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Incorporation of stay-green Quantitative Trait Loci (QTL) in elite sorghum (*Sorghum bicolor* L. Moench) variety through marker-assisted selection at early generation

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ABSTRACT

Incorporation of stay-green Quantitative Trait Loci (QTL) in elite sorghum (*Sorghum bicolor* L. Moench) variety through marker-assisted selection at early generation.

Objective: The present investigation was undertaken to develop drought tolerant varieties through introgression of stay-green QTLs in order to improve sorghum yield in semi-arid areas of Burkina Faso.

Methodology and Result: Marker-assisted selection was carried out to introgress stay-green QTLs into elite sorghum variety. A stay-green donor source, BTx642 (B35), was crossed to the elite variety (Sariaso09) to obtain F_1 progenies that were backcrossed to their recurrent parents to obtain the BC_1F_1 progenies. Seventeen flanking Simple Sequence Repeat (SSRs) polymorphic markers were used for foreground selection and 18 were used for background selection. Eighteen BC_1F_1 were heterozygous at all target loci for stay-green (stg1, stg2, stg3, stg4 and stgB), 5 progenies (S9B37, S9B43, S9B46 S9B85 and S9B13) had incorporated 3 of these QTLs. 4 plants (S9B34, S9B38, S9B73 and S9B16) incorporated double QTLs (Stg3 and StgB) and three plants (S9B44, S9B48 and S9B20) were heterozygous for Stg1.

Conclusions and applications of findings: Among BC_1F_1 generation, 30 progenies had incorporated at least one stay-green QTL. Two of the introgression lines had high levels of the recurrent parents' genomes and constitute some promising lines to develop drought tolerant varieties that will ensure sorghum production in semi-arid tropics areas and therefore, contribute to ensure food security in Burkina Faso. Despite the small number of genotypes obtained, the results showed the efficiency of Marker Assisted Back Crossing (MABC) versus the conventional backcross procedure.

Key words: sorghum, stay-green, drought, post-flowering, QTL, MABC

INTRODUCTION

Sorghum is the fifth most important cereal crop grown after wheat, maize, rice and barely (FAOSTAT, 2014). In Burkina Faso, sorghum is the main cultivated crop and constitutes the staple diet for the rural population. It is cultivated only under rain-fed conditions and over all the different climatic zones. Around 3.9 million hectares of sorghum are grown during the rainy season and about 5.6 million metric tons of sorghum grain is harvested (MASA/DGESS, 2014). In semi-arid tropics, the rainfall is very low with an erratic distribution (Zougmoré, 2003) and, therefore, crop production is regularly exposed to drought that considerably reduces yield. Drought is the main abiotic constraint to crop cultivation worldwide and the major source of yield instability and food shortage (Abdalla and Gamar, 2011). Sorghum is mainly susceptible to terminal drought stress that can reduce grain yields more than 50% (FAO, 2011). During this stage, drought causes premature leaf senescence leading to stalk lodging and stalk rot disease. It also leads to barrenness, kernel abortion or shrivelled grain, which has a negative impact on the grain yield (Prasad *et al*, 2008). However, some genotypes have the ability to cope with post-flowering drought stress due to the mechanism of stay-green. These genotypes maintain longer leaf greenness, resist precocious plant death, have normal grain filling during post-flowering drought (Tenkouano *et al*, 1993) and are recognized as stay-green genotypes. The stay-green trait has been characterized in limited number of genotypes such as BTx642 (B35), ET36-1, M35, SC56 and K19. The most common source of stay-green trait used has been BTx642. Using different stay-green sources, considerable research has been undertaken to investigate suitable molecular markers linked to post-flowering drought resistance loci, and numerous stay-green QTLs have been mapped (Subudhi *et al*., 2011; Sanchez *et al*., 2002; Harris *et al*., 2007 and Kassahun *et al*., 2009). Molecular markers linked to these QTLs are available (Harris *et al*., 2007 and Kassahun *et al*., 2009). The post-flowering drought studies identified four major stay green QTLs labelled as Stg1, Stg2, Stg3 and Stg4 which were consistently identified in a range of different environments and in different genetic backgrounds (Subudhi *et al*., 2000). Some minor QTLs for stay-green were also identified as StgA and StgB. Stg1 and Stg2 were mapped to sorghum chromosome 3, and are responsible for the phenotypic variation of about 20 and 30% (Sanchez *et al*., 2002; Harris *et al*., 2007). Stg3 and Stg4 were mapped on chromosomes 2 and 5 and explain respectively 16 and 10% of the phenotypic variation (Sanchez *et al*., 2002; Harris *et al*., 2007). After incorporation into different genetic backgrounds, a positive effect of stay-green QTLs on grain yield under drought stress has been reported (Bonnett *et al*., 2005 and Kassahun *et al*., 2009). In this study, QTLs of stay-green were introgressed into elite variety (Sariaso09) in Burkina Faso to improve its ability to cope with terminal drought through Marker-Assisted Backcross methodology.

