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Validating simple sequence repeat (SSR) markers for introgression of stay-green quantitative trait loci (QTLs) into elite sorghum lines

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Considerable effort has been made to improve drought-stress tolerance in sorghum by incorporating the stay-green trait into drought-susceptible elite sorghum varieties. Keeping track of the several genes involved in the expression of this complex trait during the breeding program is an enormous task. In this study, the fidelity of recently identified SSR markers were tested for introgression of stay-green QTLs into elite sorghum lines. Of the 102 SSR loci tested, seventy- eight (78) markers were found to be polymorphic between the donor lines (B35 and E36-1) and the recipient lines (Sekedo and Seredo). In total, 25 polymorphisms were detected in SSR loci flanking key stay-green quantitative trait loci (QTLs) from the B35 donor line, and 6 in E36-1. In B35, 5 SSR markers were linked to the QTL *StgA*, 6 linked to *StgB*, 3 linked to *Stg1*, 2 linked to *Stg2*, 4 linked to *Stg3* and 5 linked to *Stg4*. In contrast, only 6 polymorphic SSR markers were detected in the vicinity of key QTLs found in E36-1. Two were linked to LGA, 1 to LGJ and 3 to LGG. No markers were found linked to QTL LGD and LGH. Similar SSR polymorphisms were observed for markers needed to recover the recurrent parent genomes (RPG) in the subsequent backcross generations. These findings reveal the limitations of using E36-1 as a donor parent in marker-assisted selection (MAS) programmes for improvement of drought tolerance. Low hybridization efficiency (22.5%) was achieved using the anther dehiscence method. Such low hybridization efficiency requires use of molecular markers to easily identify plants harbouring the required genotypes.

Key words: Stay-green, drought tolerance, quantitative trait loci (QTL), simple sequence repeat (SSR) markers, polymorphism.

INTRODUCTION

Sorghum (*Sorghum bicolor* (L) Moench) is the fifth most important cereal crop grown worldwide (FAO and ICRISAT, 1996). In Uganda, it is ranked third in importance, following maize and finger millet (Oryokot

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and Ebyau, 1996). As such, sorghum plays an important role in food security and income of many rural households. This is mainly attributed to its ability to tolerate drought (Esele, 1988). Elsewhere, sorghum is mostly cultivated in the drier areas of the Eastern, Northern and South-western regions of Uganda, where it occupies more than 80% of the total area under cultivation (Ebiyau and Oryokot, 1996). These are dry and hot low land areas characterized by short rainy seasons with low and erratic rainfall. Despite the role played by sorghum in these areas, its production has continued to fluctuate, due mainly to abiotic and biotic stresses. Among the abiotic factors, drought is the most important single factor limiting the crop's productivity, growth and survival (Bohnert and Jensen, 1996; Rosenow et al., 1996). The negative impact on yield of post-flowering drought stress is of considerable importance to sorghum producers, especially when it occurs during the later stages of grain filling (Rosenow et al., 1996). When affected by post-flowering drought stress, sorghum plants exhibit premature death of stalk and leaves, stalk collapse and lodging, and reduced seed weight. Tolerance to drought is indicated when plants remain green and fill the grain normally (Rosenow et al., 1996; Sabadin et al., 2012).

The phenotype associated with post-flowering drought tolerance in sorghum is called stay-green. Under terminal drought conditions, plants with the stay-green trait have the ability to retain more chlorophyll in their leaves and thus to carry out photosynthesis longer than the senescent types (Harris et al., 2007). Such plants are able to remain relatively alive, continue to fill grains normally, and have increased resistance to lodging (Subudhi et al., 2000). Stay-green has been noted as the most desirable characteristic for production of sorghum under conditions of limited water. However, since it is an enormous task to keep track of the several genes involved in the expression of stay-green, molecular markers linked to these genes can be used to locate and follow the numerous interacting genes that determine such complex traits, including drought tolerance.

Considerable effort has been made to improve tolerance to drought stress in sorghum by incorporating the stay-green trait into drought-susceptible elite sorghum varieties. Several studies have reported the development of near-isogenic lines (NILs) (Harris et al., 2007) and recombinant inbred lines (RILs) (Subudhi et al., 2000) using B35 and E36-1 (Haussmann et al., 2002; Bhattramakki et al., 2000; Rajkumar et al., 2013; Reddy et al., 2014) as stay-green donor lines. Quantitative trait loci (QTL) studies for the stay-green trait resulted in identification of several genomic regions associated with resistance to post-flowering drought stress (Haussmann et al., 2002; Bhattramakki et al., 2000; Rajkumar et al., 2013; Reddy et al., 2014). Research efforts are now being focused on the fine mapping and transfer of stay-green QTL to non-stay-green elite sorghum lines through marker-assisted breeding (Rajkumar et al., 2013; Reddy et al., 2014; Subudhi et al., 2000). However, the use/usefulness of these markers in sorghum breeding is still limited because of (1) the lack of a highly-saturated sorghum map with uniformly distributed markers across the genome, (2) the lack of a common nomenclature for the sorghum chromosome, which makes it difficult and cumbersome to compare and use results obtained by different groups (Kim et al., 2005), and (3) the lack of universally valid QTL marker associations applicable over different sets of breeding materials (Babu et al., 2004). Recently, however, 220 additional sequence-tagged sites (STS or SSRs) have been added to the sorghum genetic map, greatly enhancing the potential utilization of marker-assisted selection (MAS) in sorghum improvement (Madhusudhana et al., 2012; Wu and Haung, 2006). The fidelity of these SSR markers needs to be tested in populations targeted for improvement before their deployment.

