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Introgression of Stay Green Quantitative Trait Locus (QTLS) into Elite Sorghum Variety by MABC

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Sorghum is a major staple food crop for the people in semi-arid areas of Asia. Post-flowering drought is a global constraint of sorghum production. The study aimed to improve stay-green characteristics of GS-23 sorghum variety by transferring *stg3A* and *stg3B* QTL`s respectively from donor genotypes K260 and K359w of ICRISAT by using marker assisted backcrossing. The experimental material comprised of six basic generations P_1, P_2, F_1, F_2, BC_1 and BC_2 developed from crossing GS-23 × K260 and GS-23 × K359 were genotyped using a set of 133 SNPs and 79 SSR markers. Whereas, 53 polymorphic SNPs among parents and backcross F_1s for stay green

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trait at maturity were used to track introgression of stay green trait. Similarly,10 SSR markers were found to be polymorphic were used to track introgression of stay green trait *i.e.,* stay green trait QTL`s *stg3A* and *stg3B* from donor parent K260 and K359w respectively in GS-23 background across backcross population and to identify plants that were homozygous for the desired allele. In which 02 SNPs *SnpSB0039* and *SnpSB0093* were identified as polymorphic for both K260 and K359w. Whereas 17 SNPs identified polymorphic for K260 and 34 SNPs for K359w. These SNPs were validated in both F_1 and BC_1F_1 populations of both the crosses. Similarly, out of 10 SSR markers utilized, 02 SSRs were identified polymorphic to K260 and 02 SSRs were identified for K359w. Whereas 06 SSRs were polymorphic to both the parents K260 and K359w. Prominent 02 SSRs *viz., Xtxp 141_Fam* and *Xgap84_Vic* found to be more reliable and polymorphic to both the parents. These SSRs were validated in both F_1 and BC_1F_1 populations of both the crosses. The genotypic analysis revealed the presence of favorable alleles in homozygous conditions at markers loci associated with *stg3A* and *stg3B* QTL`s in BC populations, suggesting successful introgression of stay green QTLs from the donor parents to the recurrent parent. Therefore, our study demonstrated the utility of marker-assisted backcrossing for drought tolerance improvement of locally adapted sorghum variety.

Keywords: SNP technology; breeding process; genotype; sorghum.

1. INTRODUCTION

Sorghum is the fifth most important cereal crop with a thick, waxy cuticle known to be better adapted to arid, semi-arid tropical, and sub-tropical climatic conditions, serving as a staple food for many of the world's poorest and food-insecure people. A plethora of biotic and abiotic stresses are the major constraints of sorghum, while drought is a major abiotic constraint behind the significant loss in crop productivity across the world. Delay in senescence is one of several mechanisms that can contribute to the ability of a plant to withstand drought stress. Such functional "stay-green" individuals retain green leaf area (GL) for a longer period of time, following the onset of a "drought spell", and this can be expected to have a more stable grain yield performance across sites and years, in their zones of adaptation. The best-characterized trait contributing to grain-yield maintenance under terminal drought stress/post-flowering drought tolerance is "stay-green", which is well-documented in several economically important crop plants like sorghum, maize, wheat, barley, rice, and *Arabidopsis* [1].

Post-rainy sorghum grain is highly valued for its pearly white, lustrous, bold and clean grain, 98% of which is used for food. Apart from grain, sorghum stover is an important feed in the livestock sector in India particularly in the dry seasons when other feed resources are in short supply. Thus, post-rainy sorghum plays an important role in ensuring food and fodder security for millions of rural families in the semi-arid tropics. In these areas, since rainfall is low and highly erratic, terminal drought stress is the major yield constraint. Moisture stress during post-flowering stage is the most significant yield reducing factor in the semi-arid tropics. The economic benefit of successful mitigation of drought damage by developing drought tolerant sorghum varieties was estimated to be US$ 53 million per year. In sorghum, stay-green (delayed-senescence) is a post-flowering drought response, and is well characterized by the maintenance of green leaves (upper) and green stems although the plants are under severe moisture stress conditions. The genotypes possessing the stay-green trait maintain more photosynthetically active leaf area as compared to senescent genotypes, and continue to fill their grains normally under stress conditions [2]. The sorghum stay green genotypes K260 and K359w which carries *stg3A* and *stg3B* QTL`s are used as donor parents to transfer stay green QTL`s in to the GS-23 back ground is the objective of the present study.

2. MATERIALS AND METHODS

2.1 Study Area

The experiment was conducted at Agricultural Research Station, Kalaburgi and Hagari during *rabi* season of 2017-18. Kalaburagi is situated in Deccan Plateau located at 17.33°N 76.83°E and the general elevation ranges from 300 to 750 meters above mean sea level. Kalaburgi comes under north-eastern dry-zone of Karnataka with average annual rainfall of 717 mm and black soil being predominant soil type and the average ambient temperature remains 26.9°C, varies

from 14.9°C to 42°C. The average relative humidity remains around 58.9%, varies from 14.7% to 97.9%. Hagari is situated at N 15° 9' 4"latitude and E 77° 3' 0" longitude and 495 m elevation. Hagari comes under northern dry-zone of Karnataka with average annual rainfall of 515 mm. The soil type and climatic conditions of both locations are well suited for *rabi* sorghum cultivation. Hence, these are ideal places for *rabi* sorghum for generating and evaluation of F_1, F_2, F_3, BC_1 and BC_2 generations for yield and stay green traits.

2.2 Experimental Materials

The experimental material consisted of three inbred lines of which GS-23 (P_1) is a non-stay green lines used as female parent, which are crossed with two stay green donor lines K260 (P_2) and K359w (P_3) received from ICRISAT, Hyderabad. These lines were used to develop experimental material used in present study, which comprised of six basic generations P_1, P_2, F_1, F_2, BC_1 and BC_2 (Fig 1a, 1b, 2a, 2b, 3a and 3b).

2.3 Experimental Design and Layout

At the research station Hagari, the experimental material consists of 11entries (three parents, two F_1's, two F_2's and four BC's) comprising six generations of two selected crosses *viz*., GS-23× K260 and GS-23× K359w two checks (B 35 and R16) was laid out during *rabi*, 2018 in a Randomized Block Design (RBD) with two replications. The non-segregating generations, *viz.,* parents, F_1's and checks were raised with 2 rows, while segregating generations *viz*., F_2's were raised with 10 rows and BC_1 and BC_2 populations were grown with 4 rows each. The entries were planted in rows of 4m length with spacing of 60 x 15 cm.

