

A new breeding strategy towards introgression and characterization of stay-green QTL for drought tolerance in sorghum

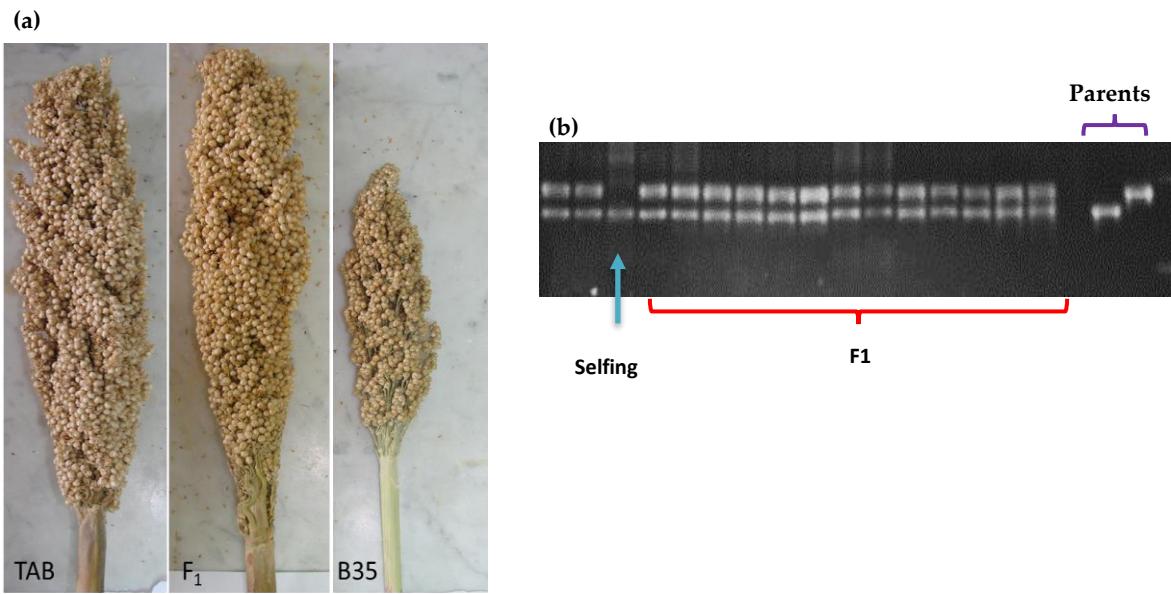
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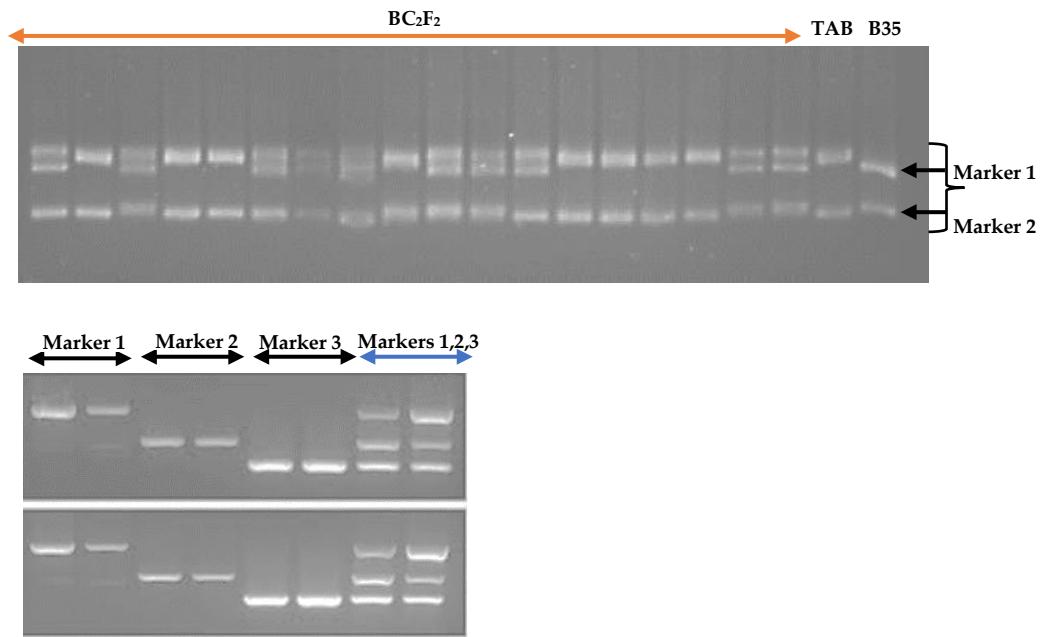
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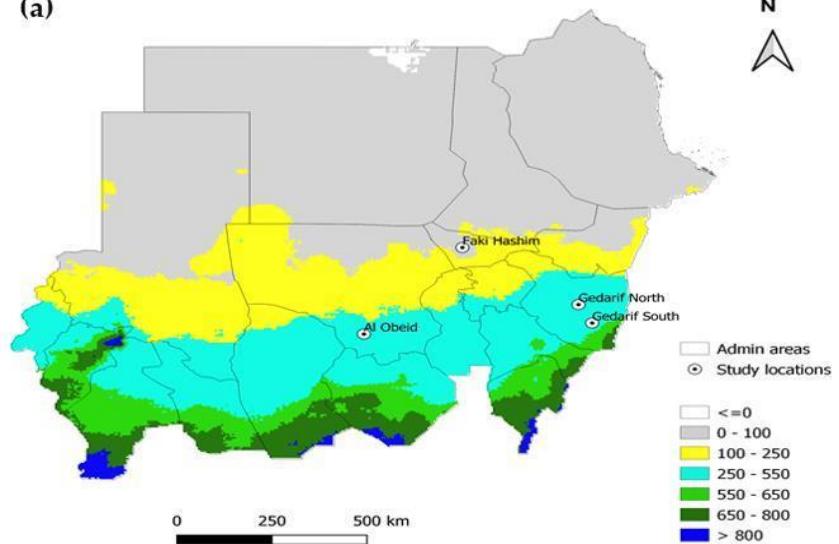