In this study, the utility of donor lines, B35 and E36-1 as sources of stay-green for introgression of stay-green QTL into elite sorghum in Uganda was tested. This included their amenability to MAS. An attempt was also made to determine the relative efficiency of hand pollination in sorghum using control of the anther dehiscence method.

MATERIALS AND METHODS

Four sorghum lines were used for this study. Two of these lines, B35 and E36-1, were obtained from ICRISAT. These are popular donors lines frequently used for research (Tao et al., 2000; Subudhi et al., 2000; Xu et al., 2000a,b) and for the development of commercial hybrids (Henzell et al., 2001). The other two lines Sekedo and Seredo are the locally adapted lines developed by the NaSARRI sorghum breeding program. Line B35 is a BC1 derivative of IS12555 dura sorghum from Ethiopia (Harris et al., 2007) and shows distinct responses to drought at both pre- and post-flowering stages (Rosenow et al., 1996), being highly susceptible to pre-flowering drought stress though highly resistant to post-flowering drought (stay-green trait), with a relatively low yield. In contrast, line E36-1 is a high-yielding breeding line assigned to the *Guinea caudatum* hybrid race of Ethiopian origin (Haussmann et al., 2002). Sekedo and Seredo are locally adapted lines released by the NaSARRI sorghum program in 1995 and 1988, respectively.

Utility of stay-green donor parents B35 and E36-1

The four sorghum lines were assessed for their agronomic performance at the National Semi-Arid Resources Research Institute (NaSARRI). Phenotypic data on plant height, days to 50% flowering, leaf senescence and grain weight was recorded on an average of 10 plants each. Data for plant height and grain weight were measured at maturity. Plant height was obtained by measuring the distance from the ground to the tip of the panicle when the panicle had fully emerged from the flag leaf. Grain weight was measured on 1,000 seeds. Leaf senescence was scored on a 1 to 5 scale, based on ICRISAT's descriptors list for *S. bicolor* (IBPGR and ICRISAT, 1993). Data were subjected to analysis of variance using GenStat. Where significant differences were observed, means were compared using Fishers protected least significant difference test.

Identification and development of molecular markers for foreground and background selection

Population development for marker assisted selection

The four parental lines were crossed in a 2 × 2 factorial design to generate the F1 population, using B35 and E36-1 as males and Sekedo and Seredo as females. "Control of anther dehiscence" method was used to control pollination. To prevent pollen contamination, the panicles of the male parents were bagged with paper bags immediately after emergence from the flag leaf (blooming stage).

Molecular analysis of SSR markers for foreground and background selection

Genomic DNA extraction

Genomic DNA was extracted from leaf tissues using a modified cetyltri-methylammonium bromide (CTAB) protocol described by Saghai-Maroof et al. (1984). The DNA was purified using a phenol:chloroform:isoamyl (25:24:1v/v) mixture. Cold isopropanol previously stored at -20°C was used to precipitate the DNA, which was decanted off leaving clear pellets. The recovered pellets were then washed twice with 70% ethanol, air dried before re-suspending them in TE buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA) and stored at -20°C.

PCR amplification and capillary electrophoresis

The PCR conditions for each primer were optimised and each PCR reaction was set up in a 10 ul reaction volume in 96-well PCR plates. Each PCR reaction contained 2 µM of the primer, 2 mM $MgCl_2$, 2 mM dNTP, 0.2 U Amplitaq gold polymerase and 1 × Mg^{2+} free PCR buffer (Applied Biosystems, Foster City CA, USA). For capillary electrophoresis, the forward primers were labelled with FAM, PET, NED or VIC (Applied Biosystems) to allow post-PCR pooling of the primers into groups of three primer products, each with a primer in a given group being labelled with a different dye. Temperature cycling was carried out using the Gene-amp PCR system 9700 (Applied Biosystems) and a touch-down PCR amplification: one 15 min denaturation cycle at 94°C followed by the first six cycles of 94°C for 30 s, 61°C for 1 min (ramp of 1°C per cycle) and 72°C for 2 min, then by 29 cycles of 94°C for 30 s, 55°C for 1 min and 72°C for 2 min. After the completion of the 35 cycles, a final extension of 20 min at 72°C was included. The PCR products were pooled post-PCR, where 1.0 µl of FAM labelled product, 1.0 µl of PET labelled product and 1.5 µl of NED or VIC labelled products were mixed with 7 µl of Formamide to make up to 10 µl reaction volume. The 10 µl reaction volume was then used for capillary electrophoresis. Formamide was made by mixing 1 ml of HIDI and 12 µl of Genescan^{TM}-500LIZ size standard (Applied Biosystems).

PCR conditions were optimized for 102 SSR primer pairs, and their ability to detect polymorphism was assessed by capillary electrophoresis PCR products of four parental lines of sorghum. The DNA fragments were denatured and size-fractioned using capillary electrophoresis on an ABI 3730 automatic DNA sequencer (Applied Biosystems). Data were generated using Gene-Mapper version 3.7 software (Applied Biosystems).

