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RESEARCH ARTICLE

Marker assisted introgression of stay green QTLs and identification of stable high yielding stay green genotypes using AMMI based selection indices in *rabi* sorghum (*Sorghum bicolor* L. Moench)

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Abstract

Sorghum is a key cereal crop in Asia and African regions, valued for its resilience in dryland regions and used for food, fodder, and biofuel. However, in the post-rainy season, sorghum production is impacted by terminal drought stress. The stay-green trait enhances its adaptability to drought, making it a vital focus in breeding efforts. This study aimed to identify stable, high-yielding stay-green sorghum lines through marker-assisted backcross breeding. Three farmer-preferred, non-stay-green varieties *viz*., SPV2217, BJV44, and SVD0806 were crossed with stay-green donors K260 and K359w, which carry the stg3A and stg3B QTLs, respectively. Analysis of the BC_2F_2 populations of six crosses revealed high heritability, variability and genetic advance for yield traits. Foreground selection with 10 SNP markers confirmed the presence of stay-green alleles. Further evaluation in the BC_2F_3 and BC_2F_4 generations validated consistent stay-green expression. In BC_2F_4 generation, positive significant correlations were seen between most of the stay-green traits and grain yield, except rate of leaf senescence, which had a negative correlation. Field trials at four locations in the northern zones of Karnataka i.e., Dharwad, Bailhongal, Hukkeri, and Vijayapura identified genotype STG 1 as the most stable, high-yielding line, based on AMMI stability indices ASTABi and ssiASTABi, making it promising for drought-prone regions in Karnataka.

Keywords: Sorghum, staygreen, QTLs, SNP, introgressed lines, AMMI.

Introduction

Drought is the most important factor limiting sorghum productivity worldwide (Borrell et al. 2008). It is certainly of great significance in the semi-arid tropics, where limited and erratic distribution of rainfall leading to around 70% grain yield losses. The lower yields due to drought contribute to hunger, poverty, malnutrition, and high costs from imports (Ejeta et al. 2007). Sorghum is one of the crops that tolerate high temperatures and limited water or precipitation since it uses C_4 photosynthesis, similar to maize and sugarcane. However, moisture stress, especially at the post-flowering stage (terminal drought), significantly diminishes grain yield in sorghum. Stress at this stage aggravates susceptibility to charcoal rot and crop lodging, leading to poor grain filling and loss of fodder quality (Prabhakar et al.2022). Sorghum faces drought stress both at the pre-flowering stage, prior to anthesis and at the post-flowering stage,during grain-filling (Harris et al. 2007). Delayed senescence (a mechanism termed as "stay green") improves adaptation to post-flowering drought stress and has been well studied in sorghum (Blum et al. 1989).

The stay-green trait, also known as delayed senescence, is a post-flowering drought response (Jordan et al. 2012). It is characterized by the retention of green upper leaves and stems even under severe moisture stress. Stay-green genotypes maintain more green foliage at maturity, allowing continued photosynthesis to support grain filling during drought. This trait is linked to several benefits, including

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resistance to charcoal rot, stronger stalks that resist lodging, improved fodder quality, and higher grain yield (Borrell et al. 2000; Jordan et al. 2012). Additionally, stay-green is associated with elevated cytokinin levels (Rosenow et al., 1983) and increased stem sugar accumulation in basal nodes (McBee 1984). Since chlorophyll degradation is a key marker of leaf senescence, the stay-green trait indicates delayed or impaired chlorophyll breakdown (Thomas and Ougham 2014). Stay-green (SG) can be categorized into two types: functional and non-functional. Functional SG genotypes are particularly valuable in agriculture as they retain their photosynthetic capacity longer than non-SG genotypes. These functional genotypes either postpone the onset of senescence (Type-A) or begin senescence at the usual time but progress through it at a slower rate (Type-B) (Thomas and Ougham 2014). In contrast, non-functional (or cosmetic) SG genotypes undergo senescence at the typical rate, but leaf greenness persists due to disruptions in the chlorophyll degradation process, leading to reduced photosynthetic activity (Type-C). Other forms of non-functional SG include pigment retention caused by freezing or desiccation, as seen in frozen spinach or herbarium specimens (Type-D), or an inherently high pigment concentration despite normal photosynthetic function, which is classified as SG (Type-E). (Shimoda et al. 2016; Zhao et al. 2019).

Progress in genetically enhancing *rabi* sorghum for drought resilience by means of conventional breeding methods has been gradual. The efficiency of selection has been constrained by the complex interplay between genotype and environment, posing a challenge to achieving steady progress. In view of these difficulties, a QTL approach is appropriate to dissect the stay-green trait at the genomic level. Overall, seven sources of the stay-green trait have been used for QTL identification: B35 (Tuinstra et al. 1997), E36-1 (Haussmann et al. 2002), QL41 (Tao et al. 2000), SC56 (Kebede et al. 2001), 296B (Srinivas et al. 2009), SC283 (Sabadin et al. 2012), and SDS 1948-3 (Habyarimana et al. 2010). Among these, B35 (BTx642, a BC1 derivative of IS12555, durra sorghum from Ethiopia) is the most commonly used source (Rosenow et al. 1983). Studies have shown that stay-green is quantitatively inherited, and the QTLs varied across environments and years. However, six major stay-green QTLs *viz*., stgC (SBI-01), stg3A and stg3B (on SBI-02), stg1 and stg2 (on SBI-03), and stg4 (on SBI-05) were reported in several studies (Tao et al. 2000; Xu et al. 2000; Haussmann et al. 2002; Sanchez et al. 2002; Harris et al. 2007). Although mapped QTLs are from different mapping populations, their physical positions are consistent across the maps (Wang et al. 2014). Different breeding programs have been introduced to incorporate stay-green QTLs into advanced breeding lines through marker assisted backcross breeding (MABB).