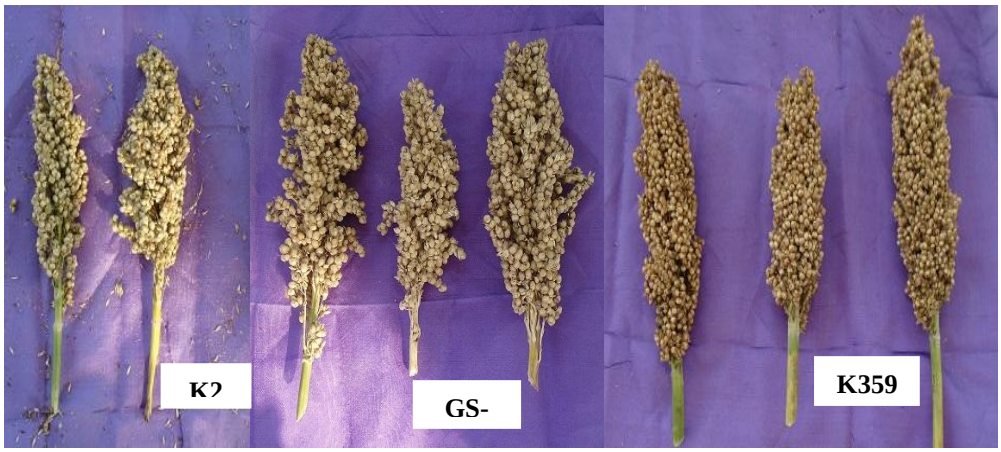

Fig. 1a. Panicle photographs of the parents used in the study

Fig. 1b. Panicles of the checks used for the study

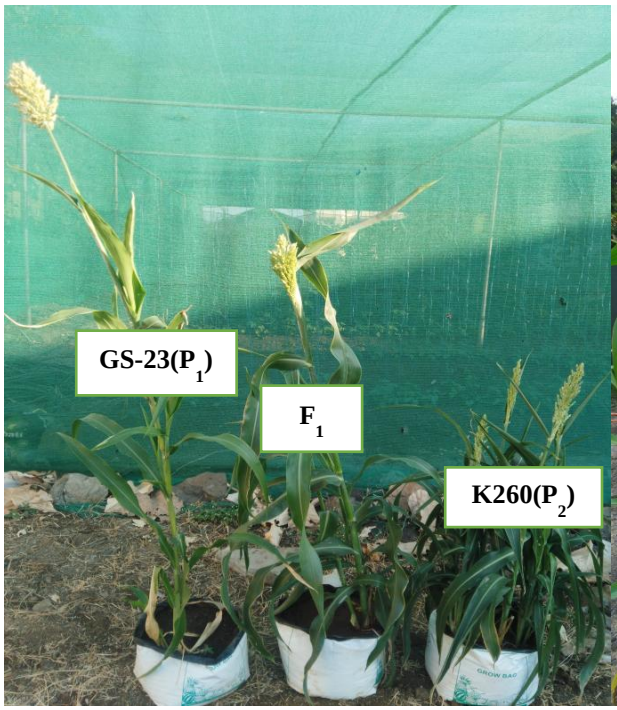

Fig. 2a. Phenotype of the plants of parents and F_1 the cross GS-23×K260

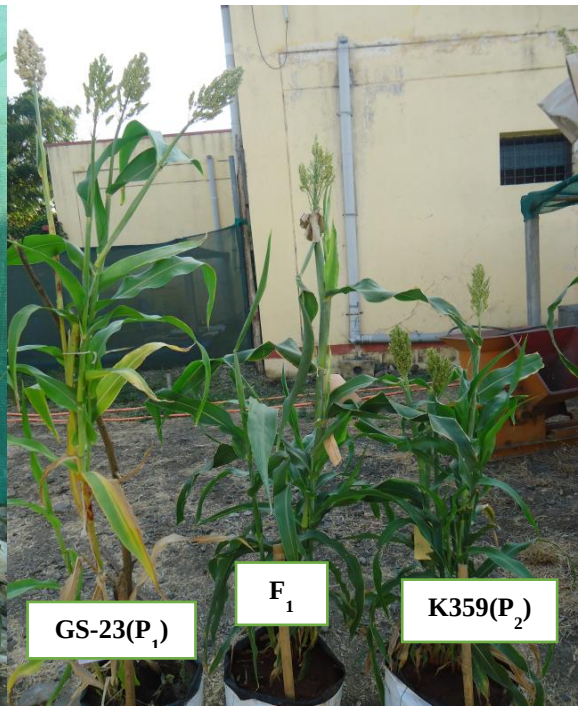

Fig. 2b. Phenotype of the plants of parents and F_1 the cross GS-23×K359w

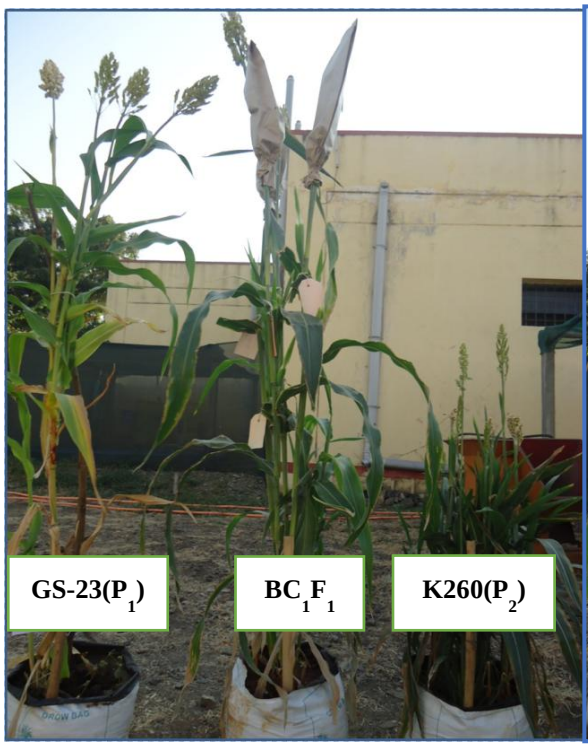

Fig. 3a. Phenotype of the plants of parents and $BC_1F_1$the cross GS-23×K260

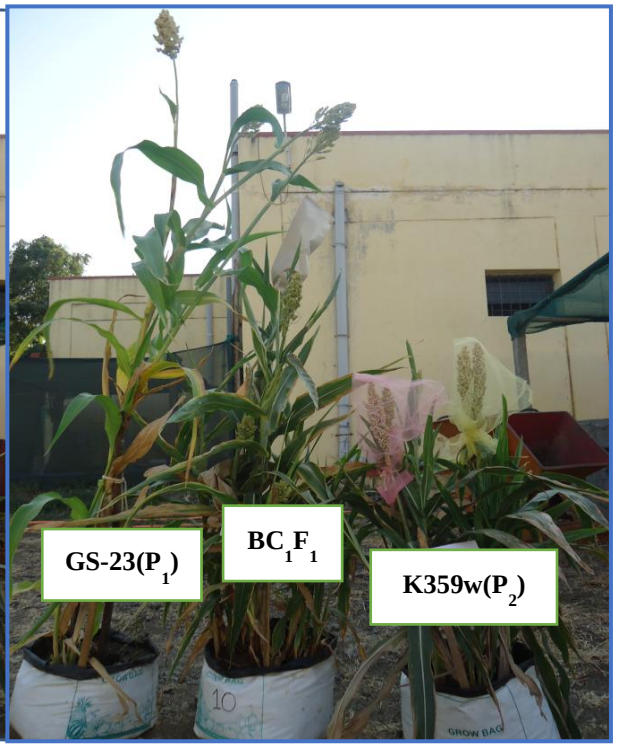

Fig. 3b. Phenotype of the plants of parents and $BC_1F_1$the cross GS-23×K359w

1.1 Development of SSR and SNP Markers to Study of Parental Polymorphism, Identification of F_1s and True Back Crossed Plants

1.1.1 DNA isolation

In the present study, DNA was isolated from three different genotypes (2 donors and 1 recipient genotype), which were used to develop two set of F_1s and two set of F_2s and also backcross population. DNA was extracted by using Qiagen DNeasy® Plant Mini Kit for assessing parental polymorphism which may serve as valuable resources for Marker Assisted Backcross Breeding (MABC) [3].

1.1.2 Assessment of genomic DNA quality with agarose gel electrophoresis

The quality of the extracted genomic DNA was assessed using gel electrophoresis. For quality, the samples were run on 0.8 *per cent* agarose gel in 1X TBE buffer (diluting 100 ml of 10X TBE buffer in 900 ml of distilled water) and stained with 5µl ethidium bromide (10 mg/ml) per 100 ml and checked for shearing of DNA, contamination of RNA and protein.

2. MATERIALS

- 3X loading dye
- 10X Tris Borate EDTA (TBE) Buffer
- 100bp Ladder

2.1 DNA Quality Assurance

DNA concentration and purity were checked based on A260/A280 ratios obtained using Nanodrop Spectrophotometer given in the Fig 4a and 4b.