Selection of SSR markers for foreground and background selection

A total of 102 SSR markers were surveyed for polymorphism among the elite parents Sekedo and Seredo and the stay-green donor parents, B35 and E36-1. Forty-five (45) of these were foreground markers that have been mapped to the vicinity of stay-green QTLs identified in the genomes of B35 and E36-1. The other fifty-seven (57) SSRs were markers that can be used for background selection during the introgression process. All the markers chosen were assigned to various linkage groups described by Bhattramakki et al. (2000) and Haussamann et al. (2002). Additionally, linkage groups and/or chromosome locations of these SSRs are as described by ICRISAT (*unpublished data*). Four stable stay-green QTL, namely, *Stg1, Stg2*, *Stg3* and *Stg4* were targeted for transfer from the stay-green donor parent B35 into the genetic background of senescent recurrent parents, Sekedo and Seredo. Similarly, the stay-green QTL LG A, LG J and LG G on chromosomes SB1-01, SBI-10 and SBI-07 (respectively) of the donor source E36-1 were also targeted for transfer into the genetic backgrounds of Sekedo and Seredo. These QTL were chosen because they were reported to have a significant contribution to the stay-green drought tolerance in sorghum (Haussmann et al., 2002).

Identification of F1 heterozygote (foreground selection at F1)

Marker segregation was tested by analysing the F_1 hybrids. To do this, 80 individual F_1 plants developed from crosses between stay-green donors B35xSekedo, B35xSeredo, E36-1xSekedo and E36-1xSeredo were screened with six foreground markers each, corresponding to stay-green QTLs from each of the donor parents (B35 and E36-1). A sample of twenty individual F_1 plants was used to represent each genotype.

RESULTS AND DISCUSSION

Utility of stay-green donor parents B35 and E36-1

The results revealed significant differences (at $P \leq 0.05$) for plant height, percentage senescence and grain yield (Table 1). All 4 parental lines attained 50% flowering at the same time. The plant height of the lines ranged from 83.3 to 137.3 cm, with an average of 118 cm. The 1000 seed weight of the lines ranged from 15.4 to 28.4 g, with an average of 22.2 g. The stay-green score ranged from 2.0 to 4.0, with an average of 2.7 (on a 1 to 5 scale, where, 1 = most green and 5 = least green) (Table 1). In general, reduced senescence rate and higher seed weights were observed in the two donor sources, namely B35 and E36-1, while average flowering time was similar in all the lines evaluated. Line B35 exhibited dwarfism tendencies under the conditions at NaSARRI. The growth habits of the stay-green donor E-36-1 were similar to that of the local cultivars used in this study, except for leaf senescence scores. In contrast, the local cultivars differed from line B35 in plant height as well as leaf senescence. The greatest differences were observed between B35 and Seredo. These two lines differed in all traits measured except grain weight per 1000 seed. It is therefore suggested that the best gain for improvement in terminal drought tolerance would be expected for programs where Seredo is used as the recipient parent. However, the combining abilities of the parental lines need to be determined before they can be fully exploited for MAS.

Table 1. Agronomic characteristics of sorghum lines used in the introgression of quantitative trait loci (QTLs) for the stay-green trait.

Parental line	Agronomic scores			
	Height (cm)	D50%F	Senescence rating	1000 seed wt (g)
B35	83.2	72.00	2.00	25.20
E36-1	137.30	72.00	2.00	28.40
Sekedo	126.00	69.00	3.00	15.40
Seredo	127.80	67.00	4.00	20.10
LSD (0.05)	13.93	4.50	1.20	5.90
%CV	3.10	1.30	9.60	7.90

D50%F = days to 50% flowering, Senescence assessed on scale of 1 to 5 based on ICRISAT's descriptors list for *sorghum bicolor (*IBPGR and ICRISAT, 1993). Data represent the mean of 4 replications.

Identification of SSR markers for foreground and background selection

Capillary electrophoresis revealed varied levels of polymorphism among the parental materials used (Figures 1 and 2). Seventy-eight (78) of the 102 SSR markers surveyed exhibited varied levels of polymorphism ranging from highly polymorphic to highly monomorphic. This represents 76.5% of the total number of SSR loci surveyed. Twenty-three (23) SSR markers were found to be monomorphic and were excluded from further analysis. In the present case, only one marker, Xgap206, did not amplify.

Molecular markers for foreground selection

Twenty-nine (29) markers were found appropriate for use in foreground screening (Table 2). Of these, 25 were found to be polymorphic between B35 and Sekedo, and between B35 and Seredo. In contrast, only six SSR loci were polymorphic between E36-1 and Sekedo, and between E36-1 and Seredo (Table 3). Flanking markers (that is, 2 pairs of markers) for each of the 4 stable stay-green QTLs, namely *Stg1, Stg2*, *Stg3* and *Stg4*, were easily identifiable for B35 genomic background (Table 2). In contrast, only one marker, in most cases, was identified for the corresponding QTLs LG A, LG J and LG G on the E36-1 background (Table 3). The flanking markers around these QTL would enable foreground selection for the target QTL and ensure that the region being transferred contains the QTL of interest. These QTL were also targeted because they were reported to have significant contribution to the stay-green drought tolerance in sorghum (Harris et al., 2007). Thus, more markers need to be placed around the E36-1 QTL to ensure that the region being transferred contains the donor QTL of interest. Flanking markers between/around/surrounding the target alleles are necessary to remove linkage drag, as the optimal distance between the target gene and the flanking markers governs the selection intensity that can be exerted. To reduce linkage drag, flanking markers should be chosen as closely linked to the target locus as possible (Hospital, 2001). Theoretically, all markers that are tightly linked to QTL should be used for marker-assisted selection (MAS). However, due to the cost of using several QTL, only markers that are tightly linked to not more than three QTL are typically used (Ribaut and Betran, 1999). As mentioned above, four stable QTL were targeted for introgression in this study. Although, up to five QTL have been introgressed into tomato and other crops using MAS (Lecomte et al., 2004; Kassa et al. 2010).

