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RESEARCH ARTICLE

Performance of *bmr* 6 and 12 Sorghum Mutants in Different Wild Backgrounds Under Salinity

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Abstract Sorghum is one of the bioenergy crops, with considerable tolerance to salinity. The current work was undertaken to assess the salinity tolerance of brown midrib (*bmr*) mutant lines and wild parents for biomass composition and potential theoretical ethanol yield (TEY). The variation for salinity levels in field plots was significant; hence, salinity screening under controlled environment was performed. The mutant line N 600 (*bmr*-12) had performed better under field screening (at 10 dS m^{-1}) with fresh stalk yield of 17.3 t ha^{-1}, dry stalk yield of 7.4 t ha^{-1}, and grain yield of 2.0 t ha^{-1}. The performance of *bmr*-6 and *bmr*-12 mutant alleles showed that *bmr*-12 allele, i.e., N 597 and N 600 had performed better than its wild types EHS and Atlas, respectively, for relative fresh and dry biomass index at 20, 40 and 80 days after imposing 150 mM salinity stress. The lines N 597 (13.05 cm^2 g^{-1}), N 596 (6.84 cm^2 g^{-1}) and N 593 (7.39 cm^2 g^{-1}) recorded the highest specific leaf area at 20, 40 and 80 days of stress, respectively. High membrane stability index was recorded in mutants N 596 (*bmr*-6-85.33%) and N 597 (*bmr*-12-84.78%) with EHS though under different genetic background under stress. Higher TEY was recorded in N 597 (2219.82 L ha^{-1}), N 600 (2159.79 L ha^{-1}), N 595 (2019.03 L ha^{-1}) and N 598 (1945.33 L ha^{-1}) under stressed conditions, with a moderate reduction of 47.85 and 47.50% in 2014 and 2015, respectively, in TEY.

Keywords Brown midrib (*bmr*) · Biomass · Salinity · Sorghum · Membrane stability index (MSI) · Theoretical ethanol yield (TEY)

Abbreviations

Bmr	Brown midrib
ICRISAT	International Crops Research Institute for the Semi-Arid Tropics
SPAD	Soil plant analysis development
SLA	Specific leaf area
MSI	Membrane stability index
SHR	Seedling height reduction
RFBI	Relative fresh biomass index
RDBI	Relative dry biomass index
NDF	Neutral detergent fiber
ADF	Acid detergent fiber
ADL	Acid detergent lignin
TEY	Theoretical ethanol yields

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Introduction

Sorghum (*Sorghum bicolor* L Moench.) is a major food and forage crop in Asia and African continents, grown under rainfed conditions (Srinivasa et al. 2009; Krishnamurthy and Mand 2011) and considered as a potential bioenergy feedstock. It is grown in environmental conditions harsh for maize. With the current global climatic changes, it has gained importance for its abiotic stress tolerance and as bio-fumigation green manure crop (De Nicola et al. 2011).

Salinity is the major stress in arid and semiarid region of the world affecting the optimal crop growth during vegetative and reproductive stage thus reducing the economic yield (Athar and Ashraf 2009). Salt stress will adversely affect the plant growth by inducing external osmotic potential, which prevents water uptake, and toxicity of Na^+ ions on plant growth (Khajeh-Hosseini et al. 2003). The magnitude of salt stress is directly proportional to decrease in the shoot length as well as total biomass accumulation (Bashir et al. 2011; Rani et al. 2012). Considering the recent increase in utilization of sorghum in bioethanol industry, the sweet and energy sorghum cultivars are particularly bred as a dedicated biofuel feedstock for fodder production (Prakasham et al. 2014). The availability of low lignin containing brown midrib (bmr) mutants is considered as boon for not only dairy industry (Dien et al. 2009; Srinivasa Rao et al. 2012; Prakasham et al. 2014) but also in biofuel industry as it significantly aids in reducing pretreatment costs while converting biomass to biofuels or platform chemicals (Guragain et al. 2015). The lines with lower reduction in biomass production under saline stress are defined as salinity tolerant (Burton and Fincher 2014). Nevertheless, the assessment of sorghum genotypes for biomass yield under saline condition has been reported by numerous researchers, (Saadat and Homaee 2015; Ali et al. 2014) but the effect of salinity on the biomass or biofuel production has not been extensively studied (Vasilakoglou et al. 2011; Srinivasa Rao et al. 2010) and no report on the effect of different background of mutation in bmr allele under saline conditions (Srinivasa Rao et al. 2012) is assessed. Thus, an attempt to study the effect of wild parents and mutational allele on salinity tolerance was carried. The bmr mutants available at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) gene bank were screened for salinity tolerance, and their efficacy under stress for bioethanol production is assessed.

Materials and Methods

Glasshouse Screening

The experiments were conducted in parallel at field (100 mM) and in glasshouse (at 100 and 150 mM) during the post-rainy season of 2014. Based on their performance for biomass yield, 14 entries including the mutants and their wild parents were selected and evaluated in glasshouse at 150 mM. The level of salinity for glasshouse screening was set to double of field (i.e., 200 mM), but no plant stand was established at 200 mM; hence, 150 mM with considerable germination required to record observations was finalized. Eight bmr mutants possessing bmr-6 and bmr-12 alleles