MATERIALS AND METHODS

Plants materials : The parental lines (varieties) used in this study were BTx642 (donor parent), formerly named B35, a low yielding BC_1 derivative of *durra* sorghum germplasm accession IS 12555, post-flowering drought tolerant from Ethiopia and an elite local improved variety, Sariaso09, *guinea* race and post-flowering drought susceptible was used as recurrent parents.

Methodology

Backcrosses generation: A recurrent parent (Sariaso09) and B35 were sown in a breeding nursery at the research centre of the Institute of Environment and Agricultural Research (INERA)/Farako-Bâ in February 2014 to develop the F_1 generation. The F_1 plants were then sown along with the recurrent parent (Sariaso09) in July 2014. At flowering, the recurrent parent was hand emasculated and artificial pollinations were done with the pollen of their respective F_1 plants to obtain the BC_1F_1 generation. A total of 107 BC_1F_1 progenies were generated and genotyping was done using CERAAS genotyping platform in 2015.

Samples collection and DNA extraction: The BC_1F_1 progenies were sown and leaf samples were collected from three weeks old plants and dried in an oven at 40°C for three days. After drying leaf, samples were ground using a Geno-grinder (RETSCH) at 500 strokes per minute for 9 minutes (3 times, 3 minutes each) and then

DNA extraction was conducted according to the Mixed Alkyl Trimethyl Ammonium Bromide (MATAB) procedure described by Frost *et al*. (2007). Determination of the quality and purity of the isolated DNA were done using agarose gels (0.8%) and the working concentration of about 5ng per µl was obtained from dilution of initial DNA solution.

Polymerase chain reaction (PCR) and polyacrylamide gel electrophoresis: PCR was performed in a 10 µl reaction volume containing 25ng of genomic DNA template, 0.1mM of dNTPs, 1x buffer, 200µM of $MgCl_2$, 0.1µM of both forward and reverse primers, 0.1µM of IRdye, 0.1U of ampliTaq polymerase enzyme and double distilled water. The PCR were realized in 35 cycles using a thermal cycler (MWG AG Biotech). Thermal cycler was programmed as followed: a denaturation at 94°C for 4 min, followed by 10 cycling composed of 45s of denaturation at 94°C and annealing at TM-5°C with a reduction of 0.5°C/cycle for 1min and extension at 72°C for 1min15s. The process was repeated in 25 cycles. Each cycle included a chain of denaturation (94°C during 45s) and, annealing (at TM- 5°C for 1min) and then an extension (at 72°C for 1min15s). Finally, the reaction was ended by 5 min extension at 72°C. PCR products were subjected to electrophoresis in 6.5% polyacrylamide gels with a licor 4300 DNA Analyser system. Electrophoresis was carried out using 20 ml of polyacrylamide 6.5% polyacrylamide, 200ml 10X TBE diluted to 1L, 175 µl ammonium per sulphate (APS), and 25µl TEMED). The gel was run at 1500V and 35mA constant power supply, for 2 hours using a licor 4300 DNA analyser unit.

Data analysis: For polymorphism assessment within parents, the bands on the gel were coded as "a", "b", "c", "d" and "e" based on their allele band size (position) compared with that of the donor parent allele. For BC_1F_1 progenies, the bands were coded as "a", "b" and "h" according to their pattern compared to those of the parents. The code "a" indicated homozygous plant for allele from non stay-green parent (Sariaso09). Code "b" indicated homozygogous plant for allele from donor parent (B35) and code "h" indicated heterozygote plant with both parental allele for marker loci. The missing data were scored as "x".

