA	115		snSB00101	CC	2
NA316C	snSB00049	GG	92		snSB000102	AA	2
	snSB00053	AA	122		snSB00103	GG	2
	snSB00072	AA	27	Wahi	snSB00049	GG	3
	snSB000102	AA	121		snSB00101	CC	2
NACO Mtama 1 (Recurrent P)	snSB00053	AA	3				
	snSB00054	GG	3				

Table 6. Favourable alleles and STG QTLs identified in BC_2F_3 populations of sorghum

SNP Markers	FA	No. of FA	QTL	Physical Location	Function
snpSB0042	T	2	STG 3A	S2_59255001	Retain STG and PCD triggering
snpSB00049	G	584	STG 3A	S2_59821923	Retain STG and N mobilisation
snpSB00053	A	707	STG 3A	S2_60059010	Retain STG and N mobilisation
snpSB00054	G	709	STG 3A	S2_60098184	Retain STG and N mobilisation
snpSB00072	A	105	STG 3A	S2_61811307	EREBPs
snpSB00089	C	729	STG 3B	S2_65453155	Retain STG
snpSB00101	C	728	STG 3B	S2_70523721	Retain STG
snpSB00102	A	727	STG 3B	S2_71360153	Retain STG

EREBPs—ethylene-responsive element binding proteins; FA—Favourable allele; No. FA—Number of favourable allele; N—Nitrogen; PCD—Programmed cell death; QTL—Quantitative trait loci, SA—salicylic acid; SNP—Single nucleotide polymorphism; STG—stay-green

Conclusions

In the present study,

- Marker-assisted selection was performed in BC_2F_1 and BC_2F_3 populations, and QTLs (STG 3A and STG 3B) associated with stay-green (STG) characteristics from the donor parent B35 **were successfully introgressed** into two farmers' preferred recurrent varieties, namely NACO Mtama 1 and Seguifa

- Three genotypes (NA307, NA316C, and SE438) **outperformed the recurrent parents** NACO Mtama 1 and Seguifa

- The SNP markers **snpSB0042**, **snpSB0049**, **snpSB0053**, **snpSB0054**, **snpSB0072** and **snpSB00089** were *associated with STG 3A QTL*

- The **snpSB0101 and snpSB0102 markers were linked with STG 3B QTL** in the BC_2F_3 populations

- The SNP markers which were linked to STG QTLs in the backcrosses in this study can be used as the reference in East Africa in the marker-assisted backcrossing for enhancing drought tolerance in sorghum

Recommendations

- Further mapping of SNP markers which are tightly linked with stay green QTLs should be done for enhancing drought tolerance in sorghum

- Sufficient backcrossing is necessary to widen the chance of transfer of the targeted trait from the donor parents to farmers' preferred recurrent parents

- The genotypes **NA307, NA316C and SE438** are recommended for multi-location evaluation before release

Acknowledgement

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