including their wild types, making to 12 lines (Table 1) were used for screening salinity tolerance in 2014 and 2015 at glasshouse conditions in ICRISAT, India. The experimental design adopted was randomized complete block design (RCBD) with 3 replications under control and saline conditions. The pots used in the experiment had following specifications top diameter 27.5 cm, bottom diameter 20.5 cm and height 23 cm. Ten seeds of each genotype were planted in each pot under control and stress, placed at equal distance, after 2 weeks the seedlings were thinned to 5 per pot. The checks used were S 35 (tolerant check) and ICSR 170 (susceptible check) (Krishnamurthy et al. 2007). The salinity level chosen was 150 mM, applied in two split doses of 75 mM NaCl, to avoid salt shock (Shavrukov 2013). The field saturation level was achieved on a volume by weight basis (v/w), i.e., 200 mL of water is required to saturate 1 kg of pot mixture (red soil: sand in the ratio 4:3); thus, 10 kg of pot mixture was saturated with 2000 mL of 75 mM saltwater at time of sowing. A week later the second split dose of 75 mM salinity was imposed with 200 mL of 75 mM saltwater. The bottom of the pots was sealed to ensure no saltwater seepage loss. Water to the pots was not provided till second split dose and later on alternate days up to 20 days after sowing and every day at later stages of growth to replace evapotranspiration losses and bring soil moisture levels to field capacity. The water requirement was determined by weighing 5 pots, and the difference in weight was replaced to maintain field capacity. On the other hand, for control (nonsaline) pots, double distilled water was used for watering and the bottom of the pots was not sealed, so that additional water will seep out.

Field Screening

The bmr mutant (8 lines provided in Table 2) were screened in the field of Agriculture Research Station (ARS), Gangavathi, University of Raichur, Karnataka, India (Location: 15°25′48″N, 76°31′48″E. The soil has natural salinity level of 8–10 dS m^{-1} and the screening was performed during post-rainy season 2014 in a RCBD with three replications following standard management practices (House 1985). Agronomic traits like days to 50% flowering, plant height (m), fresh stalk, dry stalk and grain yield (t ha^{-1}) were recorded at maturity.

The morpho-physiological observations recorded were on plant height (cm), fresh weight (g) and dry weight (g) of plants per pot. The physiological observations were soil plant analysis development (SPAD) which was measured from top third leaf with 5 replications on each leaf using chlorophyll meter (SPAD-502Plus); specific leaf area (SLA (cm^2 g^{-1})) was measured using leaf area meter (LICOR LI-3100), and membrane stability index (MSI) was adopted from Singh et al. (2008), as follows

Table 1 *bmr*-6 and *bmr*-12 mutant lines with its respective wild parent were used for salinity screening in glasshouse at ICRISAT, India

S. No.	Wild Parent	S. No.	*bmr*-6 mutant	Pedigree of mutant	S. No.	*bmr*-12 mutant	Pedigree of mutant
1	Rox Orange (RO)	5	N 592	RO × N 121	9	N 593	RO × F 220
2	Kansas Collier (KC)	6	N 594	KC × N 121	10	N 595	KC × F 220
3	Early Hegari-Sart (EHS)	7	N 596	EHS × N 121	11	N 597	EHS × F 220
4	Atlas	8	N 598	Atlas × N 121	12	N 600	Atlas × F 220

Table 2 Mean performance of mutant lines in field condition in 10 dS m^{-1} at ARS, Gangavathi, Karnataka during Post-rainy-2014 season

SI No.	Genotypes	Days to 50% flowering	Plant height (m)	Fresh stalk yield (t ha^{-1})	Dry stalk yield (t ha^{-1})	Grain yield (t ha^{-1})
1	N 592	83^{bc}	1.99^c	10.48^c	4.36^c	1.46^b
2	N 593	84^c	1.69^{ab}	8.80^a	3.26^a	1.18^a
3	N 594	82^{ab}	1.28^a	8.52^a	3.25^a	1.07^a
4	N 595	83^{bc}	1.65^{ab}	15.81^d	5.74^e	1.15^a
5	N 596	83^{bc}	1.48^{ab}	9.74^b	3.78^b	1.19^a
6	N 597	82^{ab}	1.76^{bc}	15.28^d	6.70^f	1.17^a
7	N 598	81^a	1.73^{bc}	10.28^{bc}	4.70^d	1.23^a
8	N 600	82^{ab}	1.43^{ab}	17.30^e	7.40^g	2.00^c
Mean		82.5	1.63	1.63	4.90	1.30
SEM±		0.62	0.19	0.31	0.10	0.06
LSD 5%		1.32	0.41	0.67	0.22	0.13
CV %		1.00	14.40	3.20	2.60	5.90

SEM standard error of the mean; *LSD* least significant difference; *CV* coefficient of variation, means followed by the same letter in a column do not different significantly according to Duncan's multiple range test

$$\text{MSI } (\%) = \left[1 - \left(\frac{\text{Value } A}{\text{Value } B}\right)\right] \times 100$$

Value *A*—the conductivity of the bathing solution (double distilled hot water) after incubation; Value *B*—the conductivity of the bathing solution after disks was boiled for 15 min to kill the tissue.

Shoot height reduction (SHR), relative fresh biomass index (RFBI) and relative dry biomass index (RDBI) (Kandil et al. 2012) were the other traits recorded to assess the salinity tolerance in the given experiment.

$$\text{SHR } (\%) = \frac{\text{Shoot length in control} - \text{Shoot length in salinity}}{\text{Shoot length in salinity}} \times 100$$

RFBI (%)
$$= \frac{\text{Shoot fresh weight in control} - \text{Shoot fresh weight in salinity}}{\text{Shoot fresh weight in salinity}}$$
$$\times 100$$

RDBI (%)
$$= \frac{\text{Shoot dry weight in control} - \text{Shoot dry weight in salinity}}{\text{Shoot dry weight in salinity}}$$
$$\times 100$$

All these observations were recorded at 20, 40 and 80 days of interval from stress initiation from the 10 seedlings, except MSI, at 80 days after sowing, to see the recovery of plants at physiological maturity against stress and control. The data were recorded on shoot samples, as the main objective was to study on the effect of salinity for purpose of production of biofuel; hence, no data on roots were recorded.