Among various DNA-based markers, SNP markers effectively identify genetic polymorphisms in many crops including sorghum. Compared to canonical SNP markers that rely on electrophoresis, Kompetitive Allele-Specific PCR (KASP) markers offer advantages such as a lower genotyping error rate and compatibility with automation (Steele et al. 2025). This makes them a valuable tool alongside direct field phenotyping, helping to shorten breeding cycles when combined with traditional breeding approaches. Moreover, the co-dominant property of KASP markers allows for the identification of beneficial alleles in heterozygous states, supporting the transfer of traits through marker-assisted backcross breeding (MABB), regardless of whether they exhibit dominant or recessive inheritance. Accordingly, the present investigation aimed to introgress stay-green QTLs into agronomically superior yet non–stay-green sorghum genotypes using KASP marker-based foreground selection in combination with phenotypic evaluation. Subsequently, multi-environment trials were conducted to identify stable, high-yielding stay-green genotypes with potential utility in future sorghum breeding programs targeting arid and semi-arid regions of India.

Materials and methods

Plant materials and experimental details

The current investigation evaluated introgressed lines (from BC_2F_2 to BC_2F_4 generations) derived from six stay-green crosses developed by crossing three recurrent parents *viz.,* SPV2217, BJV44 and SVD0806 with two stay green donors K260 and K359w, having “STG 3A” and “STG 3B” stay-green QTLs, respectively. The pedigree details of these donor parents are given in Table 1. A flowchart detailing the entire MABB programme followed is shown in Fig. 1. Phenotypic evaluation was done employing augmented design at AICRP on sorghum, MARS, UAS, Dharwad in *rabi* 2019-20 (BC_2F_2) and in *rabi* 2021-22 (BC_2F_4). The details of the number of introgressed lines (ILs) evaluated in each generation from BC_2F_2 to BC_2F_4 derived from six crosses were shown in Table 2.

Genotyping using KASP markers

For foreground selection, genotyping of the BC_2F_2 population of all six crosses was performed using 10 KASP markers (Table 3) associated with stay-green QTLs. Genotyping results were visualized in SNP viewer software to identify the ILs that were homozygous for favourable alleles, heterozygotes and homozygous for alternative alleles.

Phenotyping for stay green and productivity traits

In the BC_2F_3 generation 204 introgressed families, and in the BC_2F_4 generation, a total of 256 introgressed families (Table 2) were evaluated in *rabi* 2020-21 and *rabi* 2021-22, respectively. Observations were recorded for stay green and yield traits which included, SPAD meter readings at booting (SPADB), SPAD readings at maturity (SPADM), Total number of green leaves at booting (GLB), Total number of green leaves at maturity (GLM), Per cent green leaves retained at maturity (PGLM), Green leaf area at booting (GLAB), Green leaf area

Table 1. Pedigree and salient features of donor and recurrent parents used in the study

S. No.	Donor parents	Pedigree/ Origin	QTLs/Salient feature	Source
1	K260	Derived from cross R16 × B35	STG -3A/Stay green	ICRISAT, Hyderabad
2	K359w	Derived from cross R16 × B35	STG -3B/Stay green	ICRISAT, Hyderabad
S. No.	Donor parents	Pedigree/ Origin	QTLs/Salient feature	Source
1	SPV2217	Gundu Jola × IS29404	High yielding, bold seeded, shoot fly susceptible and moderate stay green	AICRP on sorghum, Dharwad
2	BJV44	(CSV-216R × DSV5) x CSV-216R	Rabi variety, grain and roti quality on par with M 35-1, fodder quality better than M 35-1, shoot fly susceptible & non stay green	AICRP on sorghum, Dharwad
3	SVD0806	RS585 × CSV14R	High yielding, bold seeded, shoot fly susceptible and non-stay green	AICRP on sorghum, Dharwad

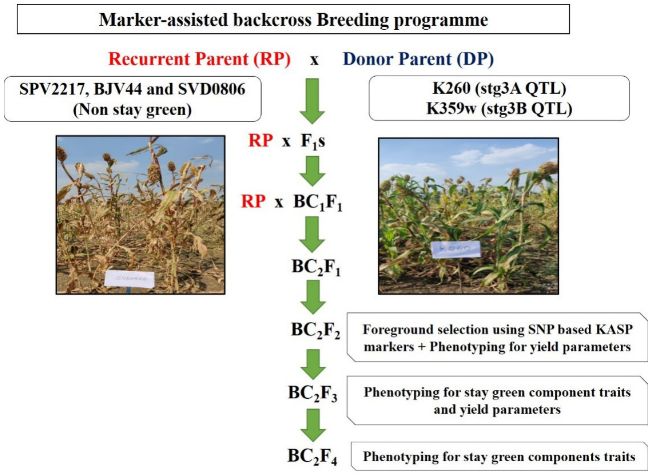


Fig. 1. A flowchart detailing the entire MABB programme

at maturity (GLAM), Per cent green leaf area retained at maturity (PGLAM), Rate of leaf senescence (RLS) in cm²/day. Yield components such as days to fifty per cent flowering (DF), days to maturity (DM), plant height (PH), panicle length (PL), panicle width (PW), panicle weight (PWT), hundred seed weight (SW) and Grain yield per panicle (GYPP) were also recorded. All observations were documented from ten randomly selected plants across two rows. Data recorded was analysed as per augmented design employing the augmentedRCBD package in R studio. Further, variability parameters, such as phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV), heritability (h^2), and genetic advance expressed as per cent of mean (GAM), were calculated as per Burton and Devane (1953), Lush (1940) and Johnson et al. (1955) respectively.Mean and variances were analyzed using the formula given by Singh and Chaudhary (1977).