The concentration of the DNA was calculated as:

DNA (µg/ ml) = OD260 ηm X 50 X dilution factor

2.2 SNP Development

Utilizing the available sequencing and resequencing data of *Sorghum bicolor* at ICRISAT, Single nucleotide polymorphisms (SNPs) were extracted from the SBI-02 covering a span of 56Mbp to 72Mbp, which resemblance to consensus QTL interval of 114 -112cM and 130 -141cM of *stg3A* and *stg3B* QTL`s respectively. SNPs for missing calls, downstream, upstream, intron, intergenic region and low coverage synonymous SNPs including SNPs falling in splicing region were filtered out. The KASPar SNP genotyping was performed according to the manufacturer's instructions (LGC Biosearch Technologies). The reaction mixture consisted of: 2.5 µL of extracted DNA, 1.25 µL of KASPar SNP genotyping reaction mix, 1.25 µL of KASPar SNP genotyping buffer and 0.5 µL of KASPar SNP genotyping primer mix. The reaction was performed in a final volume of 5 µL. The thermocycling conditions were as follows: 95°C for 3 minutes, followed by 50 cycles of 95°C for 15 seconds, 56°C for 30 seconds and 72°C for 30 seconds, with a final extension step at 72°C for 5 minutes [4].

2.3 Data Analysis

The KASPar SNP genotyping data were analyzed using the KASPar SNP genotyping data analysis software (LGC Biosearch Technologies). The software was used to generate a genotyping profile for each sample, which was then compared to the reference database to determine the genotype at each SNP locus. The genotype calls were verified by visual inspection of the fluorescence data. To ensure the quality of the data, a subset of samples was genotyped in duplicate, and the genotyping results were compared to ensure consistency. In addition, a set of positive and negative control samples were included in each genotyping run to monitor the performance of the reaction and to assess the accuracy of the genotyping results [5].

2.3.1 Selection of the SSR markers

SSRs are small repetitive DNA sequences, which are spread throughout the genome of eukaryotes, are often highly polymorphic due to variation in number of repeat units, provide the basis of PCR based multi-allelic, co-dominant marker system [6]. SSR markers linked to QTLs for stay-green (Table 1) were used for foreground selection to select the individuals presumably having the donor allele (foreground selection) at a particular target stay-green QTL. Donor parent alleles at foreground marker loci indicate the presence of the target stay-green QTL that is flanked by these marker loci. The tighter the markers are linked to the QTL, the greater the chance that the QTL mapped between a pair of flanking markers has indeed been transferred. Therefore, phenotypic testing of the final products of the MAB exercise needs to be performed in order to confirm the transfer of

stay-green QTL. In PCR two primers (short single stranded DNA sequences) are used that are complementary to opposite strand of DNA sequence to be amplified DNA thermocycler. The reactions were performed in volumes of 5µl and a touchdown PCR program (Fig 4a and 4b).

Table 1. List of SSR markers associated with STG -QTL used for genotyping

Sl. No.	Marker	Dye	Stay green donors		RP	Remarks
			K359w	K260	GS23	
1	Dsenhsbm015	B	191.71654	187.51602	191.49104	Polymorphic to K260
2	Stg3a-22	G	176.97355	176.72254		Polymorphic to both (Dominant)
3	Stg3a-24	Y	141.934770	141.935431	144.321818	Monomorphic
4	Stgnhsbm35	R	153.836056	155.727203	153.647166	Monomorphic
5	Stg3a-33	B	109.21708	109.131435	101.78917	Polymorphic to both
6	Xtxp464	G	142.2184	143.05481		Marker problem (heterozygous calls)
7	Xtxp445	Y	261.05238	261.13877	260.96857	Monomorphic
8	Dsenhsbm108	R	153.841617	153.521235	153.27326	Monomorphic
9	Xiabtp265	B	91.9310223	91.7331900	91.938208	Monomorphic
10	Xisep0913	G	232.823000	232.733281	232.825705	Monomorphic
11	Xiabtp80	Y	1952391	194.812509	197.762084	Polymorphic to both
12	Xisp10334	R	196.114541	195.923448	195.92063	Monomorphic
13	Stg3a-30	B				
14	Xtxp298	G				
15	Xisp10336	Y	173.372465	173.542577	165.661767	Marker problem (heterozygous calls)
16	Xiabtp509	R	153.45480	150.98155	153.52521	Polymorphic to K260
17	Xiabtp231	B	163.95256	160.5675	159.8260	Polymorphic to K359w
18	Xtxp008	G	104.01 183.5 1020 147	103.66 183.49 262 175	103.43 183.06 253 134	Monomorphic Monomorphic
19	Stg3a-32	Y	171.13425	171.092684	183.81963	Polymorphic to both
20	Xisp10200	R	152.93336	153.51260	153.46291	Monomorphic
21	Xtxp 214	B				
22	Stg3a-17	G	183.245265	183.145446	183.992304	Monomorphic
23	Xisep0934	Y	183.42573	183.14772	185.12357	Monomorphic
24	Xisp10278	R	297.884324	297.873097	297.861365	Monomorphic

Sl. No.	Marker	Dye	Stay green donors		RP	Remarks
			K359w	K260	GS23	
25	Stg3a-14	B	169.18 662	169.56 777		Polymorphic to both
26	Xcup29	G	250.70 265.40 20621 31383	250.89 265.37 32703 32704	250.72 265.54	Monomorphic Monomorphic
27	Xgpsb128	Y	265.75 32733	265.73 32714	265.66 32752	Monomorphic
28	Stg3a-9	R	265.4 9026	265.2 10470	265.2 845	Monomorphic
29	Stg3a-7	B	160.56 1198	160.75 1181	160.93 1403	Monomorphic
30	Stghsbm40	G	103.78 3822	103.61 3748	103.58 7827	Monomorphic
31	Xiabtp397	Y	208.2 1600	232.31 1141	208.1 366	Polymorphic to K359w
32	Stg3b-11	R	168.39 2512	207.83 643	172.41 1464	Polymorphic to both
33	Xtxp007	B				
34	Xgap84	G	103.92 366	103.58 924	103.32 106	Monomorphic
35	Xiabtp484	Y	223.04 1637	223.02 1076	214.13 787	Marker problem (heterozygous calls)
36	Xiabtp076	R	310.49 1027	310.5 688	310.6 515	Monomorphic
37	Xcup63	B	153.15 164.05 1210 2657	154.13 163.93 1007 1868	154.15 163.98 1838 2356	Monomorphic Monomorphic
38	Xtxp429	G	104.25 3849	103.98 3467	103.82 123	Monomorphic
39	Xtxp207	Y	164.23 194.23 506 749	163.57 192.03 407 384	164.34 2899	Monomorphic
40	Stg3b-5	R				
41	Xiabtp388	B	92.43 1927	92.37 1759	92.67 1505	Monomorphic
42	Stg3a-15	G	104.83 5237	104.5 4503	104.57 4157	Monomorphic
43	Stg3a-23	Y	96.31 1981	96.19 1713	96.25 1549	Monomorphic
44	Xiabtp391	R	154.53 6205	154.07 6672	154.54 5375	Monomorphic
45	Stg3b-6	B	92.06 978	92.01 483	91.99 281	Monomorphic
46	Stg3a-5	G	104.78 28989	104.61 30156	105.09 19698	Monomorphic
47	Xtxp430	Y	105.14 2107	104.61 2789	104.91 1617	Monomorphic
48	Stgnhsbm31	R	153.39 638	154.02 774	153.76 610	Monomorphic

2.3.2 Touch down PCR program used

Initial denaturation for 15 min at +94°C

1. Denaturation: for 10 sec at +94°C
2. Annealing: at 61-52°C for 20 sec (the annealing temperature for each cycle is reduced by 1°C)
3. Extension: at +72°C for 30 sec.