Supplementary Figure 1. Confirmation of the F_1 plants using (a) grain color in donor parent (B35) and (b) PCR fragments for the Xtxp12 SSR marker..

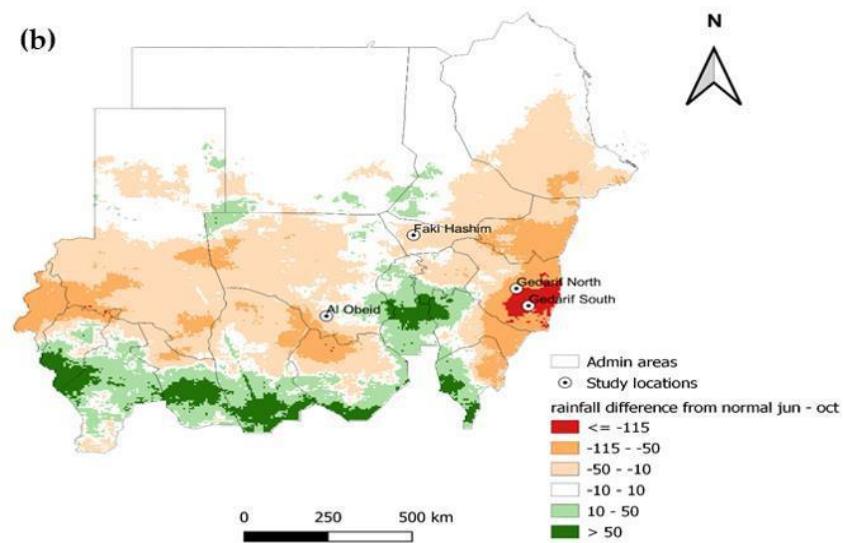


Supplementary Figure 2. Representative profiles for multiplex PCR products showing doublex of *Xtxp23* and *Xtxp15* (Marker 1 and Marker 2) (on the top) and triplex of primers *Xcup63*, *Xcup61* and *KAF1* (Marker 1, Marker 2 and Marker 3) (on the bottom).

(a)



(b)



Supplementary Figure 3. (a) Average rainfall (mm) and (b) average rainfall difference (mm) from June to the end of October in Khartoum North (Faki Hashim research farm), South and North Gedaref, and Al Obeid. (Difference from the normal means; difference of normal high rain fall from the low rain fall areas). The five environments used to test introgression lines (ILs).

Irrigated, Khartoum North (W1)



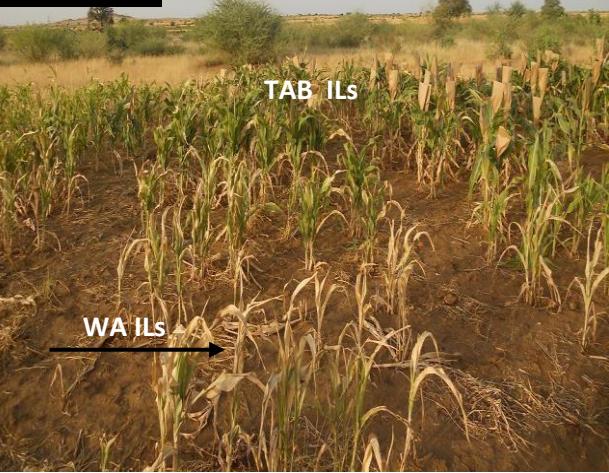
Post-flowering drought, Khartoum North (W2)



North Gedaref



Al Obeid



Al Obeid



South Gedaref



Supplementary Figure 4. The five testing environments and their variation in drought intensity and timing and the tolerance of the stay-green introgression lines.