Biomass Composition Protocol

The dry biomass from two seasons of salinity screening from glasshouse in three replications were sampled for quality analysis; the biomass samples, including stem and leaves, were chopped and oven-dried at 60 °C for approximately 3 days and then fine ground to pass through a sieve of pore size 210–800 µ. The powdered samples were stored in airtight covers until analysis to avoid moisture absorption. Biomass composition in Fibretherm apparatus (Gerhardt) was assessed at ICRISAT for the following traits—neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL), which were determined using 1 g of biomass sample. The calculation of theoretical ethanol yield (TEY) from the

total soluble sugars (cellulose and hemicellulose) was calculated (Zhao et al. 2009), and ADL is reported on an ash free basis.

$$NDF(\%) = \frac{\text{Dry weight of sample after treatment} - \text{Ash}}{\text{Initial weight of sample}} \times 100$$

$$ADF(\%) = \frac{\text{Dry weight of sample after treatment} - \text{Ash}}{\text{Initial weight of sample}} \times 100$$

$$ADL(\%) = \frac{(\text{Weight of crucible with sample} - \text{Empty weight crucible})}{\text{Initial weight of sample}} \times 100$$

$$Ash(\%) = \frac{\text{Weight of crucible with ash} - \text{Empty weight of crucible}}{\text{Initial weight of sample}} \times 100$$

From the above parameters, the cellulose, hemicellulose and lignin weights were determined:

$$Cellulose(\%) = \frac{(ADF - ADL)}{\text{weight of the sample}} \times 100$$

$$Hemicellulose(\%) = \frac{(NDF - ADF)}{\text{weight of the sample}} \times 100$$

$$Lignin(\%) = \frac{(ADL - Ash)}{\text{weight of the sample}} \times 100$$

$$\text{Theoretical ethanol yield}(Lha-1) = \frac{\text{celllulose} + \text{henicellulose}(\%) \times \text{dry biomass}(t\ ha^{-1}) \times 1.11 \times 0.85 \times 0.51 \times 0.85 \times 1000}{0.79 \times 100}$$

Statistical Analysis

The analysis of variance was performed for both field and the glasshouse data by using GENSTAT (14th edn. 2011, ver 14.1.0.5943, VSN International ltd). Model: $Yi = \mu + \alpha j[i] + \beta k[i] + ei$ with $ei \sim iid$ N (0, $\sigma 2$ e) μ = population mean across treatments, αj = deviation of irrigation method j from the mean, constrained to Pa $j = 1$ $\alpha j = 0$. βk = fixed block effect (categorical), $k = 1,\ldots,$ b constrained to Pb $k = 1$ $\beta k = 0$, or random effect with $\beta k \sim iid$ N (0, $\sigma 2\beta$). In the current experiment salinity treatment was considered as the fixed effect and genotypes as the random effect. Further the means comparison was performed using SPSS package (SPSS Inc. version 16.0)

for all sets of data and means were compared using Duncan multiple range test (DMRT) at $P = 0.05$.

Results

Glasshouse Screening

There was no significant variation observed for the interaction for SPAD at 40 days after sowing (DAS), NDF, ADF and Dry weight at 5% probability across the year; SPAD at 40 and 80 DAS was not significant in treatments. Lignin and PAD at 80 DAS were not significant for G*Y interactions; SLA at 40 DAS was significant across year and treatments interactions. Across genotype, year and treatments SLA and SPAD at 20, MSI, NDF and Lignin were not significant (Table 3).

The lowest seedling height reduction of stress plants over control was recorded for Kansas Collier (KC) with 29.6, 22.3 and 16.6% at 20, 40 and 80 days after stress, respectively, followed by Rox Orange (RO) with 32.6 and 25.3% of height reduction at 20 and 40 days after stress and at 80 days after stress N 597 (17.5%) (Table 4). The highest SPAD was recorded by the parent Early Hegari-Sart (46.7%), KC (44.8%) and in mutants N 593 (bmr-12) (45.21) and N 592 (bmr-6) (44.68) with RO background, at 80 days of salinity stress, but not higher than tolerant check S-35 (47.15) (Table 5). The SPAD value under saline conditions was lower than the control conditions; the average of 36.83 and 38.53 was recorded across two seasons under stress conditions. The SLA recorded at different intervals has shown reduction under stress conditions with crop growth. The average SLA recorded under saline conditions was 5.3 and 5.7 cm^2 g^{-1}, in 2014 and 2015, respectively, whereas under control conditions were 12.2 and 12.6 cm^2 g^{-1}. The lines N 597 (13.0 cm^2 g^{-1}), N 596 (6.8 cm^2 g^{-1}) and N 593 (7.3 cm^2 g^{-1}) have recorded highest SLA at 20, 40 and 80 days, respectively, although genotype with highest SLA was N 593 (7.4 cm^2 g^{-1}) and least was by N 595 (3.1 cm^2 g^{-1}) at 80 days after stress. Both of these two lines have the mutant bmr-12 allele in RO and EHS background, respectively. The MSI at 80 days after stress imposition was in the range of 74.5–85.3%. The lines with high MSI were N 596 (85.3%) and N 597 (84.7%) with EHS as genetic background with
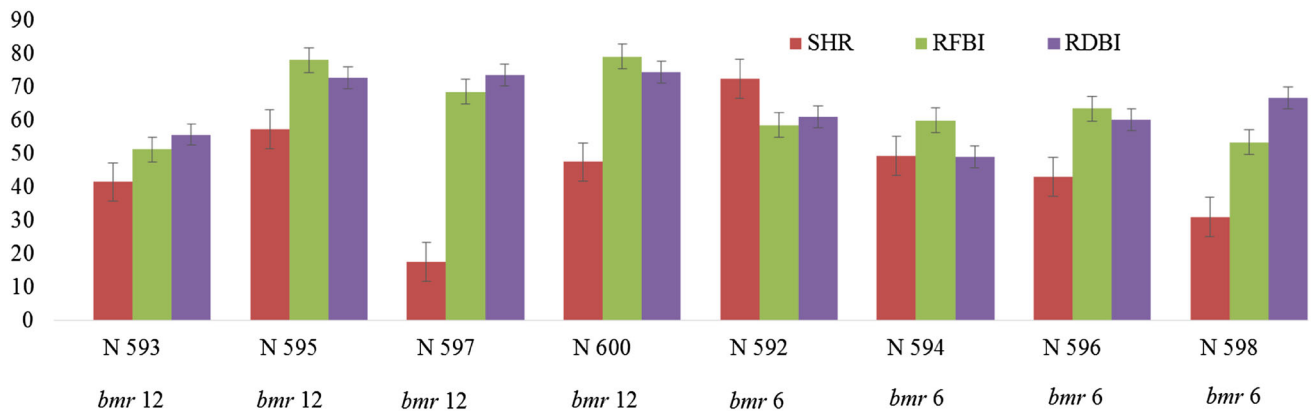
Fig. 1 Comparison across the *bmr* mutant genes containing lines for relative fresh biomass index, relative dry biomass index and seedling height reduction

