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Research Article

SiDREB2-based SNAP Marker-Assisted and Multi-Trait Selection in The Early Generation of Foxtail Millet (*Setaria italica* L. Beauv.)

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ABSTRACT

Setaria italica L. or foxtail millet is known for its nutritious grains and adaptability to unfavorable environmental conditions. High productivity, early heading, medium stature, and tolerance to drought- or salinity stress are among the breeding objectives for foxtail millet. The objective of this study was to select F_3 families of foxtail millet from the cross of Botok-10xICERI-6 by weighted selection index and assisted by *SiDREB2*-based SNAP marker. Genotyping of 178 F_3 families using the *SiDREB2*-based SNAP marker resulted in 29 A/A genotypes, 121 A/G genotypes, and 28 G/G genotypes. Further evaluation was conducted on 48 F_3 families consisting of 27 A/A genotypes and 21 A/G genotypes in an augmented randomized complete block design together with their parental genotypes (Botok-10xICERI-6) and three check genotypes (Mauliru-2, NTB-1, and Toraja). Plant height and heading time had high broad-sense heritability, whereas grain weight per plant had a moderate broad-sense heritability. Ten potential F_3 families were selected based on a weighted selection index with 20% intensity, comprised of seven A/G genotypes and three A/A genotypes with a weighted selection index ranging from 0.84 to 3.76. The F_3 family with pedigree numbers B10I6-15-136, B10I6-15-161, and B10I6-15-70 with A/A genotypes are considered putative transgressive segregants and could be continued to the next generation for further breeding process.

INTRODUCTION

Millet is a group of underutilized small-seeded cereals from the Panicoidae subfamily commonly cultivated in areas with water scarcity (Panchal et al. 2023). India, African countries, and China are the top three global millet producers (FAO 2021). One of the major millet produced globally is foxtail millet (*Setaria italica* L. Beauv) which ranks second after pearl millet (*Pennisetum glaucum*) (Panchal et al. 2023). The other members of millet include barnyard millet, finger millet, kodo millet, little millet, and proso millet (Saini et al. 2021). Foxtail millet is

considered a functional food due to its nutritional benefits, including its low glycemic index and high contents of protein, dietary fiber, and antioxidant in its grain (Arora et al. 2023). Additionally, several health benefits have been reported for foxtail millet, including cancer (Zhang & Liu 2015) and cardiovascular disease (Jali et al. 2012) prevention. Broad adaptation of foxtail millet to unfavorable environmental conditions, including drought (Xiao et al. 2021) and salinity (Ardie et al. 2015; Han et al. 2022) has increased the importance of this species in marginal areas. Despite the remarkable benefits of this species, foxtail millet is not a popular food crop in Indonesia.

Recombination breeding through hybridization is a conventional yet useful strategy for generating a superior variety (Al-Khayri et al. 2019). However, the self-pollinated nature, floral morphology, tiny size of the flower, and anthesis behavior are the main challenges in the hybridization of foxtail millet (Moharil et al. 2019; Nagaraja et al. 2023) leading to no Indonesian superior variety of foxtail millet has been released to date. Nugroho (2020) induced male sterility in foxtail millet by warm water treatment to facilitate artificial hybridization of Indonesian local foxtail millet genotypes, namely Botok-10 and ICERI-6. Botok-10 is a local foxtail millet genotype from East Nusa Tenggara with relatively high potential productivity but with tall plants, and late heading time. Meanwhile, ICERI-6 is one of the foxtail millet collections in the Indonesian Cereals Research Institute (ICERI) with moderate plant height, and early heading time but low potential productivity (Ratnawati et al. 2024). Furthermore, molecular assessment using the *SiDREB2*-based SNAP marker categorized ICERI-6 as a tolerant genotype, while Botok-10 as a sensitive genotype to salinity or drought stress (Widyawan et al. 2018). A suitable selection strategy is necessary to identify progenies with high productivity, moderate plant height, early heading, and tolerance to drought or salinity stress from the recombination of Botok-10xICERI-6.

One of the selection methods commonly applied for multiple traits is weighted index selection, in which relative weights are used for traits of interest (Moeinizade et al. 2020). A weighted index selection based on productivity, heading time, and plant height was used to select superior F_2 individuals from the crosses of ICERI-5 x Botok-10 (Sintia et al. 2023). However, this method was not able to identify F_2 individuals potentially tolerant to drought or salinity stresses. Plant abiotic tolerance evaluation requires proper experimental design and replications for an accountable result (Negrão & Julkowska 2020). Therefore, phenotypic selection for abiotic stress tolerance is impractical to be performed in the early generation of a segregating population. Marker-assisted selection (MAS) with proper molecular markers is expected to overcome such challenges in early-generation selection (Hasan et al. 2021).