RESULTS

Polymorphism assessment : The genotypes displayed a common allele at several loci. However, 35 loci out of 74 SSRs markers showed polymorphism among the parents. From 33 markers used for the screening in the QTL regions, 17 polymorphic markers were retained and from 41 markers in non-target regions of the genome, 18 polymorphic markers were retained for background selection. The list of polymorphic markers is presented in table 1 and the figure 1 illustrates the screening for polymorphism.

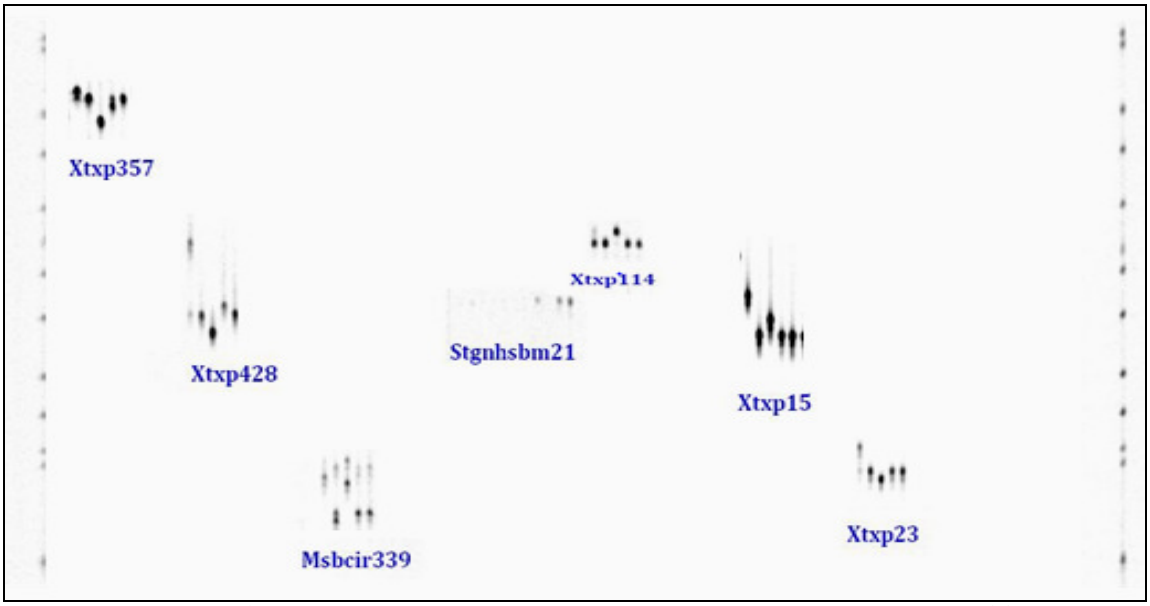

Figure 1: Illustrations of polymorphism assessment among donor and recurrent parents with 7 SSRs markers.
Legends: **Lanes 1 to 5** are B35, kapelga, Grinkan, Sariaso01 and Sariaso09 respectively.

Table 1: SSR markers (for foreground and background selection) retained after polymorphism assessment within B35 and Sarisao09