Supplementary Table 1. SSR markers selected from the consensus map of sorghum [17] used for MAB of the stay-green trait in this study.

NO.	Markers	Stg	Forward	Reverse	LG	Product length (bp)
1	Xtxp218	Stg1 and Stg2	CCGGAAAACCTGCTACTG	ACGCCGGAAGGAGAAG	C	200
2	Xtxp114	Stg1	CGTCTTCTACCGCGTCCT	CATAATCCCACCAACAATCC	C	140
3	Xtxp285	Stg1	ATTTGATTCTTCTTGCTTGCCTTGT	TTGTCATTCCCCCTTCTTCTTT	C	231-62+291
4	Xtxp34	Stg1	TGGTCGTATCCTCTCTACAG	CATATACCTCCTCGTCGCTC	C	208
5	Xtxp38	Stg1	ACAAACCGCGACGAAGTAAC	ACAAGGCAAAGCACAAAGC	C	191
6	Xtxp231	Stg2	GGAAATCCAGGATAGGGT	AGGCAAAGGGTCATCA	C	190
7	Xtxp336	Stg2	CAGCGAGCACCGACGAC	CCACCCAACCTGACCCTCT	C	171
8	Xtxp31	Stg2	TGCGAGGCTGCCCTACTAG	TGGACGTACCTATTGGTGC	C	188
9	Xtxp228	Stg2	ACAGGTTGGCGATGTTCTCT	TTCTTTTCGAATTCACTCCTTT	C	238
10	Xtxp59	Stg2	GAAATCCACGATAGGGTAAGG	GACCCAGAACATAGAAGAGAGG	C	313
11	SbAGF08	Stg3	ATGGTCGTCTGCCAGGT	CAGTTGCTAATCTTGACCG	B	176
12	Xgap84	Stg3	CGCTCTGGGATGAATGA	TAACGGACCAACTAACAAATGATT	B	200
13	XSbAGB03	Stg3	GTGTGTGTAGCTTCTGGG	ACGTAGGAGTAGTTCTAGGATT	B	200
14	Xcup63	Stg3	GTAAAGGGCAAGGCAACAAG	GCCCTACAAATCTGCAAGC	B	160
15	Xtxp1	Stg3	TTGGCTTTGTGGAGCTG	ACCCAGCAGCACTACACTAC	B	274
16	Xtxp19	Stg3	CTTCAATCGGTTCCAGAC	CTTCCACCTCCGTACTC	B	300
17	Xtxp286	Stg3	AGCAGCAGCAGCAACAG	GCGTGGTCTTGTGGTTC	B	257
18	Xtxp56	Stg3	TGTCTCGTAGTTGCGTGTG	CCGAAGGAGTGCTTGGAC	B	270
19	KAF1	Stg4	GCTTCGGCGAGCATCTTACAA	GCGGTTGGATTGCCATG	J	270
20	Xtxp15	Stg4	CACAAACACTAGTGCCTTATC	CATAGACACCTAGGCCATC	J	296
21	Xtxp225	Stg4	TTGTTGCATGTTGGTTATAG	CAAACAAAGTTCAGAAGCTC	J	310
22	Xtxp23	Stg4	AATCAACAAGAGCGGGAAAG	TTGAGATTGCTCCACTCC	J	214
23	Xtxp299	Stg4	CTCTCCCTTGTACATCCATC	TCTTCCCCACCAGGACTTCTC	J	331
24	Xtxp65	Stg4	CACGTCGTACCAACCAA	GTAAACGAAAGGGAAATGGC	J	130

Supplementary Table 2a. Means and reduction percentage (R%) in the 46 BC₂F₄ stay green introgression lines of Tabat x B35 population, carrying one, two and three QTL, and their parents evaluated under normal (W1) and post -flowering drought (W2) at Khartoum North, Agricultural Research Corporation Research Farm.