Molecular markers for background selection

Fifty-seven (57) primer pairs were found to be appropriate for background screening of recurrent parent genomes (RPG) in progeny of crosses. Forty-one (41) of the loci were polymorphic between B35 and Sekedo, and between B35 and Seredo. While, fifteen (15) SSR loci were found to be cross-polymorphic between donors B35 and E36-1, 23 SSR loci were polymorphic in E36-1 regardless of the recurrent parent used (that is, Sekedo or Seredo). Almost all the background markers used for E36-1 had been previously found appropriate for selection of key QTL in the B35 background. Details of allele sizes for each of the four parents, number of markers screened per linkage group and a list of those that were polymorphic (as revealed by capillary electrophoresis) are summarized in Tables 4 and 5. True F_1 hybrids were easily identifiable by using the identified markers (Table 6). However, the number observed per cross was low and varied with the donor line used. Out of a total of 80 F_1 individuals only 18 were identified as true F_1 hybrids, representing 22.5%. Higher numbers of true F_1 hybrids 5 and 9 were obtained in crosses where B35 was used as donor parent. This represents 25% being true F_1 hybrids from crosses between B35xSeredo, and 45% from B35xSekedo. In contrast, with E36-1 donor parent, only two individuals per cross were identified as

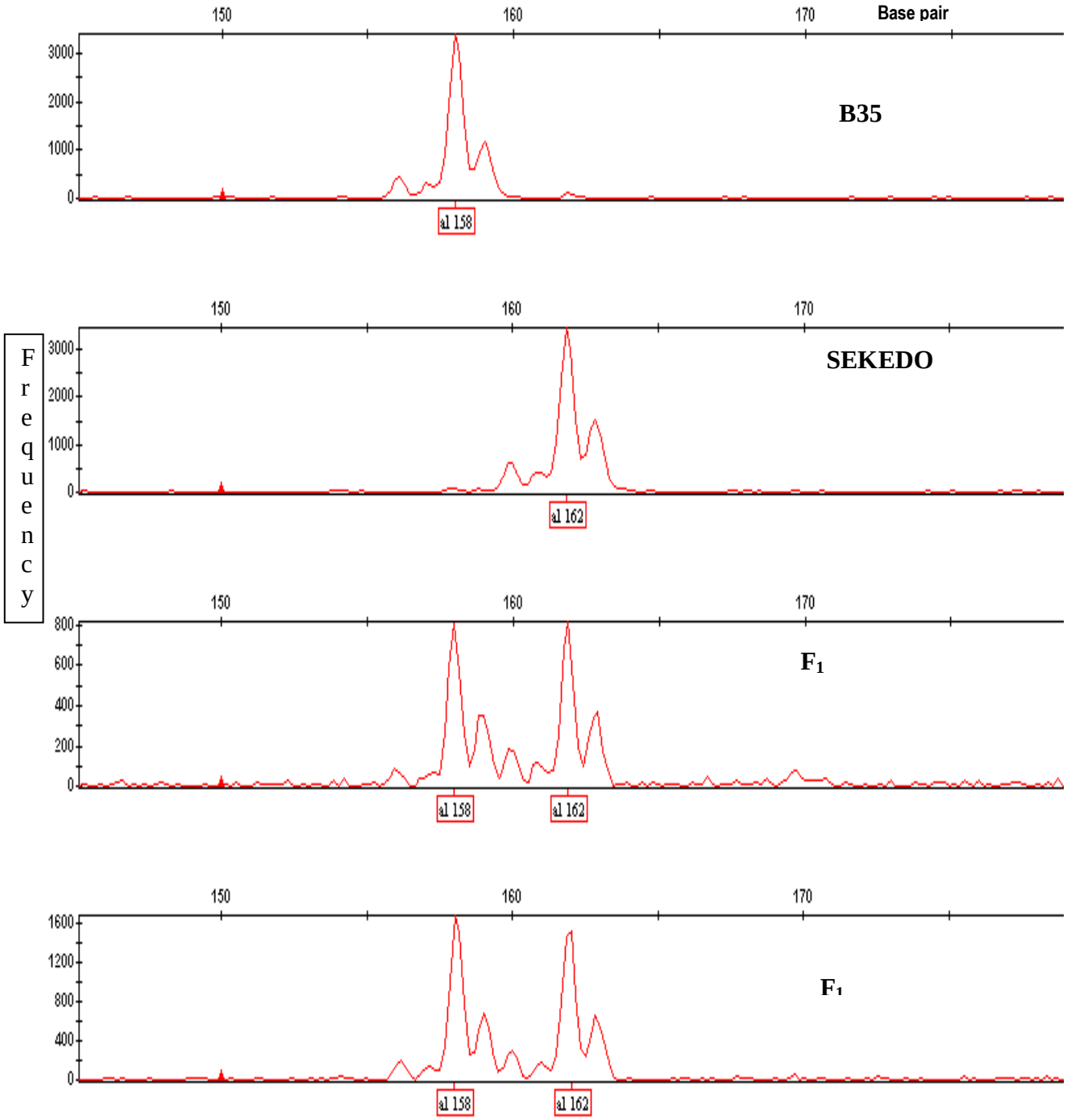

Figure 1. Typical allele sizes of PCR fragments generated from parental lines Sekedo and B35 and their F1 derivatives, analyzed using an ABI 3730 sequencer. (a) B35, Sekedo and F1 progeny of Sekedo x B35. (b) E36-1, Seredo and F1 progeny of Seredo x E36-1.

true F1 hybrids, representing 10%. Those 18 individuals have now been targeted for further backcrossing to the recurrent parent to generate the BC_1F_1 population. Low genetic variability between E36-1 and the recurrent parents used, as revealed by molecular data, may explain the low rates of recovering true F_1 hybrids from these crosses. However, it may also be due to difficulties in synchronization in anthesis during pollination of the cultivars. In most cases, the proportion of the F_1 progeny will vary based on the specific genotype used as female, the fecundity of the pollen parent, and environmental conditions (Siles et al., 2001). In general, the low numbers of

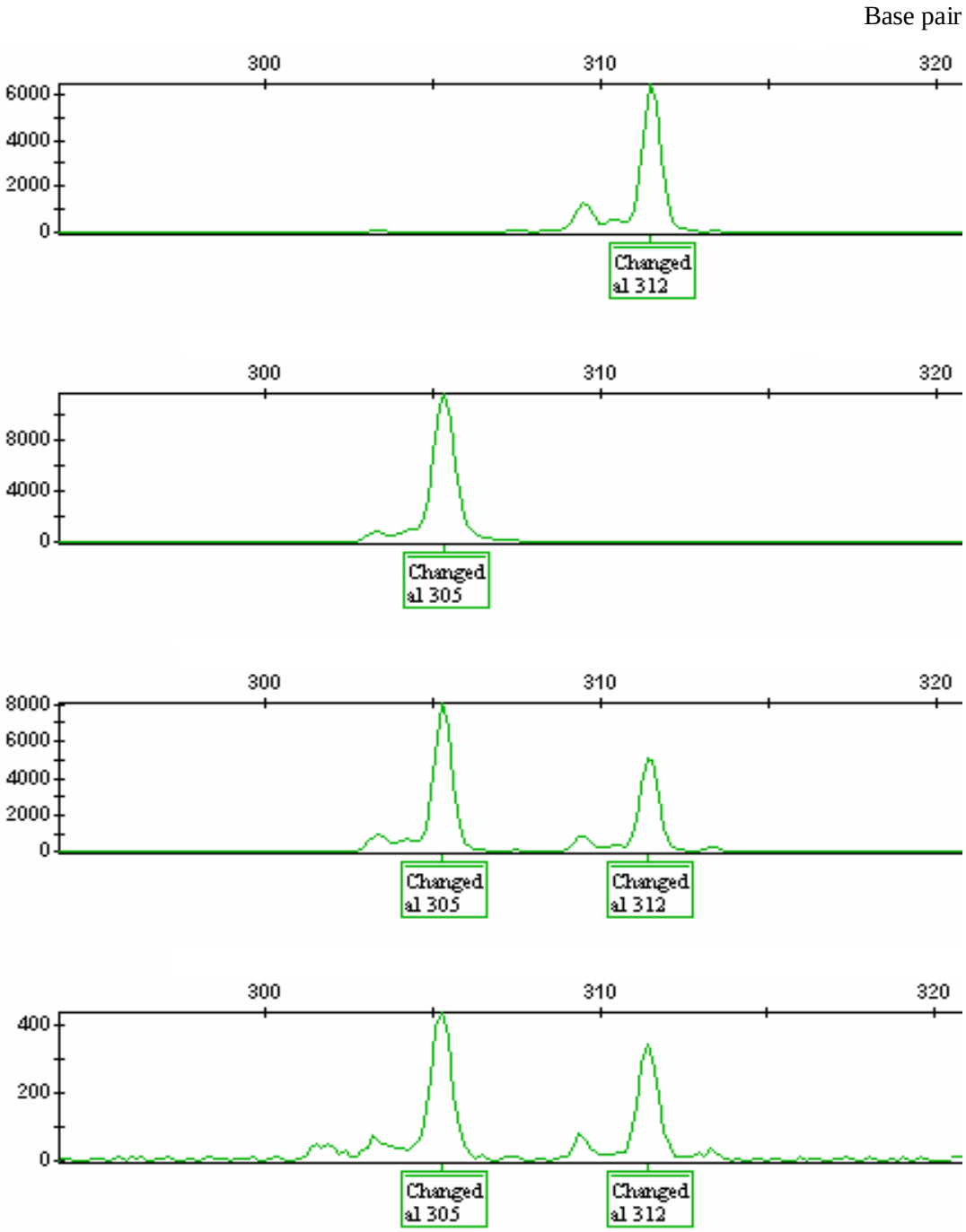

Figure 2. Typical allele sizes of PCR fragments generated from parental lines Seredo and E36-1 and their F1 derivatives, analyzed using an ABI 3730 sequencer.