The dehydration-responsive element binding (DREB) is a plant transcription factor involved in the complex regulatory tolerance mechanisms to drought and salinity stresses in many plants (Singh & Chandra 2021). A *DREB2* homolog in foxtail millet, *SiDREB2*, was reported to possess single nucleotide polymorphism (SNP) at the 558^{th} nucleotide (an A/G substitution), and this SNP was further associated with drought tolerance in foxtail millet (Lata et al. 2011). A *SiDREB2*-based single nucleotide amplified polymorphism (SNAP) marker was further developed by Widyawan et al. (2018) to estimate the tolerance level of foxtail millet to drought or salinity. As part of foxtail millet breeding through hybridization, the objective of this study was to select

F_3 families from the cross of Botok-10xICERI-6 by a weighted selection index and assisted by *SiDREB2*-based SNAP marker.

MATERIALS AND METHODS

SiDREB2-based SNAP marker-assisted selection of F_3 family derived from Botok-10xICERI-6 cross

Genetic materials

The parental genotypes Botok-10 (a local foxtail millet genotype from East Nusa Tenggara, Indonesia) and ICERI-6 (a collection of Indonesian Cereals Research Institute, ICERI), and 178 F_3 families from the cross of Botok-10 and ICERI-6 were used as genetic materials in this experiment. Seeds from each parental genotype and F_3 family were sown in two tray holes with ten seeds per hole in seedling trays containing compost and manure (1:1, v/v). The shoot parts of 14-day-old seedlings were harvested as a bulk sample (10-20 seedlings per F_3 family number) and were preserved in a 2 mL microtube containing 700 µL CTAB (Cetyl-Trimethyl Ammonium Bromide) buffer at -20°C for further DNA isolation.

Total Genomic DNA isolation and DNA amplification.

The CTAB method (Doyle & Doyle 1990) was used to extract total genomic DNA from the shoot parts of 14-day-old seedlings with slight modification namely, we exclude the use of 0.2% (v/v) 2-mercaptoethanol in the lysis buffer. The *SiDREB2*-based SNAP markers consisted of two forward primers and one reverse primer as listed in Table 1. The PCR reaction with a total volume of 10 µL consisted of genomic DNA (2.5 µL, 12 ng.µL^{-1}), forward (SD2-558-SNP-A or SD2-558-SNP-G) and reverse primer (SD2-558-SNP-Rev) (2.5 µL, 10 pmol), and 5.0 µL of 2× PCR mix (KAPA2G Fast HotStart ReadyMix, Sigma-Aldrich, Germany). The PCR was performed using Esco's Swift Maxi Thermal Cycler (Esco Technologies, Singapore) following the PCR profile reported by Ratnawati et al. (2024).

Analysis of molecular data

Successful amplification using forward primers (SD2-558-SNP-A or SD2-558-SNP-G) and reverse primer (SD2-558-SNP-Rev) resulted in a 300 bp amplicon. Amplicons were analyzed by electrophoresis at 90 volts for 40 minutes in 1x TAE buffer on 1.5% (w/v) agarose gel. The agarose gels were immersed in ethidium bromide solution (0.5 µg.mL^{-1}) prior to gel visualization using a UV transilluminator (AlphaImager® Mini). The *SiDREB2*-based SNAP marker-assisted selection was conducted by evaluating the presence of a 300 bp band for the A allele, G allele, or both A and G alleles in particular F_3 family (Figure 1). The band specific for the G allele appeared in the female parent genotype (Botok-10), while the band specific for the A allele appeared in the male parent (ICERI-6).

Table 1. The *SiDREB2*-based SNAP marker used in this experiment.