For 10 cycles

4. Denaturation: for 10 sec at +94°C
5. Annealing: at +54°C for 20 sec
6. Extension at +72°C for 30 sec.

For 35 cycles

The last PCR cycle is followed by a final extension of 20 min at +72°C to ensure amplification to equal length of both DNA strands.

Hold: 4°C forever

Along with the samples, a standard 100 bp marker ladder (50 ng/µl) was also loaded in the first and last lane of the gel to ensure proper sizing of amplified PCR fragments. Most of the markers used allowed clear differentiation of donor and recurrent parent alleles. The gel was run at 550-600 V of constant power in 0.5X TBE buffer for 3 hours using a Bio-Rad gel sequencing unit [7].

2.4 Data Collection and Analysis

2.4.1 Scanning and scoring of the gels

The gel is scanned under UMAX scanner. The gel image was saved in the computer connected to UMAX scanner, which will be useful for further reference. In case of heterozygous individual scoring of such bands is much more difficult. Automatic systems detect fluoro-labelled PCR products using a laser and capillary electrophoresis. The results are transmitted directly in to a computer database where they are available for analysis using software's such as GENESCAN and GENOTYPER (Applied Biosystems/ABI). These analysis programs provide algorithms that separate native alleles automatically from slippage products. The bands appeared on the gels were scored as A, B, H, OFF and "—" based on their pattern compared with those of the parents. "A" is referred to as the presence of allele from the recurrent parent GS-23, "B" is referred to as the presence of allele from K260 and K359w, "H" is referred as the heterozygous (*i.e.,* presence of both recurrent and donor parent alleles), "OFF" was defined as an allele neither from donor parent nor from the recurrent parent and "—" is referred as a missing sample [8].

2.4.2 Preparation of score sheet of the gels

After scoring, the data is entered in to excel spread sheet and type of the backcross population of the crosses is entered. For the samples whose product size is less than 5bp, labelled primers (FAM, HEX, NED) are used for keeping a PCR reaction. Then ABI plate is prepared by using Rox and Formamide. It is submitted to ABI prism 3700 (Perkin Elmer) DNA sequences. The data is directly obtained from the sequencer based on the peaks obtained from the graph [9].

3. RESULTS AND DISCUSSION

3.1 Marker Polymorphic Studies in Parents and F_1s

A set of 133 SNPs were identified post flowering on SBI 02 chromosome. These 133 SNPs were synthesized at KBiosciences, UK utilizing KASPar technology. A sub-set of 53 SNPs was identified for parental polymorphism and will be utilized for validation across diverse parental sets (Table 2). The polymorphic SNPs identified were utilized for identification of heterozygous lines in F_1s and evaluating BC_1F_1 population using KASPar assay. In which 02 SNPs *viz., snpSB0039* and *snpSB0093* for both K260 and K359w parent in Fig 1 and 2. Whereas 17 SNPs identified K260 and 34SNPs showed a polymorphism to K359w. These SNPs are validated in both F_1 and BC_1F_1 population of the both crosses (Table 3). These results are in line with the studies done by Mwamahonje et al., [10] where, a total of 752 individuals representing five BC_2F_1 populations and their parents were genotyped using previously reported KASP markers linked with STG 3A and STG 3B quantitative trait loci (QTL). In the BC_2F_1 populations, the maximum number of individuals with heterozygous alleles were observed in S35*Pato background (37) whereas only seven individuals derived from the B35*Wahi parents' background contained heterozygous alleles. Of

the 30 single nucleotide polymorphism (SNP) markers, favourable alleles were observed at 18 loci in BC_2F_1 populations. Similarly, a set of 79 SSRs were identified on SBI 02 chromosome.

A sub-set of 10 SSRs was identified for parental polymorphism (Fig. 5) and will be utilized for validation across diverse parental sets (Table 4). Out of 10 polymorphic SSRs identified 02 SSRs *viz., Dsenhsbm015* and *Xiabtp509* were polymorphic to parent K260. Whereas *Xiabtp231* and *Xiabtp397* were polymorphic to parent K359w. While, 06 SSRs *viz., Stg3a-22, Stg3a-33, Xiabtp80, Stg3a-32, Stg3a-14* and *Stg3b-11* were polymorphic for both K260 and K359w parent (Table 5). Similar studies were conducted by Edema and Amoding [11] where, 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.

The polymorphic SSRs were utilized for identification of heterozygous lines in F_1s and evaluating BC_1F_1 population. Polymorphic SSRs identified were utilized for identification of heterozygous lines in F_1s and two markers *viz.*, *Xtxp 141_Fam* and *Xgap84_Vic* found to be polymorphic for two crosses as shown in Table 6.

Table 2. Details of parental polymorphic SNPs for stay green at maturity between donor parents (K260 and K359w) and recurrent (GS23)

Sl. No.	Particular	Number of SNPs
1	Polymorphic to both (K260 and K359w)	02
2	Polymorphic to K260	17
3	Polymorphic to K359w	34
Total		53

Table 4. Details of parental polymorphic SSRs for stay green at maturity betweendonor parents (K260 and K359w) and recurrent (GS-23)

Sl. No.	Particular	Number of SSRs
1	Polymorphic to both (K260 and K359w)	06
2	Polymorphic to K260	02
3	Polymorphic to K359w	02
Total		10