bmr-6 and *bmr*-12 alleles, respectively, and N 592 (84.4%) with *bmr* 6 allele in RO background. The genotype N 596 (91.0%) recorded highest MSI under controlled conditions, followed by N 592 (90.1%) and wild parent KC (90.4%) (Table 4).

The highest salinity tolerance was recorded in N 596 (*bmr*-6) and N 597 (*bmr*-12) with EHS as background followed by N 592 (*bmr*-6) with RO. Furthermore, the mutants N 593 (*bmr*-12) (45.2) and N 592 (*bmr*-6) (44.6) with RO background have recorded highest chlorophyll. Thus, the allele *bmr*-12 has shown better physiological tolerance for salinity in RO background for both SLA and chlorophyll content at 80 days after stress. Similarly, in case of seedling height reduction, the Atlas has recorded higher reduction in seedling height (74.3%) compared to EHS (62.8%). The *bmr*-12 allele (N 597 and N 600) performed better than its wild types EHS and Atlas for relative dry biomass index and relative fresh biomass index at different intervals, whereas in case of seedling height reduction the *bmr*-6 mutant N 598 performed better than its wild type Atlas at 20, 40 and 80 days of stress (Fig. 1). The maximum RDBI was recorded by the mutants lines N 593 (49.0%) at 20, N 597 (63.9%) at 40 and N 600 (74.3%) at 80 days of stress. The lines with *bmr*-12 gene, N 600, N 597 and N 595 performed better over their wild parents for the relative dry biomass index. The parental line KC recorded high RFBI of 82.5% at 80 days of stress over mutants and checks and its *bmr*-12 mutant N 595 (69.97%) and RO (80.4%) ranked second at 40 and 80 days after stress, respectively (Table 5). A definite effect of background was observed for both EHS and Atlas on their mutants N 596, N 597 and N 598, N 600, respectively. This performance of *bmr*-6 and *bmr*-12 has shown that the mutants with *bmr*-12 have better agronomic superiority in terms of relative biomass accumulation and in seedling height reduction across the background of Atlas and EHS

(Fig. 2a, d). The average relative biomass accumulation was high in the mutants with EHS in their background (50.7 and 49.9% for fresh and dry biomass, respectively) compared to Atlas in the background (55.5 and 54.6%, respectively) (Fig. 2b–e).

Field Screening

There was non-significant variation for days to 50% flowering and plant height in field conditions, flowering duration ranged between 81 (N 598) to 84 days (N 593) and 1.3 m (N 594) to 1.9 m (N 592). The genotypic differences for fresh stalk, dry stalk and grain yield were significant (ANOVA not shown). The line N 600 was superior for the above agronomic traits (17.3, 7.4 and 2.0 t ha^{-1}, respectively), and N 594 was poor (8.52, 3.25 and 1.07 t ha^{-1}, respectively) (Table 2).

Biomass Compositional Traits and Theoretical Ethanol Yield (TEY)

The mutants with highest NDF in stressed conditions were N 595 (64.5%, *bmr*-12 allele and KC-genetic background) followed by N 598 (57.9%, *bmr*-6 allele in Atlas background) and N 593 (57.0%, *bmr*-12 allele in RO background). The percent reduction of NDF under stress over control in 2014 and 2015 was 13.9 and 12.9%. The ADF ranged from 32.3–44.6 and 36.4–42.7% in 2014 and 2015, respectively. The parental line Atlas (41.9%) has recorded highest ADF, followed by N 600 (41.7%) and N 598 (41.4%), with *bmr*-12 and 6 alleles with Atlas background, respectively. The percent reduction of ADF in 2014 and 2015 was 14.3 and 12.4%, respectively. The ADL ranged between 5.7 and 9.9% in 2014 and 6.0–10.1% in 2015;

Table 3 Combined analysis of variance for physiological and biomass quality parameters across two seasons