AMMI based stability analysis

For stability analysis, a total of 18 genotypes (Table 4) which include 10 BC_2F_4 stay green IL's (named as STG 1 to STG 10) and 8 checks were evaluated employing randomized complete block design (RCBD) with two replications across four locations in Northern Karnataka during *rabi* 2021-22. Details of the locations where the evaluation of promising IL's was conducted for stability analysis is shown in Table 5.The average grain yield per plant was used to assess stability. AMMI stability analysis was performed using the *metan* package in R software.

Results and discussion

Variability for productivity traits in BC_2F_2 population

Variability analysis of BC_2F_4 populations of six crosses revealed sufficient variation across all segregating populations. Across all crosses, PWT and GYPP showed high PCV and GCV (>20%), indicating strong variability and potential for selection. PL exhibited high PCV and GCV in SPV2217 × K359w, BJV44 × K260, and BJV44 × K359w, while showing moderate PCV and GCV (11–20%) in the other three crosses. PW showed high PCV and GCV in all crosses except SPV2217 × K260,

Table 2. Number of introgressed lines evaluated in different generations

S. No.	Cross	BC_2F_2 (Rabi 2019-20)	BC_2F_3 families (Rabi 2020-21)	BC_2F_4 families (Rabi 2021-22)	Stability analysis- BC_2F_4 families (Rabi 2021-22)
1	SPV2217 × K260	139	34	42	2
2	SPV2217 × K359w	164	50	42	2
3	BJV44 × K260	148	22	36	1
4	BJV44 × K359w	74	12	25	2
5	SVD0806 × K260	95	46	50	1
6	SVD0806 × K359w	40	40	61	2

Table 3. List of SNPs markers associated with STG QTLs used for genotyping.

S. No.	SNP Ids	Chromosome	QTL	Alleles	Donor Allele	Alternate allele
1	snpSB00035	SBI-02	STG 3A	C/T	C	T
2	snpSB00040	SBI-02	STG 3A	C/T	C	T
3	snpSB00049	SBI-02	STG 3A	G/A	G	A
4	snpSB00054	SBI-02	STG 3A	G/A	G	A
5	snpSB00072	SBI-02	STG 3A	G/A	G	A
6	snpSB00091	SBI-02	STG 3B	A/C	A	C
7	snpSB00095	SBI-02	STG 3B	A/G	A	G
8	snpSB00098	SBI-02	STG 3B	C/G	G	C
9	snpSB00101	SBI-02	STG 3B	C/G	C	G
10	snpSB00103	SBI-02	STG 3B	C/G	G	C

where GCV was moderate. Overall, the high PCV and GCV for most traits in the stay-green crosses suggest substantial genetic variability and limited environmental influence, favouring effective selection in BC_2F_4 populations. Further, all four traits showed high heritability (>30%) and high GAM (>20%). These findings suggest that the traits have additive gene action and strong potential for response to selection. Similar conclusions were drawn by many researchers (Amare et al. 2015; Badigannavar et al. 2017; Shivaprasad et al. 2019).

Foreground selection for stg QTLs in BC_2F_2 population with SNP markers

To confirm the presence of stay-green QTLs in the BC_2F_2 backcross ILs, foreground selection was conducted using ten KASP markers, of which 5 associated with Stg3A QTL and five with Stg3B QTL (Mwamahonje et al. 2021).As an example, the genotyping result for marker snpSB00091, associated with the Stg3B QTL is shown in Fig. 2 as visualized in SNP viewer. Here, allele A is the favourable (donor) allele, while allele C is the alternative allele. Homozygous individuals with C alleles (C:C) appear in red, heterozygous individuals with C:A alleles are represented in green, and homozygous individuals with A alleles (A:A) are shown in blue. The negative control (water added to the reaction mix) is represented in black.

K260 as a donor for 'STG 3A' QTL

In the stg3A QTL analysis, five favourable alleles were identified. The donor parent K260 carried all five favourable alleles, while the recurrent parents SPV2217, BJV44, and SVD0806 had 0, 1, and 1 favourable allele, respectively. In the SPV2217 × K260 cross, one plant was carrying four favourable alleles, all in heterozygous condition. In the BJV44 × K260 cross, six plants showed more favourable alleles than the recurrent parent BJV44 (1 allele). Of these, five plants carried five favourable alleles each, and one plant carried two favourable alleles, all in heterozygous condition. In the SVD0806 × K260 cross, nine plants showed more favourable alleles than the recurrent parent SVD0806 (1 allele). Among these, six plants carried five favourable alleles, two carried four, and one carried two. Out of nine plants one had favourable alleles in homozygous condition, while the rest were in heterozygous state. In addition to the plants with higher favourable alleles, 33, 16, and 37 plants were selected based on superior grain yield per plant in the SPV2217 × K260, BJV44 × K260, and SVD0806 × K260 crosses respectively and forwarded to the BC_2F_3 generation for further evaluation.