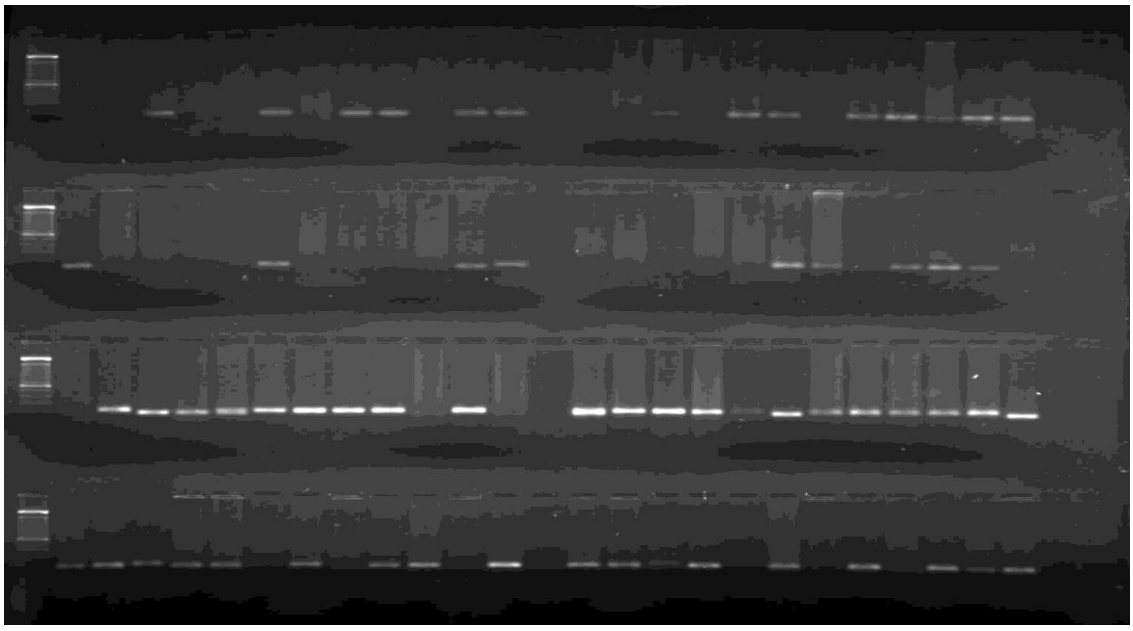

Fig. 4a. PCR analysis of GS-23×K260 BC_1F_1s

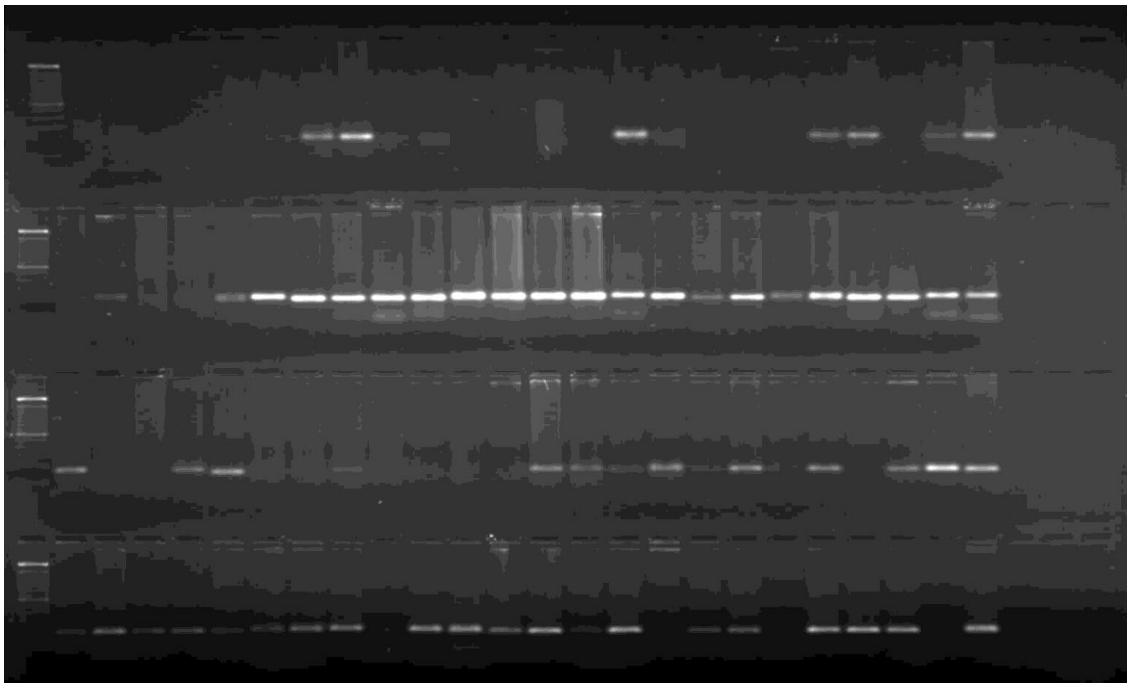

Fig. 4b. PCR analysis of GS-23×K359w BC_1F_1s

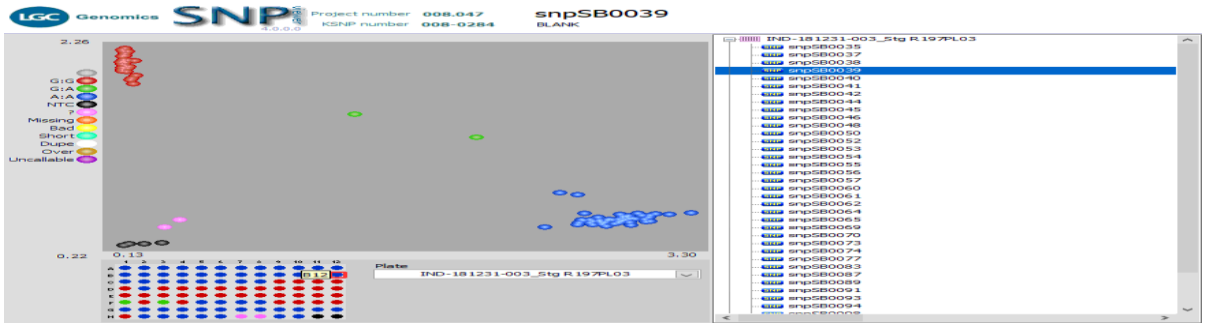

Fig. 5. KASPar assay of BC_1F_1 using SNP marker snpSB0039 green trait at maturity for cross GS-23 x K260

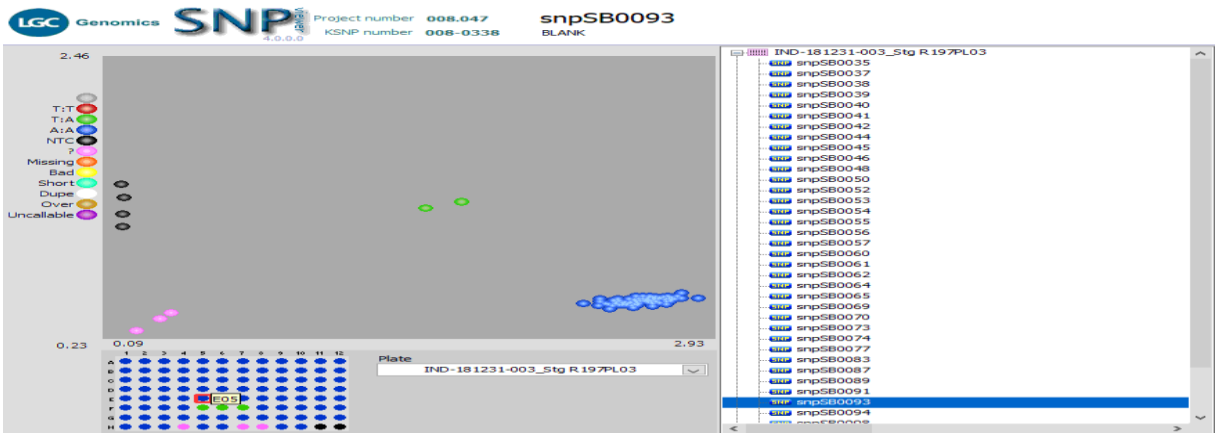

Fig. 6. KASPar assay of BC_1F_1 using SNP marker *snpSB0093* green trait at maturity for cross GS-23 x K359w

Table 3. List of SNP markers associated with STG-QTL used for genotyping

Sl. No.	SNP ID	Chromosomes	K260 (STG 3A)	K359w (STG3B)	GS-23
1	snpSB035	SBI-02	C:C	T:T	T:T
2	snpSB036	SBI-02	C:C	Uncallable	C:C
3	snpSB037	SBI-02	C:C	T:T	T:T
4	snpSB038	SBI-02	A:A	T:T	T:T
5	snpSB039	SBI-02	A:A	G:G	A:A
6	snpSB040	SBI-02	C:C	T:T	T:T
7	snpSB041	SBI-02	C:C	G:G	G:G
8	snpSB042	SBI-02	T:T	C:C	C:C
9	snpSB043	SBI-02	T:T	C:C	C:C
10	snpSB044	SBI-02	A:A	T:T	T:T
11	snpSB045	SBI-02	C:C	T:T	C:C
12	snpSB046	SBI-02	T:T	A:A	T:T
13	snpSB047	SBI-02	A:A	G:G	A:A
14	snpSB048	SBI-02	T:T	G:G	T:T
15	snpSB049	SBI-02	G:G	A:A	A:A
16	snpSB050	SBI-02	G:G	T:T	G:G
17	snpSB051	SBI-02	T:T	C:C	T:T
18	snpSB052	SBI-02	T:T	A:A	T:T
19	snpSB053	SBI-02	A:A	G:G	G:G
20	snpSB054	SBI-02	G:G	A:A	A:A
21	snpSB055	SBI-02	C:C	G:G	C:C
22	snpSB056	SBI-02	C:C	T:T	C:C
23	snpSB057	SBI-02	C:C	G:G	C:C
24	snpSB058	SBI-02	T:T	G:G	T:T
25	snpSB059	SBI-02	G:G	T:T	G:G
26	snpSB060	SBI-02	A:A	T:T	A:A
27	snpSB061	SBI-02	C:C	A:A	C:C
28	snpSB062	SBI-02	A:A	T:T	A:A
29	snpSB063	SBI-02	C:C	T:T	C:C
30	snpSB064	SBI-02	C:C	G:G	C:C
31	snpSB065	SBI-02	G:G	C:C	G:G
32	snpSB066	SBI-02	T:T	G:G	T:T
33	snpSB067	SBI-02	A:A	G:G	A:A
34	snpSB068	SBI-02	T:T	A:A	T:T
35	snpSB069	SBI-02	T:T	A:A	T:T
36	snpSB070	SBI-02	G:G	C:C	G:G
37	snpSB071	SBI-02	C:C	A:A	C:C
38	snpSB072	SBI-02	G:G	A:A	A:A
39	snpSB073	SBI-02	A:A	C:C	A:A
40	snpSB074	SBI-02	G:G	A:A	G:G
41	snpSB075	SBI-02	A:A	G:G	A:A
42	snpSB076	SBI-02	T:T	A:A	T:T
43	snpSB077	SBI-02	T:T	C:C	T:T
44	snpSB078	SBI-02	Uncallable	Uncallable	Uncallable
45	snpSB079	SBI-02	Uncallable	Uncallable	Uncallable
46	snpSB080	SBI-02	C:C	C:C	T:T
47	snpSB081	SBI-02	T:T	T:T	T:T
48	snpSB082	SBI-02	Uncallable	Uncallable	Uncallable
49	snpSB083	SBI-02	T:T	T:T	C:C
50	snpSB084	SBI-02	Uncallable	Uncallable	Uncallable
51	snpSB085	SBI-02	Uncallable	Uncallable	Uncallable
52	snpSB086	SBI-02	Uncallable	Uncallable	Uncallable
53	snpSB087	SBI-02	A:A	A:A	G:G
54	snpSB088	SBI-02	Uncallable	Uncallable	Uncallable