F_1 hybrids observed in all crosses with E36-1 as male could be attributed to the lack of perfect synchronisation in anthesis period of the cultivars and probably also to the fecundity of the pollen parent.

In addition to flowering time, factors that make it difficult to outcross easily could be physiological and/or structural, factors associated with this particular genotype, such as floral morphology. Despite the observed variation associated with pollen availability and or synchronization in anthesis, an average of about 22.5% true F_1 hybrids in sorghum suggests that the hybridisation technique was efficient. The percentage of true hybrid plants identified among the 20 F_1 individuals from each of the 4 cross-combinations (Table 6) suggests that self-fertilisation

Table 2. Target QTL, associated SSR loci and observed allele size of parents with B35 donor.

QTL	Chromosome	Marker	Allele sizes in parents		
			B35	**Sekedo**	**Seredo**
StgA	SBI-01	Xtxp032	148	150	150
		Xtxp088	121	113	113
		Xtxp149	191	187	187
		Xcup024	191	238	238
		Xtxp357	230	236	236
StgB	SBI-02	Xtxp008	181	192	192
		Xtxp100	122	126	126
		Xtxp296	186	189	189
		Xcup040	214	212	212
		Xisep733	341	350	350
		Xisep841	227	221	221
Stg1	SBI_03	Xtxp285	210	207	*
		Xtxp034	122	114	114
		Xtxp114	251	254	254
Stg2	SBI_03	Xtxp336	257	242	242
		Xtxp031	252	230	228
Stg3	SBI-02	Xtxp298	216	202	216
		Xtxp001**	200	234	200
		Xgap84=Sb6-84	227	201	201
		Xisep938	214	225	225
Stg4	SBI-05	Xisep257	257	304	304
		Xtxp015**	240	228	240
		Xtxp014	156	160	160
		Xtxp225	163	165	165
		Xtxp023	202	222	*

*Sample did not amplify, ** marker monomorphic in B35 and Seredo.

Table 3. Target QTL, associated SSR loci and observed allele size of parents with E36-1 donor.

QTL	Chromosome	Marker	Allele sizes of parents		
			E36-1	**Sekedo**	**Seredo**
LG A	SBI-01	Xtxp357**	251	236	236
		Xcup024**	**236**	**238**	**238**
LG J	SBI-10	Xgap001	258	271	271
LG G	SBI-07	Xtxp312	215	139	139
		Xisep328*	147	147	217
		Xgap342=Sb-342	285	287	287

*Marker monomorphic between E36-1 and Sekedo, **markers cross-polymorphic for B35 and E36-1. Bold also segregates with *StgA*.

Table 4. Linkage group associated SSR loci for background selection and observed allele size in B35 donor line.

Linkage group	Marker	Allele sizes of parents		
		B35	Sekedo	Seredo
LGA	Xcup53	173	195	195
	Xcup62	189	186	186
	Xcup73a	214	205	205
	Xtxp208a	256	260	260
	Xtxp302**a	181	200	181
	Xtxp320a	293	279	279
LGB	Gpsb014a	253	262	262
	Xtxp286	215	192	192
	Xtxp304	275	291	293
	mSBCIR223	124	132	132
	Xtxp050	303	293	293
LGC	Xcup32	154	142	142
	Xtxp033a	150	250	250
LGD	Xtxp012	204	194	194
	Xtxp021	189	194	194
LGE	Xtxp040	131	135	135
	Xtxp057	247	249	249
	Xtxp278	210	190	190
	Xtxp295a	169	178	*
	mSBCIR300	128	122	122
	sbAGB02	135	115	115
LGF	Xtxp10a	133	141	139
	Xtxp289a	271	290	262
LGG	Xcup07a	191	267	267
	sbAGA01a	104	102	102
	mSBCIR262	235	233	233
	mSBCIR283a	202	184	199
LGH	Xtxp273**	172	200	172
	Xtxp321	202	208	210
	mSBCIR240	202	184	199
LGI	Xgap072	211	209	209
	Xtxp145	222	215	215
	Gpsb069a	218	182	182
LGJ	Xcup57	189	199	199
	Xtxp265	144	*	164
	mSBCIR248a	190	213	213
	Xtxp299	146	149	149
Unknown	Xcup37b	202	206	206
	Xcup64b	183	212	212
	mSBCIR238b	89	106	106
	mSBCIR286b	140	130	130

[a]Markers cross-polymorphic for B35 and E36-1, *missing sample, **marker monomorphic between B35 and Seredo, [b]marker with unknown linkage group.

Table 5. Linkage group associated SSR loci for background selection and observed allele size in E36-1 donor line.