K359w as a donor for STG 3B QTL

In the stg3B QTL analysis, five favourable alleles were identified, with the donor parent K359w carrying all the favourable alleles. The recurrent parents, SPV2217, BJV44, and SVD0806 carried only two favourable alleles. In the SPV2217 × K359w cross, one plant with four favourable alleles (three in heterozygous condition) was identified. In the BJV44 × K359w cross, two plants showed three favourable alleles in heterozygous condition, exceeding the two alleles of the recurrent parent BJV44. In the SVD0806 × K359w cross, one plant among 40 genotyped plants

Table 4. Details of promising genotypes evaluated for stability analysis

Stay green introgressed lines (ILs)			Parents/Checks		
S. No.	Stay green ILs	Pedigree	S. No.	Codes	Checks
1	STG 1	BJV44 × K260	1	C1	SPV2217
2	STG 2	SVD0806 × K395w	2	C2	BJV44
3	STG 3	SVD0806 × K359w	3	C3	SVD0806
4	STG 4	SVD0806 × K260	4	C4	K260
5	STG 5	BJV44 × K359w	5	C5	K359w
6	STG 6	SPV2217 × K260	6	C6	M35-1
7	STG 7	SPV2217 × K260	7	C7	CSV22R
8	STG 8	BJV44 × K359w	8	C8	CSV29R
9	STG 9	SPV2217 × K359w			
10	STG 10	SPV2217 × K359w			

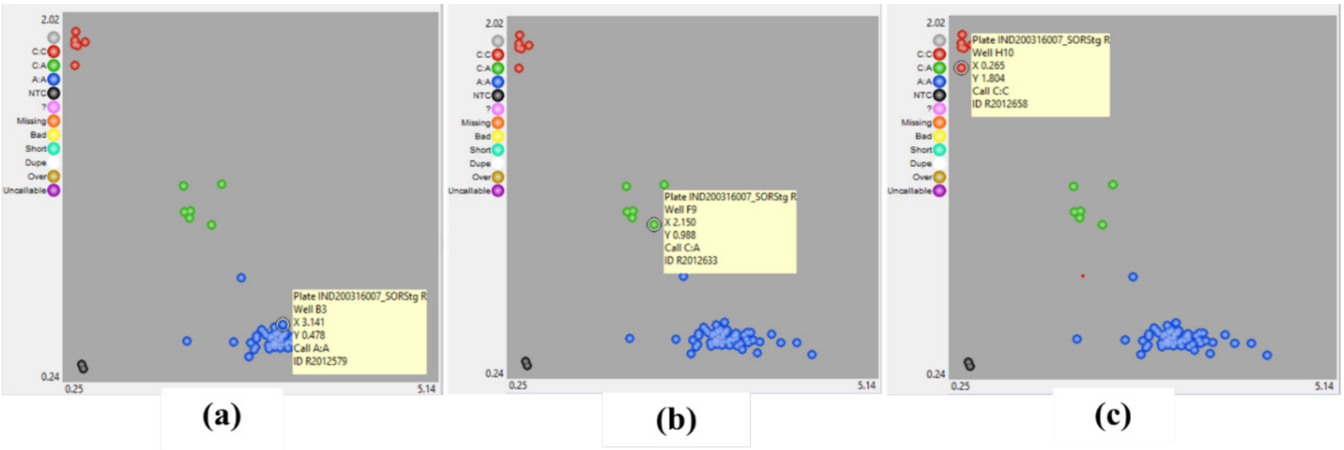


Fig. 2. The genotyping results for the marker snpSB00091, associated with the Stg3B QTL, are visualized in SNP viewer software. (a) Homozygous for donor/favorable allele, (b) Heterozygous, (c) Homozygous for alternate/unfavourable allele

exhibited four favourable alleles (two in heterozygous condition). In addition to the plants with a higher number of favourable alleles, plants showing superior grain yield per plant were also selected and forwarded to the BC_2F_3 generation for further evaluation.

Genotyping results indicated that stay-green plants derived from crosses involving the K260 and K359w donors carried more favourable SNP alleles than their recurrent parents, demonstrating successful introgression of stay-green QTLs. The stay-green nature of these BC_2F_2 plants was further confirmed phenotypically in the BC_2F_3 and BC_2F_4 generations. Similar findings were reported by Mwamahonje et al. (2021), who observed a high number of heterozygous alleles in BC_2F_1 populations involving stg3A and stg3B QTLs in sorghum.

Evaluation of BC_2F_3 and BC_2F_4 families for stay green and yield traits

The analysis of variance (Table 6) for various traits in BC_2F_3 and BC_2F_4 population revealed a significant mean sum of squares for all the seventeen traits which include stay green associated traits (GLAB, GLAM, SPADB, SPADM, GLB, GLM, PGLM, PGLAM and RLS) and yield components (DF, DM, PH, PL, PW, PWT, SW and GYPP). The estimates of mean performance and range of BC_2F_3 and BC_2F_4 families derived from the six crosses.