Sl. No.	SNP ID	Chromosomes	K260 (STG 3A)	K359w (STG3B)	GS-23
55	snpSB089	SBI-02	C:C	C:C	A:A
56	snpSB090	SBI-02	Uncallable	Uncallable	Uncallable
57	snpSB091	SBI-02	C:C	A:A	A:A
58	snpSB092	SBI-02	C:C	G:G	G:G
59	snpSB093	SBI-02	T:T	A:A	A:A
60	snpSB094	SBI-02	C:C	T:T	C:C
61	snpSB095	SBI-02	G:G	A:A	G:G
62	snpSB096	SBI-02	G:G	A:A	A:A
63	snpSB097	SBI-02	T:T	G:G	G:G
64	snpSB098	SBI-02	C:C	G:G	C:C
65	snpSB099	SBI-02	T:T	C:C	C:C
66	snpSB100	SBI-02	Uncallable	Uncallable	Uncallable
67	snpSB101	SBI-02	G:G	C:C	C:C
68	snpSB102	SBI-02	G:G	A:A	A:A
69	snpSB103	SBI-02	G:G	G:G	C:C

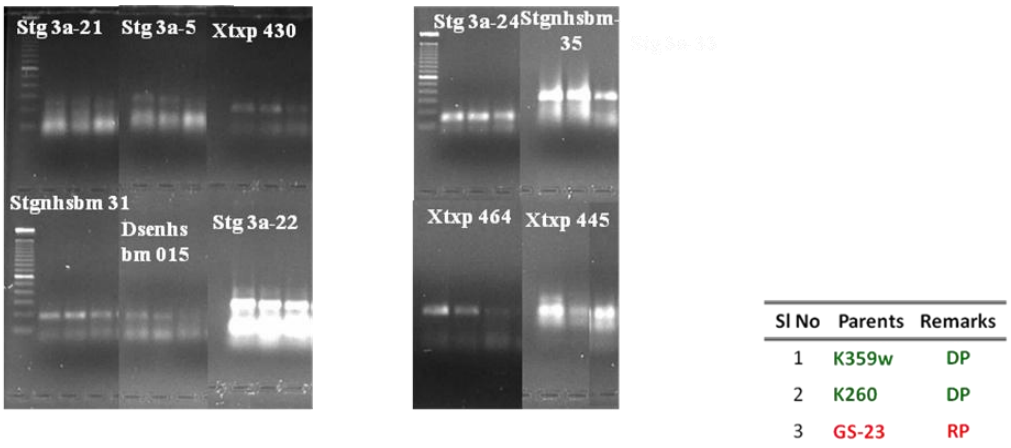

Fig. 7. Sorghum stay-green parental polymorphism studies using SSRs

Table 5. F_1 Confirmation with *Xtxp 141_Fam* and *Xgap84_Vic*

F_1 Confirmation with *Xtxp 141_Fam* and *Xgap84_Vic*		
Traits	UAS Raichur Crosses	Set-1*
Stay green trait at maturity	GS 23 × K 359w	11/15
	GS 23 × K 260	7/12

3.1.1 Fore ground selection studies in backcross population by using SNPs and SSRs

The use of SNPs and SSRs in marker assisted backcross breeding in sorghum has become an increasingly popular method for improving the genetic quality of this important crop. In the present study, the available genomic resources of sorghum at ICRISAT were used to identify the polymorphic SNPs and SSRs among parents GS-23, K260, K359w and F_1s. Where SNPs were further used to evaluated backcross breeding population. The results demonstrate the potential of SNP technology to significantly improve the efficiency and accuracy of the breeding process. Among the 53 polymorphic SNPs identified were used for fore ground selection of stay green trait in the backcross populations of both the crosses of the study. study revealed that most of the back cross plants were showed the presence of stay green trait QTL`s in the back ground of GS-23 as depicted in Fig 3, 4, 5 and 6. These plants showed stay greenness under drought conditions. One of the major advantages of using SNPs in backcross breeding is the ability to identify and select for desirable traits with greater precision. By using SNP markers, breeders can quickly and accurately determine plants carrying target trait and eliminate those that do not, which significantly reduces the time and resources required for traditional breeding methods [12]. Additionally, SNPs can also be used to track

inheritance of desirable traits through multiple generations of breeding, providing a valuable tool for monitoring the progress of the breeding program. Similarly, the 10 polymorphic SSRs identified were used for fore ground selection of stay green trait in the backcross populations of both the crosses of the study. Study revealed that most of the back cross plants showed the presence of desired stay green trait in the GS-23 background as depicted in Fig. 12a and 12b. In the present study, 10 SSR markers were found to be polymorphic among parents and backcross F_1s for stay green trait at maturity. Polymorphic SSR markers identified were used to track introgression of stay green trait *i.e.,* stay green

Fig. 8. Annotated graphical representation of PCR products of MABC$_1$F$_1$s of GS23× K260

Fig. 9. Annotated graphical representation of PCR products of MABC$_1$F$_1$s of GS23× K359w

Table 6. Stay green QTL polymorphism using *Xtxp 141, Xgap 84* markers in GS-23. K260, K359w and their F_1s