Markers for foreground selection					**Markers for background selection**				
			Alleles size					**Alleles size**	
Stay-green	**Markers**	**LG**	**B35**	**Sar09**	**Markers**	**LG**	**Position**	**B35**	**Sar09**
Stg3	*mSBCIR*238	Sb02_1	90	95	gpsb158	Sb01_1	10.41076	290	305
StgB	Xtxp72	Sb02_1	125	95	*mSBCIR*243	Sb01_1	68.2923	200	204
StgB	Xtxp55	Sb02_1	224	232	gpsb129	Sb01_1	164.6245	264	280
StgB	Xtxp428	Sb02_1	264	237	gpsb178	Sb02_1	11.88764	300	310
Stg3	*mSBCIR*339	Sb02_1	196	202	gpsb14	Sb02_1	120.0082	145	160
Stg3	Stgnhsbm36	Sb02_1	213	208	Xcup11	Sb03_1	3.445377	180	200
Stg2	*mSBCIR*222	Sb03_1	120	125	Xcup14	Sb03_2	136.17	225	230
Stg2	Sb5-236	Sb03_1	190	194	*mSBCIR*188	Sb04_1	19.02932	170	200
Stg1&2	*mSBCIR*314	Sb03_1	215	210	gpsb098	Sb04_1	111.6645	247	260
Stg1	Xtxp285	Sb03_1	268	174	gpsb136	Sb06_1	93.19133	120	130
Stg2	*mSBCIR*225	Sb03_1	142	145	Xtxp159	Sb07_1	14.36734	-	-
Stg4	Xtxp225	Sb05_1	194	184	Sbagb02	Sb07_1	95.6	113	142
Stg4	Xtxp15	Sb05_1	239	229	gpsb041	Sb08_1	5.39999	-	-
Stg4	Xtxp23	Sb05_1	204	198	gpsb123	Sb08_1	92.3587	264	304
Stg4	Xtxp14	Sb05_1	156	150	*mSBCIR*191	Sb09_1	16.437	160	174
Stg4	Xtxp123	Sb05_1	278	304	gpsb079	Sb09_1	83.78589	160	174
Stg4	gpsb32	Sb05_1	280	278	Xcup49	Sb10_1	0.535191	155	174
					Xcup43	Sb10_1	145.7377	260	270

Marker-Assisted Introgression of Stay-green QTLs: Foreground selection was focused on five consistent stay-green QTLs (Stg1, Stg2, Stg3, Stg4 and StgB) located on three linkage groups (SB02-1 on LG2, SB03-1 on LG3 and SB05-1on LG 5) and 17 SSRs flanking markers were used. Out of 107 backcross progenies, 30 had incorporated at least one QTL from the donor parent (B35). Eighteen progenies derived from Sariaso09 (S9B1, S9B40, S9B52, S9B68, S9B74,S9B88, S9B39, S9B50, S9B51, S9B7, S9B66, S9B67, S9B18, S9B65, S9B91, S9B24, S9B97 and S9B107) were heterozygous at all targeted marker loci and had incorporated the five target QTLs (Stg1, Stg2, Stg3, Stg4 and StgB). Five progenies (S9B37, S9B43, S9B46 S9B85 and S9B13) had incorporated 3 of these QTLs. Four plants (S9B34, S9B38, S9B73 and S9B16) incorporated double QTLs (Stg3 and StgB) and three plants (S9B44, S9B48 and S9B20) were heterozygous for Stg1. Examples illustrating the genotyping screening for foreground selection of backcross progenies are presented in **Tables 2 and 3** and heterozygous individuals with the donor and the recurrent parent alleles of the marker Xtxp123 are shown in the **Figure 2**.

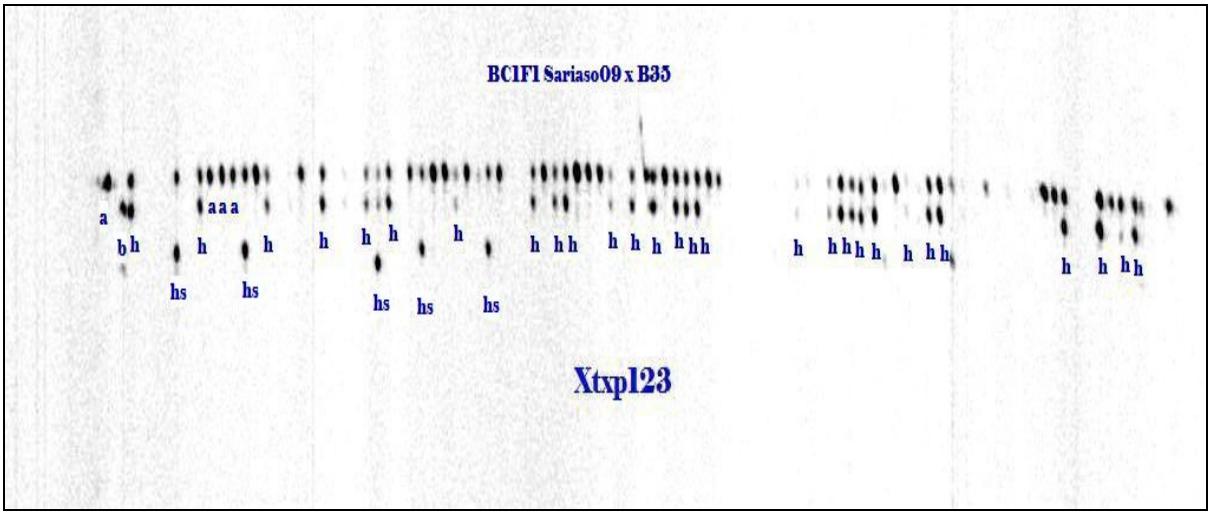

Figure 2: Output showing heterozygous individuals with the donor and the recurrent parent alleles of the marker Xtxp123. Legends: a= allele of Sariaso09, b=allele of B35, h=heterozygote, hs= off type.