SPAD reading at booting and maturity stage

SPAD meter reading reflects total leaf chlorophyll content, with elevated values at booting indicating delayed senescence, sustained photosynthesis under water stress and improved grain filling (Bekavac et al. 2007). Similarly, Borrell et al. (2000) reported increased SPAD chlorophyll content in stay-green hybrids at booting compared to non-stay-green types. Further, Xu et al. (2000) also established a positive correlation between SPAD readings at maturity and the stay-green trait. Hence, SPAD values can be used as a reliable indicator for stay green phenotype. In the BC_2F_3 generation, the highest SPAD values were observed in families 30 (54.25), 33 (54.20) and 18 (54.20). In the BC_2F_4 generation, ILs 8 (56.40), 11 (55.20) and 17 (52.14) showed high SPAD readings at booting across all crosses. These ILs have exhibited higher SPAD values than the recurrent parents. At maturity, SPAD values influence photosynthesis and grain filling. Rajashekhara et al. (2025) have also showed positive association between SPAD at maturity and grain yield under drought stress in sorghum. In the BC_2F_3 generation, the highest SPAD values at maturity were observed in family 30 (40.28) for SPV2217 × K260, family 33 (44.59) for SPV2217 × K359w, family 18 (41.52) for BJV44 × K260, family 10 (40.61) for BJV44 × K359w, family 23 (46.66) for SVD0806 × K260, and family 8 (40.97) for SVD0806 × K359w, surpassing the donor parents. In BC_2F_4, ILs 11 (40.25),

Table 5. Details of the locations for evaluation of promising ILs for stability analysis

S. No.	Location	Code	Agro climatic Zone	Latitude	Longitude	Altitude	Soil type
						(Above MSL)	
1	Dharwad	E1	Zone 8	15.45⁰N	75.00⁰E	750.0 m	Deep Black
2	Vijayapura	E2	Zone 3	16.49⁰N	75.43⁰E	593.8 m	Deep Black
3	Hukkeri	E3	Zone 8	16.22⁰N	74.59⁰E	631.0 m	Shallow Soil
4	Bailhongal	E4	Zone 8	15.81⁰N	74.85⁰E	699.0 m	Deep Black

10 (39.56) and 16 (40.50)exhibited high SPAD values, with IL 8 (42.15) in SPV2217 × K260 and IL 17 (38.60) in BJV44 × K260 showing slightly lower SPAD readings than the donor parents but higher than the recurrent parents.

Number of green leaves and green leaf area at Booting and maturity stage

Another important component of the stay-green trait is the number of green leaves retained, along with the green leaf area. Rama Reddy et al. (2014) reported a strong positive correlation between GLB and GLAB, both of which were significantly associated with grain yield, underscoring their role in sink filling under terminal drought. While higher GLB and GLAB at booting enhance early photosynthetic potential, sustained GLM and GLAM at maturity are more critical for yield under drought stress. Abebe et al. (2021) reported increased GLB and GLAB in ILs derived from the stay-green donor B35. Similarly, Borrell et al. (2000) observed a more rapid decline in GLM and GLAM in senescent hybrids compared to stay-green types under drought conditions. These patterns are postulated to be attributed to delayed degradation of chlorophyll and proteins, which supports enhanced nitrogen uptake and chlorophyll retention in stay-green genotypes (Kassahun et al. 2010; Kamran et al. 2014).These points highlights that, the number of green leaves and leaf area is important features of stay green genotype. In the BC_2F_3 generation, higher GLB and GLAB values were observed in families 30 (10.64, 2057.60 cm^2), 33 (10.20, 2106.50 cm^2) and 18 (10.20, 2140.60 cm^2). In the BC_2F_4 generation, the ILs showed similar results, 8 (10.40, 2096.50 cm^2), 11 (10.80, 2140.50 cm^2) and 17 (10.60, 2108.60 cm^2) across various crosses. These values surpassed the donor parents but showed no significant increase over the recurrent parents. GLAM and GLM are strong indicators of the stay-green trait and essential for selecting drought-resistant sorghum varieties. In the BC_2F_3 generation, higher GLAM and GLM values were observed in families 30 (6.09, 1140.50), 33 (5.60, 1250.60) and 18 (5.00, 1240.50). In the BC_2F_4 generation, ILs 8 (6.00, 1324.14), 11 (5.60, 1240.60) and 17 (6.00, 1348.20) from crosses SPV2217 × K260, SPV2217 × K359w and BJV44 × K260 confirmed their role in delaying senescence.

Performance of BC_2F_3 and BC_2F_4 families for yield and yield component traits

In the BC_2F_3 and BC_2F_4 generations, promising stay-green lines from six crosses showed no significant difference in plant height compared to recurrent parents, likely due to phenotypic selection for recurrent parent traits in backcross and subsequent generations. In BC_2F_3, 7 families outperformed their parents in grain yield. In BC_2F_4, at least one ILs showed higher yield than recurrent parents in their respective crosses. Panicle characteristics such as length, width, weight, and hundred-grain weight also showed higher values in selected families compared to recurrent parents. The selected best performing BC_2F_4 ILs from all crosses were derived from BC_2F_3 families, which in turn came from BC_2F_2 plants with higher favourable alleles for stay-green QTLs. In conclusion, SPV2217 × K260 and SPV2217 × K359w exhibited higher mean values for SPAD, green leaf related traits at maturity, slower leaf senescence, and higher grain yield compared to other crosses involving BJV44 and SVD0806. This may be primarily due to the SPV2217's inherent stay-green nature with additional complementary effects from K260 and K359w's stg3A and stg3B QTLs.