Sample ID	Well No	*Xtxp 141_Fam*				Remarks	*Xgap84_Vic*				Remarks	Xtxp141:Xgap84
		Size 1	Height 1	Size 2	Height 2		Size 1	Height 1	Size 2	Height 2		
R17110001	A9	169.63	32749			Non-parental allele	169.63	12449	226.52	16860	True F_1	Heterozygus
R17110002	B9	169.55	32745			Non-parental allele	169.55	13305	227.44	15148	True F_1	Heterozygus
R17110003	C9	168.65	330			Non-parental allele			224.35	5977	Self	Not-confirmed
R17110004	D9	169.35	9494			Non-parental allele			226.39	30693	Self	Not-confirmed
R17110005	E9	169.41	31931			Non-parental allele	169.41	5939	226.46	31932	True F_1	Heterozygus
R17110006	F9	168.55	1164			Non-parental allele			226.54	14153	Self	Not-confirmed
R17110007	G9	169.41	30661			Non-parental allele	169.41	1908	226.43	26385	True F_1	Heterozygus
R17110008	H9	169.56	32741			Non-parental allele	169.39	11685	226.68	28408	True F_1	Heterozygus
R17110009	A10	169.47	31183			Non-parental allele	169.47	3997	226.79	27633	True F_1	Heterozygus
R17110010	B10	169.48	13362			Non-parental allele	169.48	828	226.69	4222	True F_1	Heterozygus
R17110011	C10	166.56	779			Non-parental allele			224.18	10631	Self	Not-confirmed
R17110012	D10	169.44	2428			Non-parental allele			226.34	31470	Self	Not-confirmed
GS 23	E10	159.38	604				169.39	11694	218.06	6502		
K 260	F10	164.6	708						228.64	18289		
R17111001	G10	169.43	13714			Self	212.55	7003	226.56	31596	True F_1	Heterozygus
R17111002	H10	169.58	9569			Self	212.57	3139	226.83	12504	True F_1	Heterozygus
R17111003	A11					No Amplification			227.11	854	Self	Not-confirmed
R17111004	B11	169.23	124			Self	213.14	457	226.8	4221	True F_1	Heterozygus
R17111005	C11	168.65	419			Self	213.04	2282	226.76	5192	True F_1	Heterozygus
R17111006	D11	169.54	26722			Self	213.11	6296	226.75	21841	True F_1	Heterozygus
R17111007	E11	169.53	17234			Self	212.98	5865	226.82	31692	True F_1	Heterozygus
R17111008	F11					No Amplification	212.87	442	226.76	3889	True F_1	Heterozygus
R17111009	G11	165.14	168	178.31	570	True F1			226.7	1222	Self	Heterozygus
R17111010	H11	166.5	526	179.83	589	True F1	211.4	121	226.98	1619	True F_1	Confirmed
R17111011	A12					No Amplification			227.23	460	Self	Not-confirmed
R17111012	B12	169.69	11909			Self	212.66	1962	227.12	10419	Non-parental allele	Not-confirmed
R17111013	C12	169.66	3756			Self			226.96	3872	Self	Not-confirmed
R17111014	D12	169.67	1542			Self	200.64	143	227.02	4402	True F_1	Heterozygus
R17111015	E12	166.04	363			Self	207.94	202	226.89	847	True F_1	Heterozygus
GS 23	F12	168.62	1298	177.07	254		204.66	799	218.39	6186		
K 359w	G12	163.78	143	172.41	238				224.55	676		

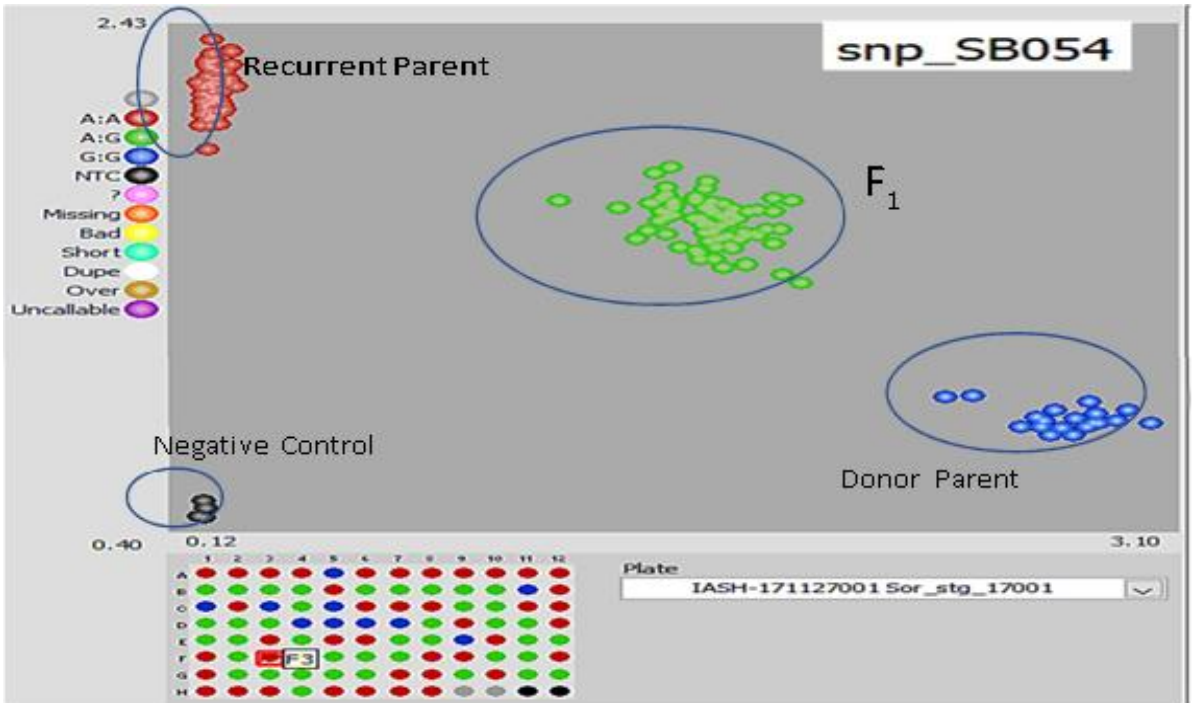

Fig. 10. KASPar assay of BC_1F_1s using SNP marker *snp_SB054* for stay green trait at maturity for cross GS 23 × K 260

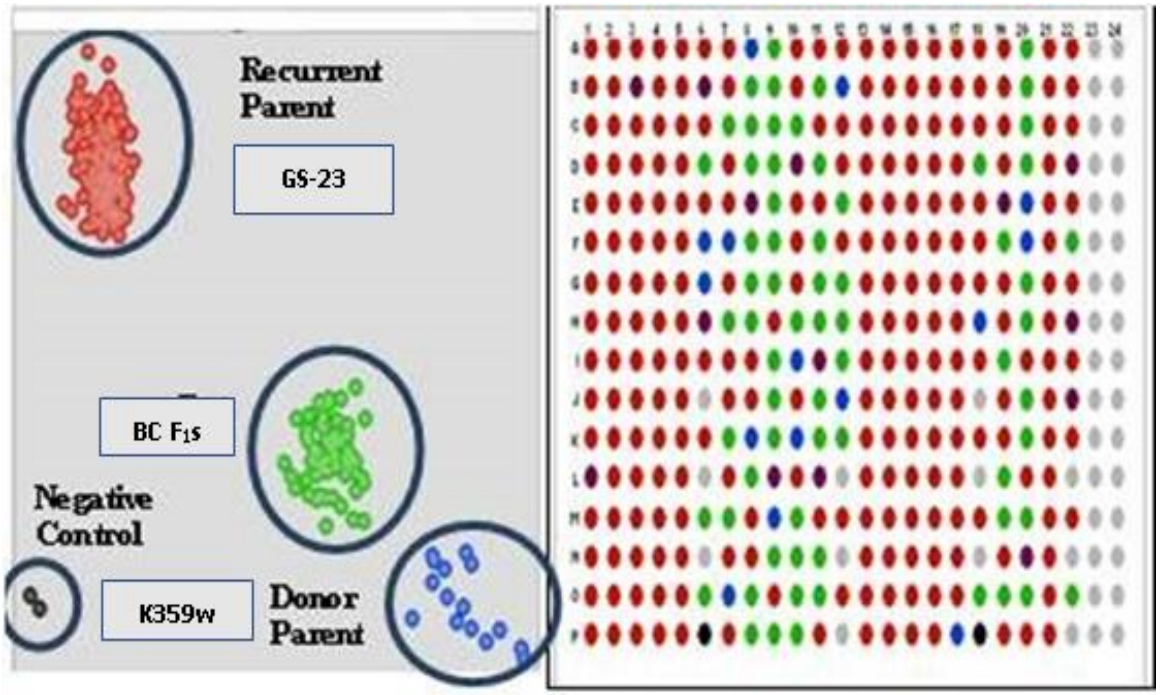

Fig. 11. KASPar assay of BC_1F_1s using SNP marker *snp_SB055* for stay green trait at maturity for cross GS 23 × K 359w