Marker-Assisted background selection: Progenies with 5, 3, 2 or 1 stay-green QTLs also incorporated a part of the non-target regions of the donor parent (B35) chromosome. Background genotyping was used to determine the recovery rate of the recurrent parent genome of BC_1F_1 progenies having incorporated 5, 3, 2 or 1 stay-green QTL. Eighteen markers (two in each linkage group) were used. One marker was located at or near the one end and the other was located at or near the other end of each linkage group. Among the BC_1F_1 progenies, 28 (S9B1, S9B40, S9B52, S9B68, S9B74, S9B88, S9B39, S9B50, S9B51, S9B7, S9B66, S9B67, S9B18, S9B65, S9B91, S9B24, S9B97, S9B107, S9B37, S9B43, S9B46 S9B85, S9B13, S9B34, S9B38 S9B16, S9B44 and S9B20) descendants from Sariaso09 had a low recovery rate (below 75%). Two progenies (S9B73 and S9B48) had recovery rates between 75-83%. The results are presented in **Tables 4**.

Table 4: Background screening of BC_1F_1 progenies

Sample Name	No. of Heterozygous loci	No. of RcP loci	% of RcP loci
S9B1	9	9	50
S9B7	9	9	50
S9B16	9	9	50
S9B17	9	9	50
S9B18	9	9	50
S9B24	8	10	56
S9B34	10	8	44
S9B37	8	10	56
S9B38	8	10	56
S9B39	9	9	50
S9B40	9	9	50
S9B41	8	10	56
S9B43	7	11	61
S9B44	8	10	56
S9B46	9	9	50
S9B48	4	14	78
S9B50	7	11	61
S9B51	10	8	44
S9B52	8	10	56
S9B64	7	11	61

S9B65	**8**	**10**	**56**
S9B66	**7**	**11**	**61**
S9B67	**10**	**8**	**44**
S9B68	**11**	**7**	**38**
S9B73	**3**	**15**	**83**
S9B74	**9**	**9**	**50**
S9B85	**10**	**8**	**44**
S9B88	**10**	**8**	**44**
S9B91	**9**	**9**	**50**
S9B96	**9**	**9**	**50**
S9B97	**8**	**10**	**56**
S9B107	**7**	**11**	**61**