Some promising stay-green lines which were observed in BC_2F_3 (e.g., families 12, 19 from BJV44 × K260 and families 3, 6, 24 from SVD0806 × K260) and BC_2F_4 (e.g., ILs 21, 32 from SPV2217 × K260, IL 26 from SPV2217 × K359w, ILs 6, 31 from BJV44 × K260, IL 20 from BJV44 × K359w, IL 49 from SVD0806 × K260, IL 43 from SVD0806 × K359w) were better for stay-green traits but slightly lower in yield. These lines can be used as potential stay-green donors for future breeding programs. The highest number of promising stay-green lines was observed in the cross BJV44 × K260 (5 lines) and SVD0806 × K260 (8 lines) in BC_2F_3. In BC_2F_4, more promising lines were obtained in the cross SPV2217 × K260 (3 lines) and BJV44 × K260 (3 lines). Ten BC_2F_4 lines, derived from the best performing BC_2F_3 families, were selected and named STG 1 to STG 10 (Table 4). These lines, with higher favourable SNP alleles for stay-green, were forwarded for multi-location yield stability trials, which will be further assessed for their potential in breeding programs without yield penalties.

Correlation among stay green traits and grain yield

A correlation analysis was conducted to evaluate the contribution of stay-green traits to grain yield per plant in ILs (Fig. 3). Most stay-green traits showed positive, significant correlations with grain yield, except for SPAD readings at booting and the number of green leaves at booting, which had non-significant correlations. Among the stay-green traits, GLM exhibited a positive correlation with grain yield (0.36), highlighting its key role in supporting grain filling under terminal drought stress. GLAB had a positive correlation with GLAM (0.33), and both were positively associated with GY (GLAB with GY = 0.18, GLAM with GY = 0.50). A higher number of green leaves or increased green leaf area at maturity improves photosynthesis and ensures better food reserve availability, contributing enhanced grain filling and yield under drought conditions. Further, RLS (rate of leaf senescence) showed a positive correlation with GLAB (0.43) but negative correlations with GLAM (-0.70), GLM (-0.60), PGLM (-0.61), PGLAM (-0.78), and GY (-0.36). Rama Reddy et al. (2014) also reported similar trend in association among stay green and grain yield.

Table 6. Analysis of variance of augmented block design for stay green and yield traits in 204 BC_2F_3 families along with five checks of sorghum

Source	df	GLAB (cm^2)	GLAM (cm^2)	SPADB	SPADM	GLB	GLM	PGLM (%)	PGLAM (%)	DFF
Treatment (Eliminating block effect)	208	44370.21**	77047.12**	27.45 **	122.59**	0.33	1.85**	142.56**	170.17**	6.11**
Checks	4	534452.95**	805666.35**	100.65**	241.45**	3.41**	7.32**	416.50**	1932.11**	108.00**
Checks vs ILs	1	2898.42	1799989.04**	138.70**	1202.71**	0.72	46.11**	2991.27**	4295.99**	175.06**
ILs	203	34917.71**	54202.70**	25.46**	114.93**	0.27	1.53**	123.13**	115.13**	3.27**
Block (Eliminating treatment effect)	3	2752.74	1384.32	0.23	1.02	0.18	0.15	9.07	2.30	0.18
Error	12	2167.46	2477.33	0.62	2.08	0.18	0.09	5.00	3.13	0.12

Source	*df*	*DTM*	*RLS (cm^2 day^{-1})*	*PH (cm)*	*PL (cm)*	*PW (cm)*	*PWT (g)*	*GYPP (g)*	*100 GW (g)*
Treatment (Eliminating block effect)	208	7.56**	41.63**	567.22**	10.42*	1.10*	360.96**	330.42**	0.12*
Checks	4	210.80**	940.06**	4099.08**	7.19	4.85**	1771.89**	1729.49**	0.21*
Checks vs ILs	1	517.57**	532.33**	2679.49**	87.61**	0.88	1220.18**	926.27**	0.66**
ILs	203	1.05**	21.51**	487.22**	10.10*	1.02*	328.93**	299.92**	0.12*
Block (Eliminating treatment effect)	3	0.45	0.21	47.77	8.22	0.27	8.82	11.64	0.02
Error	12	0.56	0.94	46.13	3.19	0.34	20.25	7.84	0.04

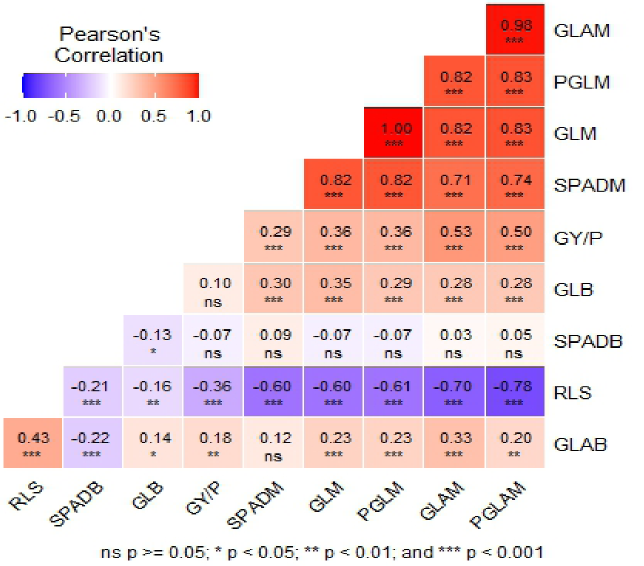
* - Significant at 5 % level of probability, **- Significant at 1 % level of probability, green leaf area at booting (GLAB), green leaf area at maturity (GLAM), SPAD at booting (SPADB), SPAD at maturity (SPADM), Number of green leaves at booting (GLB), number of green leaves at maturity (GLM), per cent green leaves at maturity (PGLM), percentage green leaf area at maturity (PGLAM), days to 50% flowering (DFF), days to maturity (DTM), plant height (PH), rate of leaf senescence (RLS), panicle length (cm), panicle width (cm), panicle weight (g), 100 grain weight (g) and grain yield per plant (g)

Stability analysis of promising ILs for grain yield over locations

Despite the development of stay-green cultivars GEI often affects performance (Reddy et al. 2011; Xu et al. 2013). Multi environmental trials are thus crucial for evaluating stability (Tadese et al. 2024; Kondombo et al. 2024). In this study, 10 stay-green genotypes and 8 checks were tested in four locations during *rabi* 2021-22. The average grain yield per plant was highest at Dharwad, followed by Vijayapura, Bailhongal and Hukkeri. Among the evaluated lines, STG 1 (from the cross BJV44 × K260) exhibited the highest grain yield per plant at Dharwad (94.95 g) and across all locations among 10 tested genotypes (Table 8).