Genotypes	GY			Chlo.GF			Chlo.M			GM			GLAM (cm ²)			
	<u>QTL</u>	<u>W.1</u>	<u>W.2</u>	<u>R%</u>	<u>W.1</u>	<u>W.2</u>	<u>R%</u>									
1 QTL		32.0	23.8	25.6	3.7	3.8	-2.2	4.6	2.5	45.0	74.0	37.0	50.0	3316.0	1481.0	55.3
2 QTL		27.0	24.5	9.3	3.8	3.9	-3.6	4.4	2.5	42.5	73.0	37.0	49.3	3058.0	1475.0	51.8
3 QTL		28.0	26.0	7.1	4.1	4.2	-2.7	4.6	3.8	17.1	75.0	43.0	42.7	3275.0	1847.0	43.6
B35		22.0	20.6	6.4	5.0	5.0	0.0	5.0	4.7	6.7	82.0	79.0	3.7	3208.0	3292.0	-2.6
TAB		56.0	15.3	72.7	4.0	3.0	25.0	4.5	1.0	77.8	60.0	8.0	86.7	3125.0	450.0	85.6
SE±		4.2		0.5			0.3			4.2			309.5			
MS (Q)		315.7		3.0388			5.2496			1669.0			1971978			
P value		*		*			**			**			**			
MS (T X Q)		402.2		0.4483			3.5826			543.7			1877767			
P value		**		ns			**			**			*			

Supplementary Table 2b. Means and reduction percentage (R%) in the 54 BC₂F₄ stay green introgression lines of Wad Ahmed x B35 population, carrying one, two and three QTL, and their parents evaluated under normal (W1) and post -flowering drought (W2) at Khartoum North, Agricultural Research Corporation Research Farm.

Genotypes		GY			Chlo.GF			Chlo.M			GM			GLAM (cm ²)		
QTL		W1	W2	R%	W1	W2	R%	W1	W2	R%	W1	W2	R%	W1	W2	R%
1 QTL		28.0	21.0	25.0	4.2	4.0	5.9	3.8	1.5	59.9	55.0	28.0	49.1	1697.0	1122.0	33.9
2 QTL		24.0	17.0	29.2	4.4	4.2	3.4	3.9	2.0	49.4	62.0	35.0	43.5	1831.0	1328.0	27.5
3 QTL		21.0	19.0	9.5	4.0	4.3	-8.3	3.5	2.2	37.1	59.0	36.0	39.0	1829.0	1540.0	15.8
B35		22.0	60.6	6.4	5.0	5.0	0.0	4.7	3.7	21.4	80.0	75.0	6.3	2627.0	2496.0	5.0
WA		47.0	16.0	66.0	4.0	3.0	25.0	3.7	0.6	84.5	36.0	8.0	77.8	1099.0	356.0	67.6
SE±		3.9			0.3			0.2892			4.7			233.4		
MS (Q)		575.9			5.03			3.7324			2405.9			3111921		
P value		**			**			**			**			**		
MS (T X Q)		286.9			1.19			1.4926			213.4			120561		
P value		*			ns			*			ns			ns		

Supplementary Table 3. ReML analysis for the scored parameters in introgression lines derived from Tabat X B35 and Wad Ahmed X B35 carrying one, two or three QTL combinations evaluated under rain fed condition at South Gadaref (SG), North Gadaref (NG) and Al Obeid (OB).

Genotypes/Locations	Tabat population					Wad Ahmed population					% Over TAB		
	SG	NG	OB	SG	OB	SG	OB	SG	NG	OB	SG	OB	
	GM	GM	GM	GY (g)	GY	GM	GM	GM	GY (g)	GY (g)	WA		
1 QTL	68.6	48.86	23.4	19.9	12.9	65.0	89.0	39.9	33.20	7.3	21.5	–	54.0
2 QTL	65.6	58.38	12.8	16.5	6.0	58.0	76.4	39.7	35.52	6.2	18.0	–	46.0
3 QTL	65.5	51.54	37.0	10.0	15.9	32.0	91.1	42.8	31.26	7.3	13.2	–	26.0
B35	76.0	55.50	51.0	27.5	21.1	75.0	93.3	76.0	55.50	51.0	27.5	21.1	64.0
WA	–	–	14.3	–	1.4	30.0	–	15.3	29.86	–	9.8	–	–
TAB	43.2	33.21	2.5	6.8	–	–	–	–	–	–	–	–	–
Wald statistic	85.64	54.69	28.11	37.41	12.02	–	–	58.74	33.85	26.61	42.05	–	–
F statistic	10.7	6.83	3.51	4.67	1.5	–	–	5.34	3.08	2.42	3.82	–	–
Wald/d.f.	127	131.4	131.3	55.6	131.4	–	–	144	145.6	89.2	144.3	–	–
F probability	<0.001	<0.001	0.001	<0.001	0.163	–	–	<0.001	<0.001	0.011	<0.001	–	–
SE±	3.2	5	8.72	0.76	7.51	–	–	4.9	6.8	7.096	6.1	–	–