Linkage group	Marker	Allele sizes of parents		
		E36-1	SEKEDO	SEREDO
LGA	Xcup73	202	205	205
	Xtxp302**	200	200	181
	Xtxp320	283	279	279
	Xtxp208	256	260	260
LGB	gpsb014	264	262	262
	Xcup26	219	225	225
	Xtxp207	206	196	196
	Xtxp050	295	293	293
	Xtxp304	263	291	293
	Xtxp001**	234	234	200
	Xtxp007	249	239	239
	Xisep841	233	221	221
	Xtxp298	219	202	216
LGC	Xtxp031***	228	230	228
	Xtxp033	246	250	250
	Xtxp285	190	207	*
	Xcup14	204	206	206
LGD	-			
LGE	sbAGB02	119	115	115
	Xtxp295	169	178	*
	Xtxp225	163	165	165
LGF	Xcup02	194	191	191
	Xtxp10	147	141	139
	Xtxp289	294	290	262
LGG	sbAGA01	93	102	102
	Xcup07	270	267	267
	mSBCIR283**	184	184	199
LGH	-			
LGI	gpsb069	206	182	182
	Xtxp057	247	249	249
	Xtxp065	131	133	133
	mSBCIR329	131	129	129
	Xtxp299	159	149	149
	Xtxp057	247	249	249
LGJ	gpsb123	311	305	305
	Xtxp273***	172	200	172
	Xtxp321	204	208	210
	mSBCIR240	202	184	199
	Xtxp015	224	228	240
	mSBCIR248	210	213	213
Unknown	mSBCIR286b	126	130	130

Table 6. Recovery of true F_1 hybrids from 4 populations involving crosses between 2 stay-green donors (B35 and E36-1) and 2 local sorghum cultivars (Sekedo and Seredo).

Female	Parent		True hybrids	
	Male	Total plants	Number	%
Sekedo	B35	20	9	45
Sekedo	E36-1	20	2	10
Seredo	B35	20	5	25
Seredo	E36-1	20	2	10
	Total	80	18	22.5

does occur and varies between 55 and 95%.

Conclusion

Lines B35 and Seredo were observed to have the greatest agronomic contrasts. These two lines differed in all the other traits measured except grain weight per 1000 seed. It is therefore suggested that the best gain for improvement in terminal drought tolerance would be expected for programs where Seredo is used as the recipient parent and B35 as the donor. However, the combining ability of these lines needs to be determined before they can be fully exploited for drought tolerance. More polymorphic markers flanking key stay-green QTL were observed in crosses involving the donor source B35 (25 in total) than in E36-1 (6 in total). In B35, 5 SSR markers were linked to the QTL StgA, 6 linked to StgB, 3 linked to Stg1, 2 linked to Stg2, 4 linked to Stg3 and 5 linked to Stg4. In contrast, only 6 polymorphic SSR markers were detected in the vicinity of key QTL found in E36-1. Two were linked to LGA, 1 linked to LGJ and 3 linked to LGG. No markers were found linked to QTL LGD or LGH. Similar SSR polymorphisms were observed for markers needed to recover the recurrent parent genomes (RPG) in the subsequent backcross generations. These findings reveal the limitations of using E36-1 as a donor parent in MAS programs for improvement of drought tolerance.

Additional SSR markers will need to be placed on the genetic map of E36-1 to enhance its utility. Alternatively, the use of other marker systems, such as single nucleotide polymorphisms (SNPs) that can lead to the development of ultra-high density maps should be evaluated. For reasons yet to be determined, low hybridisation efficiency was achieved using the anther dehiscence method. Cross pollination was estimated at 22.5%. Since sorghum is a self-fertilising plant many self-escapes can be expected with any artificial pollination method employed. The anther dehiscence method is time-saving and appropriate when large experimental fields under replicated trials are needed. However, at such a low level of efficiency, verification with molecular markers is crucial to easily identify plants harbouring the required genotypes. Two other methods, namely hand emasculation and hot water for emasculation, are known to be effective for artificial hybridisation. It is important to perform a comparative analysis of these pollination techniques to better determine the most efficient and economical method to use in sorghum breeding.

However, the preliminary results obtained here show that when markers are available, the anther dehiscence method is easy to use.

This study has identified SSR markers that will ease the transfer of important stay-green QTL to sorghum cultivars of interest. An F_1 hybrid population has been developed, and 18 lines confirmed to contain the appropriate QTL. Subsequent studies should focus on evaluating the effect of the introgressed stay-green QTL. This can be achieved by evaluating the BC_2 or BC_3 generations. The resulting BC_2/BC_3 progeny will then have to be evaluated for the expression of this trait under drought stress.

Conflict of interests

The author(s) did not declare any conflict of interest.

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Abbreviations

RPG, Recurrent parent genomes; **NILs,** near-isogenic lines; **RILs,** recombinant inbred lines; **QTL,** quantitative trait loci; **SNPs,** single nucleotide polymorphisms; **CTAB,** cetyltrimethylammonium bromide.