AMMI analysis and biplots

The mean squares of GEI in AMMI analysis showed significant difference ($p<0.05$) for the grain yield per plant. Genotypes, environments and GEI effect accounted for 67.96%, 6.74%, and 10.74% of the total sum of squares, respectively. AMMI analysis revealed two significant interaction principal component axes (IPCA) (Table 8). To analyze the main effects and interactions across different environments, AMMI 1 and AMMI 2 biplots were generated for grain yield per plant (Fig. 4). In the AMMI 1 biplot (Fig. 4a), means of genotype and environment are plotted against their respective IPCA 1 scores, while the AMMI 2 biplot (Fig. 4b) displays the



Where, GLAB: Green leaf area at booting (cm2), GLM: Number of green leaves at maturity, GLAM: Green leaf area at maturity (cm2), PGLM: Percentage green leaves at maturity (%), SPADB: SPAB at Booting. SPADM: SPAB at maturity, RLS: Rate of leaf senescence, PGLAM: Percentage green leaf area at maturity (%)GLB: Number of green leaves at booting, GY/P: Grain yield per plant (g)

Fig. 3. Phenotypic correlation between stay green traits and grain yield per plant in *rabi* sorghum

Table 8. AMMI analysis of variance of ten sorghum genotypes for grain yield per plant pooled over four locations

Source	Degrees of freedom	Sum of squares	Mean sum of squares	F value	Pr(>F)	Proportion	Accumulated
ENV	3	2537.71	845.90	19.76	0.01		
REP(ENV)	4	171.23	42.81	2.29	0.07		
GEN	17	25589.83	1505.28	80.62	0.00		
GEN:ENV	51	4042.97	79.27	4.25	0.00		
PC1	19	2653.92	139.68	7.48	0.00	65.60	65.60
PC2	17	1034.51	60.85	3.26	0.00	25.60	91.20
PC3	15	354.54	23.64	1.27	0.25	8.80	100.00
Residuals	68	1269.72	18.67				
Total	194	37654.44	194.10				

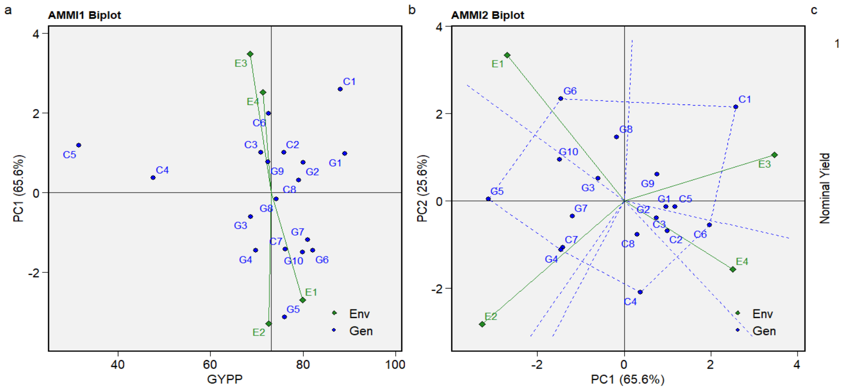


Fig. 4. (a) AMMI 1 and (b) AMMI 2 biplots for grain yield per plant

relationship between IPCA 1 and IPCA 2 scores. In the AMMI 1 biplot, differences among genotypes in terms of both direction and magnitude along the X-axis (yield) and Y-axis (IPCA 1 scores) are critical.

The genotypes C8 (CSV29R) and G9 (STG 9) demonstrated high yield with IPCA 1 scores close to zero, indicating minimal interaction with the environment. Among them, C8 (CSV29R) emerged as the overall best-performing genotype (Fig. 4a). Conversely, G1 (STG 1), C1 (SPV2217), and G2 (STG 2) exhibited high yields above the grand mean but had positive IPCA 1 scores, suggesting greater interaction with environmental factors. From AMMI 2 biplot analysis, G1 (STG 1), G2 (STG 2), G9 (STG 9), C5 (K359), G8 (STG 8) and C3 (SVD 0806) fall nearer to origin so they were identified as stable genotypes (Fig. 4b). Whereas genotype G6 (STG 6) were best for Dharwad location, G4 (STG 4) was identified as ideal for Vijayapura, C1 (SPV2217) for Hukkeri and C6 (M35-1) for Bailhongal location. Similarly, Enyew et al. (2021) used AMMI analysis to assess 320 landraces and 4 improved varieties across three Ethiopian environments and Kumari et al. (2025) used AMMI model to decipher G×E interaction and adaptability of multi-cut forage genotypes.