SV	DF	Specific leaf area			SPAD_20 DAS			MSI_80 DAS	NDF	ADF	Lignin	Dry weight	TEY	TEY
		20DAS $cm^2\ g^{-1}$	40DAS	80DAS	20DAS μmol per m² of leaf	40DAS	80DAS	%				(tons per ha)	(Liters per ha)	(Liters per ton)
G	13	137.42ᵃ	7.98ᵃ	10.43ᵃ	81.368ᵃ	49.921ᵃ	52.42ᵃ	96.60ᵃ	120.15ᵃ	67.10ᵃ	12.50ᵃ	12.08ᵃ	1,127,851ᵃ	3357.97ᵃ
Y	1	18.30 (0.003)	4.27ᵃ	4.03ᵃ	87.769ᵃ	205.957	91.89ᵃ	54.08ᵃ	0.07 (0.881)	7.05 (0.035)	5.04ᵃ	0.00 (0.989)	637,621ᵃ	6906.37ᵃ
T	1	2989.18ᵃ	1849.53ᵃ	1328.70ᵃ	786.92ᵃ	55.63 (0.014)	84.39 (0.009)	2142.46ᵃ	3326.67ᵃ	1538.71ᵃ	225.68ᵃ	1001.19ᵃ	116,233,763ᵃ	47,162ᵃ
G × Y	13	6.927ᵃ	3.15ᵃ	3.84ᵃ	16.371ᵃ	10.681ᵃ	6.26 (0.106)	17.27ᵃ	15.01ᵃ	12.48ᵃ	0.79 (0.024)	1.3519 ᵃ	134,680ᵃ	538.31ᵃ
G × T	13	61.15ᵃ	12.19ᵃ	14.91ᵃ	46.065ᵃ	42.219ᵃ	44.77ᵃ	22.21ᵃ	31.68ᵃ	9.60ᵃ	3.15ᵃ	12.72ᵃ	981,366ᵃ	603.27ᵃ
Y × T	1	3.56 (0.180)	4.43ᵃ	0.10 (0.390)	1.228 (0.573)	0.013 (0.947)	1.78 (0.506)	0.03 (0.890)	5.21 (0.223)	9.92 (0.013)	0.03 (0.760)	0.08 (0.378)	91,680 (0.017)	6.92 (0.783)
G × T × Y	13	4.85 (0.005)	3.64ᵃ	2.10ᵃ	7.426 (0.033)	11.256ᵃ	12.16ᵃ	1.56 (0.599)	16.87	9.14ᵃ	0.97 (0.005)	9.39ᵃ	187,444ᵃ	623.77ᵃ
Residual	108													

G genotypes; Y years; T treatments; DF degrees of freedom; SLA specific leaf area; SPAD soil–plant analysis development; MSI membrane stability index; NDF neutral detergent fiber; ADF acid detergent fiber; TEY theoretical ethanol yield; DAS days after stress

ᵃ Highly significant @ 5%, values in parenthesis are non-significant P value

Table 4 Mean of genotypes under stress conditions and different intervals of stress for agronomic traits

SI No.	Genotype	Seedling height reduction (%)			Relative dry biomass index			Relative fresh biomass index		
1	Atlas (NSL 3986)	143.53[g]	124.92[e]	99.50[g]	38.08[cd]	45.18[ab]	51.20[b]	42.68[ef]	44.22[b]	48.42[b]
2	Early Hegari-Sart	163.37[h]	112.91[e]	58.01[e]	19.33[a]	46.35[ab]	49.94[b]	34.36[bc]	51.76[def]	54.28[cde]
3	ICSR 170	78.93[c]	72.7[c]	63.51[e]	35.13[cd]	44.03[a]	44.35[a]	35.81[cd]	36.44[a]	42.47[a]
4	Kansas collier	29.66[a]	22.32[a]	16.66[a]	39.55[d]	61.54[ef]	68.49[ef]	46.14[f]	71.32[h]	82.50[h]
5	N 592	89.81[d]	74.8[c]	72.43[f]	35.80[cd]	55.89[cd]	61.00[d]	41.51[e]	46.8[b]	58.52[def]
6	N 593	86.86[d]	51.43[b]	41.44[c]	49.03[e]	52.70[c]	55.68[c]	40.02[de]	49.61[cde]	51.13[bc]
7	N 594	101.29[e]	74.11[c]	49.27[d]	35.66[cd]	45.56[ab]	49.02[b]	33.58[bc]	52.73[ef]	59.85[def]
8	N 595	140.53[g]	97.92[d]	57.28[e]	38.53[cd]	52.92[c]	72.66[fg]	50.91[g]	69.96[h]	78.02[h]
9	N 596	160.82[h]	51.91[b]	43.05[cd]	34.00[c]	45.38[ab]	60.00[cd]	30.18[ab]	48.79[cd]	63.48[fg]
10	N 597	66.29[b]	37.68[ab]	17.56[a]	27.31[b]	63.90[f]	73.62[g]	27.44[a]	61.04[g]	68.48[g]
11	N 598	105.2[e]	52.79[b]	31.0[b]	37.99[cd]	58.88[de]	66.58[e]	36.76[cd]	50.50[de]	53.36[bc]
12	N 600	112.88[f]	96.49[d]	47.44[cd]	36.31[cd]	59.15[de]	74.31[g]	55.65[h]	58.54[g]	79.02[h]
13	Rox Orange	32.58[a]	25.34[a]	18.62[a]	46.07[e]	48.62[b]	67.41[e]	52.59[gh]	54.56[f]	80.42[h]
14	S-35	33.29[a]	27.47[a]	15.06[a]	56.93[f]	77.06[g]	84.49[h]	56.38[h]	75.38[i]	78.25[h]
	Mean	96.08	66.66	45.07	37.84	54.07	62.77	41.72	55.12	64.16
	Maximum	163.37	124.92	99.5	56.98	77.06	84.5	56.39	75.38	82.51
	Minimum	29.66	22.32	15.06	19.34	44.04	44.35	27.45	36.44	42.48
	LSD @ 5%	7.7	13.23	6.97	4.59	3.96	4.33	4.29	3	5.18
	SEM±	2.73	4.68	3.49	1.63	1.4	1.53	1.52	1.06	1.83

SEM standard error of the mean; *LSD* least significant difference, means followed by the same letter in a column do not different significantly according to Duncan's multiple range test

ADL % was high in mutant N 592 (10.0%, *bmr*-6 allele in RO background) followed by N 593 (8.6%, *bmr*-12 allele in RO background). The lower ADL was recorded in parents, KC and Atlas (6.3 and 6.5%, respectively) (Table 4).