Primer name	Nucleotide sequence (5'-3')	Tm (°C)	Primer type
SD2-558-SNP-G	GCAAGTCCGTGGAGGTACTACAG	58.8	Forward
SD2-558-SNP-A	AAGTCCGTGGAGGTACTGCAA	58.3	Forward
SD2-558-SNP-Rev	AGGAACTCAACACACAGGACAACT	57.9	Reverse

Source: Widyawan et al. (2018)

Weighted index selection of Botok-10xICERI-6 derived F_3 family

Plant materials

Fifty F_3 family numbers were further selected from the above 178 F_3 families for further field evaluation. These 50 F_3 families consisted of 29 A/A genotypes and 21 A/G genotypes based on *SiDREB2*-SNAP marker. However, two F_3 family numbers with A/A genotypes failed to grow in the field and further analyses were conducted on the remaining 48 F_3 family. Five check genotypes used include the parental genotypes (Botok-10 and ICERI-6) and three local genotypes (Toraja, NTB-1, and Mauliru-2). The Toraja genotype originated from Sulawesi, the NTB-1 genotype originated from West Nusa Tenggara (NTB-1), and the Mauliru-2 genotype originated from East Nusa Tenggara.

Procedure

The experiment was conducted from January to May 2023 in the Cikabayan Bawah Experimental Station of IPB University, Bogor, Indonesia (6°33'24.23"S, 106°43'33.4"E). The agro-climates conditions during this experiment were recorded to be 21.43°C average temperature, 87.82% average humidity, and 1,100 mm per month average rainfall (BMKG 2023). This experiment was arranged in an augmented randomized complete block design with five replicates. Each replicate was a 40 m x 0.8 m size block consisting of ten F_3 family numbers and five check genotypes. Each F_3 family number and check genotype were planted in three rows, resulting in 45 planting rows per block. Each row consisted of eight plants, with plant spacing of 75 cm x 10 cm.

Seeds were subjected to hot water treatment to reduce the risk of seed-borne fungi as described by Parlindo et al. (2022). Treated seeds were then directly sown in planting holes containing 3% Carbofuran. Fertilizers of SP-36 (150 kg.ha^{-1}) and KCl (75 kg.ha^{-1}) were applied two weeks after planting (WAP), while urea was applied two times at 2 and 6 WAP with the rate of 150 kg.ha^{-1} at each application. A plant net was installed at 2 WAP to prevent crop loss due to birds.

The observation was conducted for 11 characters according to the UPOV descriptor (UPOV 2013) on the following characters: plant height (cm), the length and width of flag leaf (cm), stem diameter (mm), heading time (DAP), harvest time (DAP), 100-grain weight (g), the length (cm) and weight (g) of main panicle, main panicle grain weight (g), and grain weight per plant (g).

Data analysis

The obtained phenotypic data were analyzed for variance components estimates, phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), broad-sense heritability (h^2_{bs}), and weighted selection index using SAS, Minitab 19, and Microsoft Excel software. The variance component estimates include phenotypic variance (σ^2_p), environmental variance (σ^2_e), and genetic variance (σ^2_g) as described by Mahmud & Kramer (1951). The PCV and GCV were divided into three categories according to Knight (1979), namely low (0-10%), moderate (10 -20%), and high (>20 %). The classification of broad-sense heritability followed the classification of Stansfield (1991): high (50% ≤ h^2_{bs} < 100%), moderate (20% ≤ h^2_{bs} < 50%), and low (0 ≤ h^2_{bs} < 20%). An equation reported by Sintia et al. (2023) was used to calculate the weighted selection index (SI) as follows: SI = – plant height – heading time + (3 x grain weight per plant).

Scatter plots were built using Microsoft Excel based on the selection index (Y-axis) and means of standard deviation of the three

targeted traits (X-axis). The mean of the standard deviation of five check genotypes is indicated by the vertical dashed line, while the mean of SI calculated from 48 F_3 families is shown by the horizontal dashed line. Only 25 F_3 families with a minimum of 12 observable plants per family were mapped in the plot.

RESULTS AND DISCUSSION

SiDREB2-based SNAP marker-assisted selection of F_3 family derived from Botok-10xICERI-6 cross

Molecular markers have been widely used to improve abiotic stress tolerance in crops (Younis et al. 2020). Our study suggests that marker-assisted selection using the *SiDREB2*-based SNAP marker is a simple method to select potentially drought- or salinity-tolerant lines in an early segregating population. Figure 1 shows the representative visualization of amplicons using a particular primer pair. The A/G genotype was indicated by 300 bp amplicons produced by both SD2-558-SNP-A/Rev and SD2-558-SNP-G/Rev primer pairs. The A/A genotype only showed the 300 bp amplicon produced by SD2-558-SNP-A/Rev primer pair, while the G/G genotype only showed the 300 bp amplicon produced by SD2-558-SNP-G/Rev primer pair. The 300 bp amplicons produced by the SD2-558-SNP-A primer indicate tolerant genotypes, while amplicons produced by the SD2-558-SNP-G primer indicate sensitive genotypes (Widyawan et al. 2018; Ratnawati et al. 2024). Drought- or salinity-tolerance estimation using *SiDREB2*-based SNAP markers on 178 F_3 families derived from Botok-10xICERI-6 cross resulted in 29 A/A genotypes, 121 A/G genotypes, and 28 G/G genotypes.