Stability indices based on AMMI

To better quantify stability across all PCA axes, Rao and Prabhakaran (2005) proposed the ASTABi index. This method was successfully used in many crops (Hanamaratti et al. 2010; Cheloei et al. 2020; Krishna et al. 2022). The ASTABi value reflects genotype stability, where a lower ASTABi score indicates greater stability across locations. Further, the ssiASTAB (simultaneous selection index of ASTAB) combines the grain yield component with the stability component of ASTABi, facilitating the selection of high-yielding and stable genotypes. The values and rankings for both yield and stability parameters for the studied genotypes are presented in Table 9. Genotype G1 (STG 1) exhibited the highest mean yield, followed by C1 (SPV2217) and C6 (STG 6). Genotypes G3 (STG 3) and G2 (STG 2), with the lowest ASTABi scores and ranks of 1 and 2, respectively, are identified as stable according to ASTABi. The genotypes with the lowest and highest ranks of ssiASTAB represent the most stable and the least stable genotypes, respectively, when considering both yield and stability**.** Based on the ssiASTAB rankings, G1 (STG 1), G2 (STG 2), G7 (STG 7), and G3 (STG 3) were identified as best genotypes. These genotypes were recognized for their optimal balance of yield and stability. All stay green introgressed lines evaluated for stability analysis had more favourable alleles for stay green QTLs compared to their recurrent parents (Table 9).So, the stable and high yielding genotypes STG 1 (Fig. 5) and STG 2 (Fig. 6) were also superior for stay-green expression and showed higher grain yield than their recurrent parents across environments.

In summary, improved versions of the recurrent parents carrying the stay-green trait were successfully developed, exhibiting high yield potential. The introgression lines

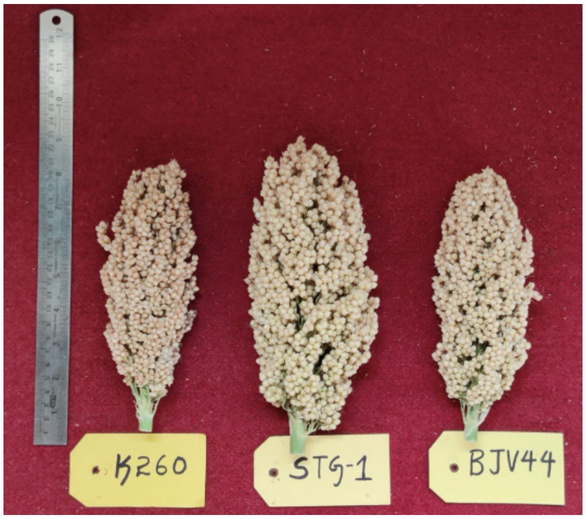


Fig. 5. **Comparison of panicle in introgressed line 'STG1' and its parents**

Fig. 6. **Comparison of panicle in introgressed line 'STG2' and its parents**

Table 9. **Ranks of selected genotypes based on grain yield, ASTAB (stability) and ssiASTAB (both yield and stability) index**

Genotypes	Pedigree	Number of favourable SNP alleles	QTL Present	Yield (g per plant)	Yield Rank	ASTAB Index	ASTAB Rank	ssiASTAB Index
STG 1 (G1)	BJV44 × K260	5	STG 3A	89.05	1	0.96	5	6
STG 2 (G2)	SVD0806 × K359w	5	STG 3B	80.08	5	0.72	3	8
STG 3 (G3)	SVD0806 × K359w	4	STG 3B	68.62	16	0.63	1	17
STG 4 (G4)	SVD0806 × K260	4	STG 3A	69.75	15	3.39	13	28
STG 5 (G5)	BJV44 × K359w	3	STG 3B	76.01	9	9.81	17	26
STG 6 (G6)	SPV2217 × K260	4	STG 3A	82.15	3	7.59	16	19
STG 7 (G7)	SPV2217 × K260	4	STG 3A	80.98	4	1.55	9	13
STG 8 (G8)	BJV44 × K359w	3	STG 3B	74.21	11	2.16	10	21
STG 9 (G9)	SPV2217 × K359w	4	STG 3B	72.45	13	0.95	4	17
STG 10 (G10)	SPV2217 × K359w	4	STG 3B	79.94	6	3.13	11	17
SPV2217 (C1)	Recurrent parent	0 2	STG 3A STG 3B	88.13	2	11.28	18	20
BJV44 (C2)	Recurrent parent	1 2	STG 3A STG 3B	75.89	10	1.47	7	17
SVD0806 (C3)	Recurrent parent	1 2	STG 3A STG 3B	70.89	14	1.47	8	22
K260 (C4)	Stay green donor (STG 3A QTL)	5	STG 3A	47.64	17	4.54	15	32
K359 (C5)	Stay green donor (STG 3B QTL)	5	STG 3B	31.5	18	1.42	6	24

generated in this study are expected to expand the sorghum varietal base and enhance climate change adaptation, particularly in semi-arid environments. These lines constitute valuable genetic resources for the development of climate-resilient sorghum cultivars. Collectively, the findings confirm that marker-assisted backcrossing, when integrated with systematic field evaluation, is an efficient strategy for introgressing target traits from donor parents while effectively recovering superior recurrent genetic backgrounds.

Authors' contribution

Conceptualization of research (NGH, RM, BDB); Designing of the experiments (NGH, BDB, RM, SEDR); Contribution of experimental materials (NGM, DSS, BDB, RM); Execution of field/lab experiments and data collection (SEDR, RK, RKM, RRM); Analysis of data and interpretation (SEDR, NGH, DSS, RKM); Preparation of the manuscript (NGH, SEDR, DBD, RM).

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