REFERENCES

Babu R, Nair SK, Prasanna BM, Gupta HS (2004). Integrating marker-

assisted selection in crop breeding: - Prospects and challenges. Curr. Sci. 87:607-619.
Bhattramakki D, Dong J, Chhabra AK, Hart GE (2000). An integrated RLPF and SSR linkage map of sorghum bicolor (L) Moench. Genome 43:988-1002.
Bohnert HJ, Jensen RG (1996). Strategies for engineering water- stress tolerance in plants. Trends Biotechnol. 14:89-97.
Esele JP (1988). Current status of sorghum and millet research in Uganda. East Africa research on sorghum and millets: Proceedings of the 6th regional workshop 20-27 July, 1988. Mogadishu, Somalia. pp. 318-335.
FAO and ICRISAT (1996). The world of sorghum and millet economies, facts, trends and outlook.
Harris K, Subudhi PK, Borrel A, Jordan D, Rosenow D, Nguyen H, Klein P, Klein R, Mullet J (2007). Sorghum stay-green QTL individually reduce post-flowering drought-induced leaf senescence. J. Exp. Bot. 58:327-338.
Haussmann BIG, Hess DE, Seetharama N, Welz HG, Geiger HH (2002). Construction of a combined sorghum linkage map from two recombinant inbred populations using AFLP, SSR, RFLP and RAPD markers, and comparison with other sorghum maps. Theor. Appl. Genet. 105:629-637.
Henzell RG, Hare BR, Jordan DR, Fletcher DS, McCosker AN, Bunker G, Persley DS (2001). Sorghum breeding in Australia: public and private endeavours. In: Borell AK, Henzell RG (eds.), Proceedings of the Australian sorghum conference Kouralbyn, Australia.
Hospital F (2001). Size of donor chromosome segments around introgressed loci and reduction in linkage drag in marker-assisted backcross programs. Genetics 158:1363-1379
IBPGR and ICRISAT (1993). Descriptors of sorghum *bicolor* (L.) Moench. International Board for Plant Genetic Resources, Rome, Italy, International Crops Research Institute for the Semi-Arid Tropics, Patancheru, India.
Kassa S, Åsmund B, Yunbi X (2010). The genetic dissection of quantitative traits in crops. Electronic J. Biotechnol. DOI: 10.2225/vol13-issue5-fulltext-14.
Kim JS, Klein P, Klein RR, Price HJ, Mullet JE, Stella DM (2005). Chromosome identification and nomenclature of sorghum bicolor. Genetics 169:1169-1173.
Lecomte L, Duffe P, Burett M, Servin B, Hospital F, Causse M (2004). Marker-assisted introgression of five QTL controlling fruit quality traits in tomato lines reveal interactions between QTL and genetic backgrounds. Theor. Appl. Genet. 109(3):658-668.
Madhusudhana R, Balakrishna D, Rajendrakumar P, Seetharama N, Patil JV (2012). Molecular characterization and assessment of genetic diversity of sorghum inbred lines. Afr. J. Biotechnol. 11(90):15626-15635.
Oryokot J, Ebyau J (1996). In: Mukiibi (Eds), Agriculture in Uganda. Crops 2001(3):42-43.
Rajkumar BF, Kavil SP, Girma Y, Arun SS, Dadakhalandar D, Gurusiddesh BH, Patil AM, Thudi M, Bhairappanavar SB, Narayana YD, Krishnaraj PU, Khadi BM, Kamatar MY (2013). Molecular mapping of genomic regions harbouring QTLs for root and yield traits in sorghum (*Sorghum bicolor* L. Moench). Physiol. Mol. Biol. Plants 19(3):409–419.
Reddy NR, Madhusudhana R, Murali MS, Seetharama N, Jagannatha VP (2014). Detection and validation of stay-green QTL in post-rainy sorghum involving widely adapted cultivar, M35-1 and a popular stay-green genotype B35. BMC Genomics 15(909):1471-2164.
Rosenow DT, Ejeta G, Clark LE, Gilbbert ML, Henzell RG, Borrell AK, Muchow RC (1996). Breeding for pre-flowering and post-flowering drought stress in sorghum. In: proceedings of the international conference on genetic improvement of sorghum and pearl millet. Lubbock, Texas. INTSORMIL and ICRISAT publication No. 97-5. pp. 400-411.
Sabadin PK, Malosetti M, Boer MP, Tardin FD, Santos FG, Guimara˜es, CT, Gomide RL, Andrade CLT, Albuquerque PEP, Caniato FF, Mollinari M, Margarido GRA, Oliveira BF, Schaffert RE, Garcia AAF, van Eeuwijk FA, Magalhaes JV (2012). Studying the genetic basis of drought tolerance in sorghum by managed stress trials and adjustments for phenological and plant height differences. Theor. Appl. Genet. 124(8):1389-402.
Siles MM, Baltensperger DD, Nelson LA (2001). Techniques for artificial hybridization of Foxtail millet (*Setaria italica* (L.) Beauv. Crop Sci. 41:1408-1412
Subudhi PK, Rosenow DT, Nguyen HT (2000). Quantitative Trait Loci for stay green trait in sorghum (*Sorghum bicolor* (L.) Moench; consistency across genetic backgrounds and environment. Theor. Appl. Genet. 101:733–741.
Tao YZ, Henzel RG, Jordan DR, Butler DG, Kelly AM, McLntyre CL (2000). Identification of genomic region associated with stay green in sorghum by testing RILs in multiple environments. Theor. Appl. Genet. 100:1125-1232.
Wu YQ, Haung Y (2006). An SSR genetic map of *sorghum bicolor* (L.) Moench and its comparison to a published genetic map. Genome 50:84-89.
Xu W, Rosenow DT, Nguyen HT (2000a). Stay-green in sorghum: relationship between visual rating and leaf chlorophyll content. Plant Breed. 119:365-367.
Xu W, Subdhi PK, Crasta OR, Rosenow DT, Mullet JE, Nguyen HT (2000b). Molecular mapping of QTL conferring stay-green in grain sorghum (*Sorghum bicolor* (L) Moench) Genome 43:461-469.