RcP: Recurrent parent

DISCUSSION

The allelic pattern indicated that there were at least two alleles per locus and the presence of common alleles in several loci meant that donor and recurrent parents' genomes did not exhibit polymorphism at these precise loci. However, the polymorphism revealed at 35 loci indicated dissimilarity between genotypes and exhibited the presence of genetic distance that is important for QTLs introgression. According to Langridge *et al*. (2001), useful markers in breeding should reveal polymorphism in different populations derived from a wide range of different parental genotypes. Foreground selection was conducted to identify the B35 marker allele (s) at the target loci in the heterozygous state in the progenies until the backcross procedure was completed. The number of progenies was low but the majority had incorporated the target QTLs from the donor parent. Only a few did not carry chromosomal regions with stay-green QTLs at the BC_1 generation. Ngugi *et al*. (2010) reported that 22 genotypes in BC_1F_1 were enough to capture one QTL with about 95% confidence. Results from this study revealed rates beyond the expected and beyond that obtained by Ngugi *et al*. (2010). Supposedly, all the QTLs contributing to a trait of interest (stay-green) could be taken into account (Collard *et al*., 2005), but, concerning MAS for multiple genes or QTLs, it was suggested to limit the number of genes in a selection procedure to three or four when the QTLs selected are on the basis of linked markers, and five to six if they are known select loci directly (Hospital, 2003). Ribaut and Bertrand (1999) reported that three QTLs are appropriate and feasible for MAS, although, Lecomte *et al*. (2004) used five QTLs in the improvement of fruit quality traits in tomato via marker-assisted introgression. Kassahun *et al*. (2009) used B35 as the donor parent and found backcross families that carried four putative stay-green (Stg1, Stg3, Stg4 and StgB) QTLs in the BC_2F_1 generation. Ngugi *et al*. (2010) used another donor parent (E36-1) and identified at BC_2F_1 generation, individuals with double and single introgression of stay-green QTL (Stg1 and or Stg2). In this study, emphasis is given to progenies that have the major QTLs (Stg1, Stg2 and Stg3). Previous studies have revealed that Stg1 and Stg2 incorporated in different genetic backgrounds (Subudhi *et al*., 2000) were able to explain about 20 and 30% of the phenotypic variance respectively (Sanchez *et al*., 2002; Harris *et al*., 2003). Stg1 and Stg2 interactions resulted in explanation of 49.8% phenotypic variation (Subudhi *et al*., 2000). Stg3 and Stg4 accounted for 16% and 10% of the phenotypic variation, respectively (Sanchez *et al*., 2002). The majority of BC_1F_1 progenies had a recovery rate of the recurrent parent genome below 75%. Only a few had adequately recovered the recurrent parents' genome (75-83%). Plants carrying single or double QTLs had incorporated less of the donor parent genome compared to progenies with more QTLs (five). This reduces the chance to identify plants carrying all donor alleles with high background genomes of the recurrent parent. This limits the number of progenies to be selected in the next generation. Sebolt *et al.* (2000) reported that the rate of success decreases when large numbers of QTLs are selected. According to Newbury (2000), if many QTLs are incorporated, the proportion of undesirable genes will be larger due to linkage drag. Bonnett *et al*. (2005) reported that efficient implementation of MAS involves several issues, e.g. breeding systems or schemes, population sizes, number of target loci. Their strategies include F_2 enrichment, backcrossing and inbreeding. In case of conventional selection, breeders need a larger number of individuals at each backcross generation to be sure that there are sufficient plants for background selection after the foreground and recombinant selection has been performed. This procedure requires the evaluation of the

material at each generation to select those progeny carrying the target QTLs or gene and, therefore, it is time consuming whereas MAS requires fewer generations.

CONCLUSION

The screening allowed the selection of 40 BC_1F_1 progenies from Sariaso09 with at least one QTL of stay-green. Eighteen progenies incorporated all five QTLs of stay-green (Stg1, Stg2, Stg3, Stg4 and StgB). Seven plants have incorporated two QTLs of the stay-green and 15 have single QTL for stay-green. The use of background markers allowed the identification of BC_1F_1 with high proportion (75 to 83%) of the recurrent parents' genomes. The introgression of these QTLs into local elite variety is an important step to obtain promising lines with ability to withstand terminal drought and surely to ensure food security in rural drone prone areas.

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Table 2: Genotyping data for foreground selection in population one (BC_1F_1 progenies derived from cross of Sariaso09 x B35)