The range of TEY (L ha^{-1}) for *bmr* mutants and their wild parents was from 1228.6 to 2219.8 and 2738.7 to 4287.1 L ha^{-1} under stress and controlled condition, respectively (Fig. 3a). Under stressed conditions, TEY recorded higher in *bmr* mutants than their parents for N 597, N 600, N 595 and N 598 with 2219.8, 2159.7, 2019.0 and 1945.3 L ha^{-1}, respectively, whereas at controlled conditions, the Atlas (4287.1 L ha^{-1}) and EHS (3890.5 L ha^{-1}) yield higher TEY than other wild types and mutant lines, followed by the *bmr*-6 and *bmr*-12 mutants of Atlas, N 598 (3660.9 L ha^{-1}) and N 600 (3610.7 L ha^{-1}), respectively. The TEY in terms of liters per ton of dry weight (L t^{-1}) for *bmr* mutants and their wild parents ranged from 241.3 to 298.0 L t^{-1} and 264.3 to 337.9 L t^{-1} under stress and control conditions, respectively (Table 4). The high TEY (L t^{-1}) observed for the mutant line N 595 over other wild types and mutant lines at both stress and controlled conditions with 298.0 and 337.9 L t^{-1}, respectively, and N 597 ranks second in controlled condition with TEY of 304.2 L t^{-1}. The maximum TEY per ton under stress was recorded for tolerant check S-35 with 301.0 L t^{-1} (Fig. 3b).

Discussion

The field salinity level will not be uniform throughout the experiment plot; hence to study the treatment effect the glasshouse screening was performed in parallel (Vadez and Tom 2007). The effect of salinity is not significant from the ANOVA across years, but significant variations were observed for genotypic differences and at different crop growth periods (Hassanein et al. 2010; Munns and James 2003; Ramesh et al. 2005). The leaf area has reduced under stress conditions by 64% (Netondo et al. 2004; Rishi and Sneha, 2013). This reduction could be due to the damage caused by salinity at initial stages affecting the cell growth, division and cell wall expansion (Giaveno et al. 2007; Munns and James 2003; Vile et al. 2005). The mutant N 593 has recorded highest SLA with *bmr*-12 allele and RO background under stress condition. Thus, the effect of mutant allele *bmr*-12 in different background is noteworthy (Dien et al. 2009), as in the mutant N 595 has *bmr*-12 allele but with EHS as parent. Similar effect of salinity on chlorophyll content (SPAD value) was observed earlier (Meloni et al. 2003).

The osmotic stress caused by the salinity in seedling stage could lead to membrane damage similar to drought stress; thus, MSI was used as a criteria to evaluate the recovery of the plants from stress at later stages of plant growth (Saravanavel et al. 2011; Munns and Tester 2008).

Table 5 Performance of genotypes for physiological and biomass quality parameters under stress and control conditions across two seasons

Season	Salinity	2014				2015			
Parameters		Mean	Maximum	Minimum	LSD @ 5%	Mean	Maximum	Minimum	LSD @ 5%
SLA_20 DAS	Stress	6.67	12.07	3.53	2.23	7.62	14.40	2.76	2.35
	Control	15.40	33.40	11.22		15.77	27.55	11.49	
SLA_40 DAS	Stress	4.37	6.59	2.54	0.68	4.37	7.09	2.90	0.63
	Control	10.68	12.43	9.05		11.33	16.83	9.23	
SLA_80 DAS	Stress	4.89	7.65	3.15	0.67	5.15	7.53	3.17	0.54
	Control	10.47	12.39	8.98		10.82	15.51	8.03	
SPAD_20 DAS	Stress	27.86	38.25	22.84	2.58	29.48	34.44	24.01	3.70
	Control	32.36	39.13	27.82		33.63	38.27	26.22	
SPAD_40 DAS	Stress	39.53	45.47	33.91	2.98	41.73	45.32	39.14	2.59
	Control	40.66	46.92	32.54		42.89	50.37	37.43	
SPAD_80 DAS	Stress	43.09	48.51	38.07	3.37	44.39	48.48	41.41	3.17
	Control	44.41	49.62	33.81		46.10	53.17	39.79	
MSI_80 DAS	Stress	79.81	85.63	74.11	2.38	80.92	85.25	75.02	1.97
	Control	86.93	91.03	78.19		88.09	91.16	82.15	
NDF %	Stress	57.17	69.34	52.25	3.43	57.57	63.78	52.43	2.55
	Control	66.43	73.48	60.64		66.12	75.54	59.97	
ADF %	Stress	39.12	44.64	32.33	1.86	39.20	42.73	36.42	2.15
	Control	45.66	49.53	42.67		44.77	49.64	40.29	
Lignin %	Stress	7.87	9.89	5.75	0.98	7.56	10.15	6.01	1.03
	Control	10.22	13.24	8.28		9.84	11.91	7.91	
TEY (L ha^{-1})	Stress	1863.67	2949.04	1216.45	227.60	1787.17	2535.50	1240.86	174.95
	Control	3573.96	4977.09	2763.40		3404.03	4253.03	2714.13	
TEY (L t^{-1})	Stress	268.84	327.23	236.43	17.31	255.61	297.08	239.26	13.32
	Control	301.94	331.92	273.50		289.53	343.88	255.18	

SLA specific leaf area; *SPAD* soil–plant analysis development; *MSI* membrane stability index; *NDF* neutral detergent fiber; *ADF* acid detergent fiber; *TEY* theoretical ethanol yield; *LSD* least significant difference; *DAS* days after stress

The MSI under stress conditions recorded were lower than under controlled conditions at 80 days after stress, indicating that the plants have recovered from the saline stress (Macharia et al. 1994) or might have acclimatized to saline conditions. A significant variation was observed among genotypes under stress and control, but the difference of percent membrane stability between salinity stress and control conditions is trifling (Ali et al. 2009). The RFBI and RDBI of *bmr* mutants and wild types have decreased significantly under the stress conditions with concomitant seedling height reduction, because of a salt accumulation in stem cells decreased the biomass production (Kandil et al. 2012; Incesu et al. 2014). The chlorophyll content and dry biomass accumulation have shown significant positive correlation at 20 and 40 days under stress conditions ($R^2 = 0.595$ and 0.527, respectively). Thus, chlorophyll content and the biomass accumulation are positively correlated and both reduced with increase in stress at vegetative stage (data not shown). The mean performances of *bmr*-12 mutant alleles viz., N 600, N 597 and N 595 across two seasons were greater than their wild parents (Atlas,

EHS and KC, respectively). The check S-35 has recorded the maximum dry and fresh biomass index (relative basis) (Krishnamurthy et al. 2007). The wild parent KC has recorded the maximum relative fresh biomass index than their mutant lines and tolerant check at 80 days of stress.