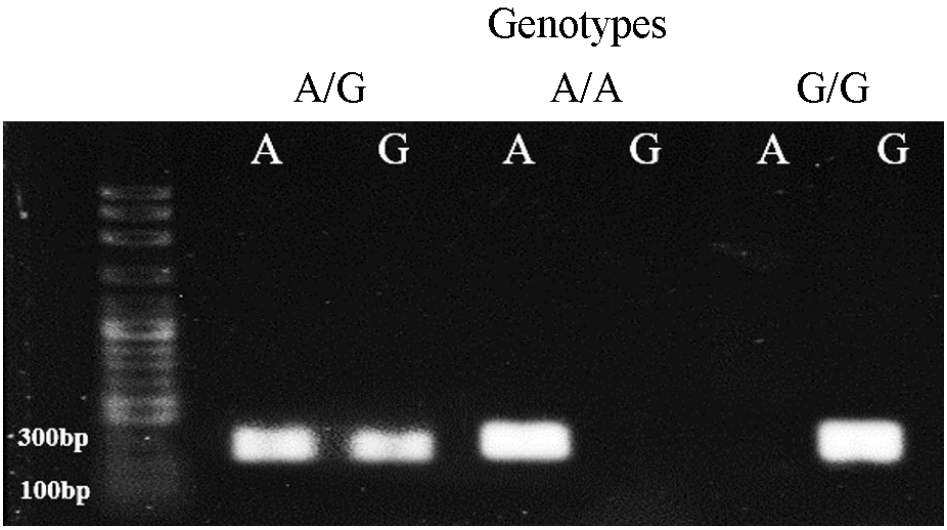

Figure 1. Representative gel electrophoresis result of A/G, A/A, and G/G genotypes using the *SiDREB2*-based SNAP marker.

There were no significant differences in plant height, heading time, and grain weight per plant between the A/A, A/G, and G/G genotypes (Table 2), indicating that there were no associations between the 558th base variation of the *SiDREB2* gene and the observed phenotypic traits at the F_2 generation under non-stress conditions. The selection index calculated based on the three previously mentioned traits showed that the A/A genotype's selection index ranged between -3.69 to 12.67, while the A/G and G/G genotypes' selection index ranged from 5.79 to 15.39 and -5.69 to 11.13, respectively. Lata et al. (2011) reported that *SiDREB2* gene expression increased under drought or salinity conditions, indicating that the effect of the *SiDREB2* allele would be more

pronounced under stress conditions. Therefore, the effect of the 558th base variation of the *SiDREB2* gene needs to be further evaluated under stress- and no-stress conditions in the later generation. Further field evaluations were then conducted on 50 F_3 family numbers having the A allele (A/A or A/G genotypes) with the highest selection index from the 178 F_3 family numbers evaluated above.

Weighted index selection of Botok-10xICERI-6 derived F_3 family

Some of the major traits targeted in the foxtail millet breeding program are high yield, early heading time, medium stature, and tolerance to drought/ salinity stress. Shorter plants and earlier heading times in comparison to the female parent (Botok-10) were observed in the F_3 population (Table 3). Furthermore, the F_3 population showed higher grain weight per plant than the male parent (ICERI-6), indicating that there are potential segregants with higher yields in the F_3 population. The F_3 population showed higher average values of the length and width of flag leaf, and 100-grain weight than both parents, while the remaining traits showed average values between the two parents.

The F_3 population in this study showed a lower standard deviation for plant height than the two parents, while the standard deviation for heading time, and grain weight per plant were in between the two parents. This indicates that although the phenotypic variation for these target traits was lower than at least one of the parental genotypes, further selection is still necessary for more uniform performances in the next generation.

In order to better understand the extent of genetic variability in the F_3 population, the variance components, phenotypic and genotypic coefficient of variation, and broad-sense heritability were calculated and presented in Table 4. Traits with high GCV values indicate high levels of

Table 2. The effect of A/A, A/G, and G/G genotypes on plant height, heading time, and grain weight per plant of F_2 generation derived from the Botok-10xICERI-6 cross.