Markers	Stg	Chro	Position	Sar09	B35	S9B1	S9B40	S9B52	S9B68	S9B74	S9B88	S9B39	S9B50	S9B51	S9B7	S9B66	S9B67	S9B18	S9B65
Msbcir238	**Stg3**	Sb02_1	62.01687	a	b	h	h	h	h	h	h	h	h	h	h	h	h	h	h
Xtxp72	**StgB**	Sb02_1	70.50211	a	b	h	h	h	h	h	h	h	h	h	h	h	h	h	h
Xtxp55	**StgB**	Sb02_1	71.86763	a	b	h	h	h	h	h	h	h	a	h	h	x	h	h	h
mSBCIR 339	**Stg3**	Sb02_1	117.4172	a	b	h	h	h	h	h	h	h	h	h	h	h	h	h	h
Sb5-236	**Stg2**	Sb03_1	80.19823	a	b	h	h	h	h	h	h	h	h	h	h	h	h	x	h
*mSBCIR*314	**Stg1&2**	Sb03_1	116.8419	a	b	h	h	h	h	h	h	h	h	h	h	h	h	h	h
Xtxp285	**Stg1**	Sb03_1	145.4819	a	b	h	h	h	h	h	h	h	h	a	h	h	h	h	a
Xtxp225	**Stg4**	Sb05_1	60.07417	a	b	h	h	h	h	h	h	h	h	h	h	a	a	a	a
Xtxp15	**Stg4**	Sb05_1	76.06783	a	b	h	h	h	h	h	h	h	h	h	h	h	h	h	h
Xtxp23	**Stg4**	Sb05_1	78.50456	a	b	h	h	h	h	h	h	h	h	h	x	h	h	h	h
Xtxp14	**Stg4**	Sb05_1	79.23723	a	b	h	h	h	h	h	h	x	h	h	a	h	h	h	h
Xtxp123	**Stg4**	Sb05_1	90.92021	a	b	h	h	h	h	h	h	h	h	h	h	h	h	h	h
Gpsb32	**Stg4**	Sb05_1	113.2587	a	b	h	h	h	h	h	h	h	h	h	h	h	h	h	h
Stgnshsmbm36	**Stg3**	Sb02_1		a	b	h	h	h	h	h	h	h	h	h	h	h	h	h	h
Total						**14 5QTLs**	**14 5QTLs**	**14 5QTLs**	**14 5QTLs**	**14 5QTLs**	**14 5QTLs**	**13 5QTLs**	**13 5QTLs**	**13 5QTLs**	**12 5QTLs**	**12 5QTLs**	**13 5QTLs**	**12 5QTLs**	**12 5QTLs**

Table 3 continued: Genotyping data for foreground selection in population one (BC_1F_1 progenies derived from cross of Sariaso09 x B35)

Markers		S9B91	S9B24	S9B37	S9B43	S9B46	S9B85	S9B64	S9B13	S9B34	S9B38	S9B44	S9B48	S9B73	S9B20	S9B30	S9B61	S9B90	S9B16
	Stg																		
*mSBCIR*2																			
38	**Stg3**	h	x	a	h	h	h	h	h	h	h	h	a	h	x	x	h	h	a
Xtxp72	**StgB**	h	h	a	h	h	h	a	h	h	h	a	a	h	x	a	x	a	a
Xtxp55	**StgB**	x	x	a	h	h	x	h	x	h	h	h	a	a	x	x	x	x	a
*mSBCIR*3																			
39	**Stg3**	h	h	h	h	a	h	a	a	h	h	a	a	a	a	h	a	a	a
Sb5-236	**Stg2**	h	h	h	a	a	h	h	h	a	a	a	a	h	a	x	h	h	h
*mSBCIR*3	**Stg1&**																		
14	**2**	h	h	h	a	h	h	a	h	h	a	a	h	h	x	x	x	a	h
Xtxp285	**Stg1**	h	h	h	h	a	a	a	a	h	a	a	a	x	h	h	x	h	h
Xtxp225	**Stg4**	a	x	h	h	a	a	x	a	a	h	x	a	a	x	x	x	x	a
Xtxp15	**Stg4**	h	h	h	h	h	h	h	a	a	h	h	h	h	h	h	h	a	a
Xtxp23	**Stg4**	x	x	a	h	a	x	x	x	a	a	h	h	a	x	a	x	h	a
Xtxp14	**Stg4**	h	h	h	h	h	x	h	a	a	x	x	h	x	h	x	x	a	a
Xtxp123	**Stg4**	h	h	h	a	h	h	h	h	a	a	h	h	h	h	h	h	h	a
Gpsb32	**Stg4**	h	h	h	a	h	h	h	h	a	a	a	h	h	x	x	x	a	h
Stgnshsm																			
36	**Stg3**	h	h	a	h	h	h	a	h	h	h	h	a	a	h	h	h	a	a
Total		**11**	**10**	**9**	**10**	**9**	**9**	**7**	**7**	**7**	**7**	**6**	**6**	**7**	**5**	**5**	**5**	**5**	**4**
		5QTLs	**5QTLs**	**5QTLs**	**3QTLs**	**3QTLs**	**3QTLs**	**1QTLs**	**3QTLs**	**2QTLs**	**2QTLs**	**1QTLs**	**1QTLs**	**5QTLs**	**1QTLs**	**0QTLs**	**0QTLs**	**0QTLs**	**2QTLs**