The significant reduction in theoretical ethanol yield in stressed condition is attributed to lower ADF, NDF and RDBI values (de Lacerda et al. 2005; Vasilakoglou et al. 2011). The NDF and ADF levels were slightly high in wild types than mutants, and also in *bmr*-12 mutants than *bmr*-6 mutants (Scully et al. 2015); these may be due to the abundance cell wall polysaccharides. The percent ADL reduction in mutant lines of EHS (N 596 and N 597) and Atlas (N 598 and N 600) was 25 and 29%, respectively, compared to the parents (Scully et al. 2015; Sattler et al. 2014; Cherney et al. 1988; Porter et al. 1978), suggesting that significant reduction of lignin upon the mutation of alleles, with 22–24% reduction across *bmr*-6 and *bmr*-12 mutants (Dien et al. 2009). The TEY (L ha^{-1}) decreased with the effect of salt stress, since the plants exposed to salinity would decrease the carbohydrate accumulation in
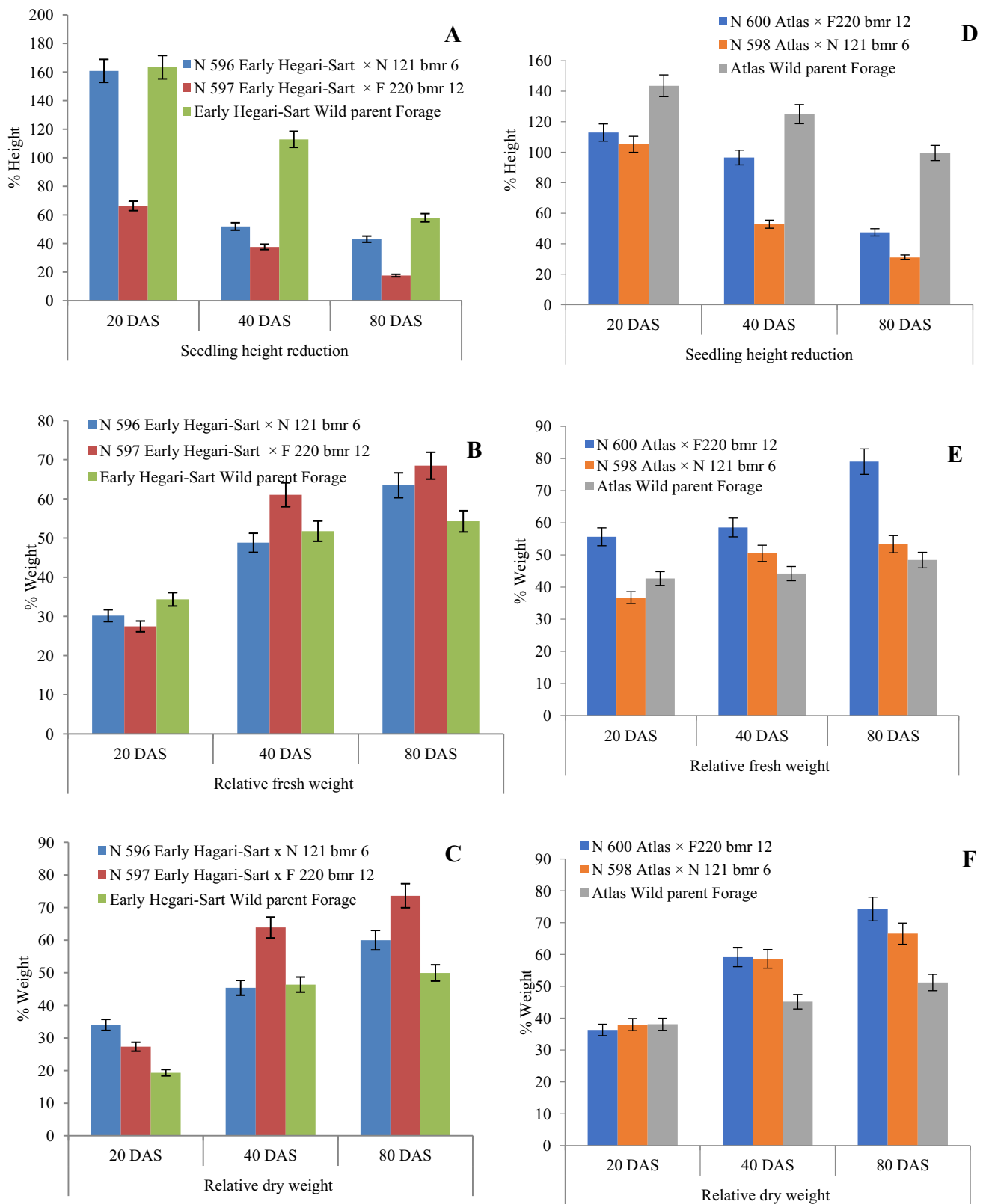
Fig. 2 Effect of background under stressed conditions across two seasons 2014–15. **a** Seedling height reduction, **b** relative fresh biomass index; **c** relative dry biomass index; in the background of Early Hegari-Sart, **d** seedling height reduction, **e** relative fresh biomass index; **f** relative dry biomass index in the background of Atlas
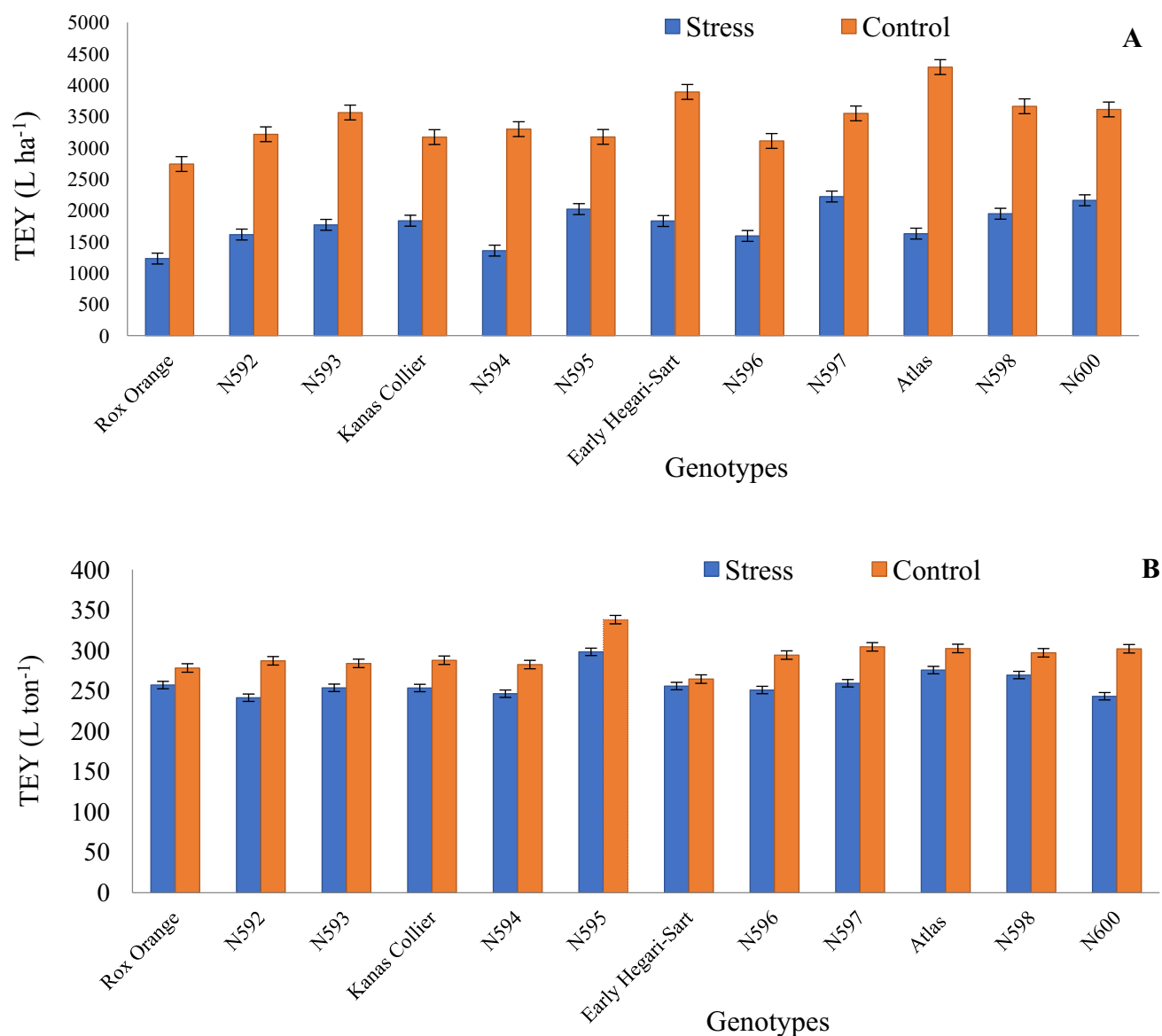
Fig. 3 Mean performance of genotypes for theoretical ethanol yield (TEY) under stress and control conditions. **a** TEY (L ha^{-1}) and **b** TEY (L t^{-1})