Traits	Genotype			Kruskal-Wallis test
	A/A	A/G	G/G	
Plant height (cm)	69.30	69.28	69.21	ns
Heading time (DAP)	122.43	122.83	127.75	ns
Grain weight per plant (g)	13.20	13.47	14.50	ns

Note: DAP: days after planting, number of F_3 families: A/A=29, A/G=121, G/G = 28; ns = not significant

Table 3. Mean value and standard deviation of Botok-10, ICERI-6, and F_3 population from the cross of Botok-10 and ICERI-6.

Traits	Mean and standard deviation		
	Botok-10	ICERI-6	F_3 (Botok-10xICERI-6)
Plant height (cm)	211.04 ± 26.68	116.36 ± 21.67	125.30 ± 20.24
Flag leaf length (cm)	31.74 ± 5.21	34.03 ± 4.80	35.53 ± 6.40
Flag leaf width (cm)	2.34 ± 0.37	2.36 ± 0.41	2.46 ± 0.52
Stem diameter (mm)	6.29 ± 0.83	5.24 ± 1.08	5.80 ± 1.15
Heading time (DAP)	95.69 ± 6.58	64.90 ± 2.61	76.08 ± 4.89
Harvest time (DAP)	127.00 ± 0.00	112.03 ± 2.95	109.78 ± 6.17
100-grain weight (g)	0.22 ± 0.07	0.26 ± 0.09	0.30 ± 0.10
Main panicle length (cm)	22.26 ± 3.24	21.28 ± 3.05	21.46 ± 5.19
Main panicle weight (g)	11.36 ± 3.63	2.47 ± 0.65	4.83 ± 0.54
Main panicle grain weight (g)	7.49 ± 3.09	1.36 ± 0.38	3.56 ± 1.86
Grain weight per plant (g)	7.49 ± 3.09	1.47 ± 0.44	3.74 ± 2.00

Note: DAP: days after planting

genetic variability, whereas traits with low GCV values demonstrate low levels of genetic variability. Meanwhile, the extent of the differences between the PCV and GCV implies the relative significance of genetic and environmental influences on a given trait, with large differences indicating a significant environmental influence and small differences indicating a significant genetic influence (Xu 2021). Therefore, successful selection for targeted traits can be expected from traits with high GCV and with minimum differences between PCV and GCV. Moderate GCV and a small difference between PCV and GCV (6.64) were recorded for plant height, while low GCV and a small difference between PCV and GCV (2.54) were recorded for heading time. Grain weight per plant showed high GCV and a relatively greater difference between PCV and GCV (20.47). These results indicate that environmental influence was more pronounced for grain weight per plant, while genetic influence was more dominant for plant height and heading time. Moreover, moderate to high GCV estimates indicate that selection can be performed based on plant height and grain weight per plant, while the heading time was relatively less varied between F_3 families. Sintia et al. (2023) also reported moderate to high GCV estimates for the plant height and grain weight per plant of an F_2 population derived from the ICERI-5xBotok-10 cross of foxtail millet.

Heritability also needs to be considered in determining effective selection traits. A high value of broad-sense heritability on a particular trait indicates that the total variability of the trait is under genetic control, and selection based on this trait would be advantageous for trait improvement (Schmidt et al. 2019). As shown in Table 4, plant height and heading time have high broad-sense heritability, while grain weight per plant has moderate broad-sense heritability. A previous study of an F_2 population derived from the ICERI-5xBotok-10 cross of foxtail millet by Sintia et al. (2023) showed high broad-sense heritability for grain weight per plant and moderate broad-sense heritability for plant height and heading time. Meanwhile, Anuradha and Patro (2020) reported that flowering time, plant height, and grain yield had the highest heritability values based on their study on eight foxtail millet genotypes in India. These different heritability estimates might be due to different parental genotypes as well as different breeding generations. Altogether, the PCV, GCV, and broad-sense heritability values in this study indicate that a weighted selection index based on the three main target traits would be effective and can be performed accordingly.

Table 4. Variance component, phenotypic coefficient of variation, genotypic coefficient of variation, and broad-sense heritability of F_3 population from the cross of Botok-10 and ICERI-6.