trait QTL`s *stg3A* and *stg3B* from donor parent K260 and K359w respectively in GS-23 background across backcross population and to identify plants that were homozygous for the

desired allele. Similarly, 53 polymorphic SNP markers identified were used to track introgression of stay green trait at maturity in the GS-23 background across backcross population. The results showed that the use of markers allowed for the efficient and accurate selection of plants carrying the target trait, resulting in a significant reduction in the number of generations required to completing the backcross breeding process. The plants which had desired allele showed stay greenness at maturity in field conditions. These results and study are in accordance with earlier reports of Hospital and Charcosset [13], Haussmann, et al., [14], Keshava Reddy [15], Sunil Puranik, [16] and Kamal, et al ., [17]. Similarly, Sukumaran [18] in a cross between Tx436 (food grain type) and 00MN7645 (drought tolerant) developed 248 recombinant inbred lines (RILs) and identified six QTLs for stay green, two on chromosome 4 and one each on chromosome 5, 6, 7, and 10 under drought conditions.

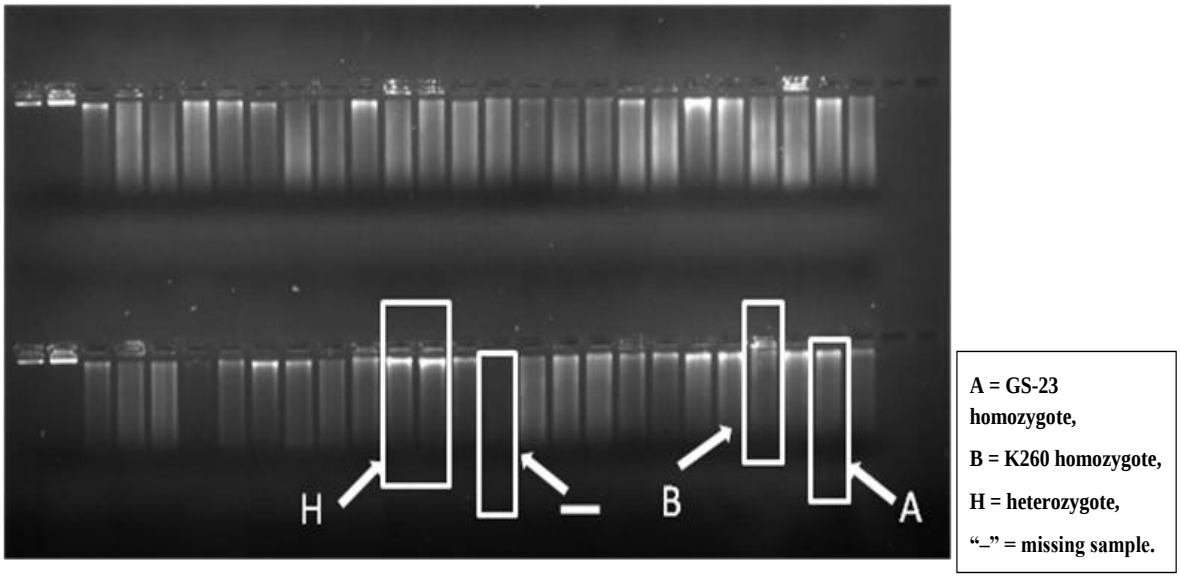

Fig. 12a. Scoring of SSR marker locus *stg3A* for the BC_1F_1 generation of GS23×K260

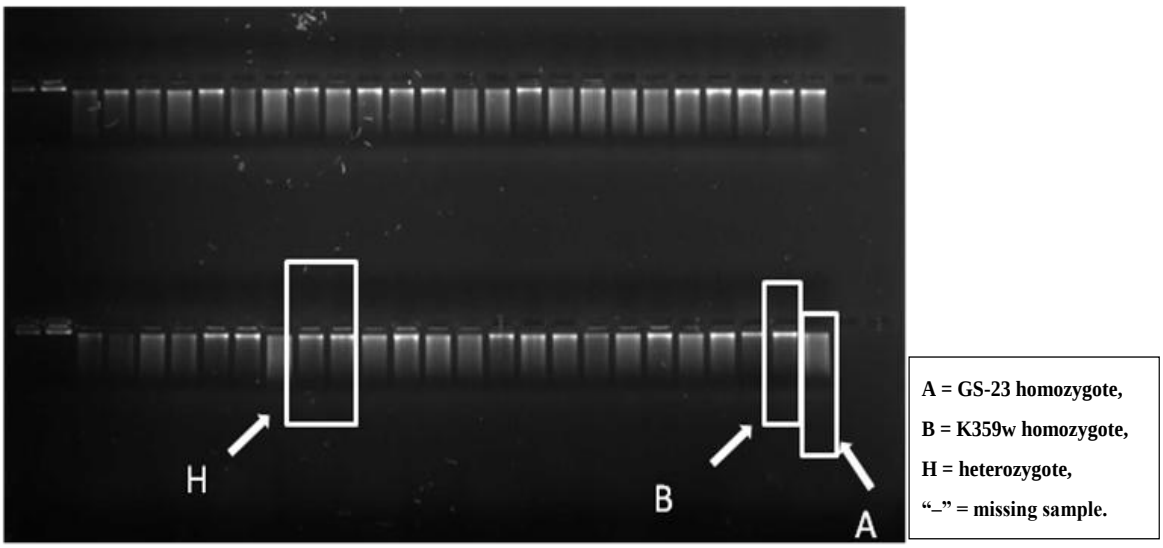

Fig. 12b. Scoring of SSR marker locus *stg3B* for the BC_1F_1 generation of GS23×K359w

4. CONCLUSION

Marker polymorphic studies utilized 133 SNPs out of which 53 SNPs were identified for polymorphism. In which 02 SNPs *SnpSB0039* and *SnpSB0093* were identified as polymorphic for both K260 and K359w parents. Whereas 17 SNPs identified polymorphic for K260 and 34 SNPs for K359w. These SNPs were validated in both F_1 and BC_1F_1 populations of both the crosses. Similarly, out of 10 SSR markers utilized, 02 SSRs were identified polymorphic to K260 and 02 SSRs were identified for K359w parent. Whereas 06 SSRs were polymorphic to both the parents K260 and K359w. Prominent 02 SSRs *viz., Xtxp 141_Fam* and *Xgap84_Vic* found to be more reliable and polymorphic to both the parents. These SSRs were validated in both F_1 and BC_1F_1 populations of both the crosses.

In conclusion, the results of this study demonstrate the potential of SNPs and SSRs as a powerful tool in marker assisted backcross breeding in sorghum. The high level of accuracy and precision achieved in this study demonstrates that KASPar is a reliable and efficient method for identifying sorghum hybrids. This is especially important in the context of seed production, where it is crucial to confirm the purity of hybrid seed to maintain the desired heterotic pattern. SSR markers can also greatly improve the speed and accuracy of the breeding process and contribute to the development of improved sorghum cultivars with higher yields and greater resistance to abiotic and biotic stresses. The results of this study provide valuable insights into the use of SNPs in backcross breeding and lay the foundation for further research into this exciting and rapidly evolving field. Fine mapping of the individual stay-green QTL can be achieved in BC_5F_1 by backcrossing selected BC_1F_1 individuals heterozygous for markers flanking single stay-green QTL and homozygous for recurrent parent alleles at all other marker loci tested, following field evaluation of the corresponding BC_1F_2 progenies to confirm that they are segregating in the expected Mendelian manner for the stay-green phenotype.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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