stem cells (Almodares et al. 2008; Vasilakoglou et al. 2011). Salinity negatively affected biomass quality and quantity that resulted in significant TEY reductions. Such fluctuations in biomass quality and yield may have significant consequences for developing lignocellulosic biorefineries. The *bmr* mutants (N 595, N 597, N 598 and N 600) have significantly higher TEY compared to wild parents (KC, EHS and Atlas), due to the decrease in dry biomass yield of wild parents under saline conditions (Vasilakoglou et al. 2011), whereas in control conditions, mutants TEY was lesser than their parents, contrasting to that of saline conditions. Similar trend have been predicted in TEY (L t^{-1}), that the mutants with *bmr*-12 in the wild type KC background under stressed and control conditions

have recorded higher yield. When reduction in quality and quantity were combined, TEYs decreased by 26–59%. In a related study, we determined that lignin structure and crystallinity of cellulose have a pronounced effect on actual and theoretical ethanol yields from biomass mutants (Guragain et al. 2014)

Conclusion

This study revealed that the salinity stress has caused considerable amount of decrease in the plant height and biomass accumulation in the mutants and wild parents. Among *bmr*-6 and *bmr*-12 mutant alleles studied, *bmr*-12

(N 600, N 597 and N 595) have better agronomic superiority in terms of biomass accumulation and TEY (L ha^{-1}) at both field and glasshouse conditions. The wild parent EHS has shown better agronomic superiority in its mutants (N 596 and N 597) and in particularly for *bmr*-12 (N 597). The lignin percent reduction under stress condition was up to 25% in EHS background. The *bmr* lines were found to be moderately tolerant under stress conditions, the mutant lines (N 597) outperformed the wild types under salinity, and it can be concluded that these lines can be used for bioethanol production on marginal lands and/or for feeding the livestock.

Compliance with Ethical Standards

Conflict of interest All the authors declare that there is no conflict of interest in publishing performance of bmr 6 and 12 sorghum mutants in different wild backgrounds under salinity in Sugar Technology.

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