Traits	σ^2_p	σ^2_e	σ^2_g	PCV (%)	GCV (%)	h^2_{bs} (%)	Category of h^2_{bs}
Plant height (cm)	986.423	628.802	532.288	25.024	18.382	53.961	High
Flag leaf length (cm)	37.554	23.222	20.783	17.326	12.889	55.341	High
Flag leaf width (cm)	0.071	0.053	0.033	10.978	7.461	46.192	Moderate
Stem diameter (mm)	0.768	0.720	0.248	15.083	8.568	32.271	Moderate
Heading time (DAP)	65.597	38.097	38.082	10.672	8.132	58.055	High
Harvest time (DAP)	78.324	67.285	29.729	8.037	4.951	37.957	Moderate
100-grain weight (g)	0.004	0.003	0.002	21.511	15.823	54.105	High
Main panicle length (cm)	22.304	27.641	2.341	22.028	7.137	10.497	Low
Main panicle weight (g)	8.456	6.275	3.924	60.456	41.184	46.407	Moderate
Main panicle grain weight (g)	4.541	3.629	1.920	60.025	39.030	42.280	Moderate
Grain weight per plant (g)	4.441	3.641	1.811	56.648	36.181	40.792	Moderate

Note: σ^2_p: phenotypic variance, σ^2_e: environmental variance, σ^2_g: genetic variance, PCV: phenotypic coefficient of variation, GCV: genotypic coefficient of variation, h^2_{bs}: broad-sense heritability, DAP: days after planting

A weighted index selection with an intensity of 20% of 48 F_3 families from the cross of Botok-10 x ICERI-6 resulted in ten F_3 families (Table 5). The top ten F_3 families comprised three A/A genotypes and seven A/G genotypes, with the selection index ranging from 0.84 to 3.76. The selection indexes of the top ten F_3 families were higher than the parental genotypes and all check genotypes, except the Mauliru-2 genotype. The Mauliru-2 genotype showed a considerably high selection index (3.03). Ratnawati et al. (2024) also identified Mauliru-2 as a potential high-yielding genotype compared to the other seven Indonesian foxtail millet genotypes. Given that Mauliru-2 has the G allele for the *SiDREB2* gene, this genotype is potentially developed further as a superior foxtail millet variety through pure line selection for non-stressed areas.

The variability between F_3 individuals within a particular F_3 family can be seen from the mean standard deviation of the three target traits. The top ten F_3 families still showed a greatly varied mean standard deviation from 4.64 to 13.35. The F_3 family with a high selection index and a low mean standard deviation is desirable to be selected and can be classified as putative transgressive segregants. Considering the check genotypes were planted in five blocks, while each F_3 family number was planted in only one block, the mean standard deviation of the check genotypes could be used as a suitable comparison to identify putative transgressive segregants in the F_3 population derived from Botok-10xICERI-6 cross. The scatter plot in Figure 2 shows the distribution of 25 F_3 families based on their selection index and the mean standard deviation of the three target traits used to develop the selection index. Genotypes in quadrants (I) and (IV) are those with selection index values lower than the mean selection index of all F_3 families observed, thus they were considered not potential to be selected further. Meanwhile, genotypes in quadrants (II) and (III) are those with selection index values higher than the mean selection index of all F_3 families observed. The F_3 family with pedigree numbers B10I6-15-136, B10I6-15-161, and B10I6-15-70 with A/A genotypes are considered putative transgressive segregants since they are located in quadrant (I). These three F_3 families have a higher selection index than the mean selection index of all F_3 families and a lower mean standard deviation than the mean standard

Table 5. Weighted index selection results in the F_3 population of Botok-10xICERI-6 cross.

Genotype	F_3 family	Mean and standard deviation			Selection index	Mean SD
		Heading time (DAP)	Plant height (cm)	Grain weight per plant (g)		
A/G	B10I6-15-177	75.34 ± 4.32	83.60 ± 19.49	5.21 ± 3.32	3.76	9.36
A/G	B10I6-15-104	62.11 ± 12.47	86.99 ± 13.70	3.79 ± 1.93	3.45	9.75
A/G	B10I6-15-48	72.47 ± 5.19	117.84 ± 22.89	5.09 ± 2.41	2.79	10.38
A/A	B10I6-15-136	79.17 ± 3.06	139.56 ± 9.50	6.17 ± 2.05	2.65	5.00
A/G	B10I6-15-180	70.19 ± 6.50	127.91 ± 30.96	4.90 ± 1.57	2.48	13.35
A/G	B10I6-15-74	71.71 ± 4.28	136.56 ± 18.45	5.00 ± 1.49	2.11	8.40
A/A	B10I6-15-161	79.95 ± 1.80	129.26 ± 9.30	5.24 ± 2.28	1.51	4.64
A/A	B10I6-15-70	83.94 ± 4.29	188.62 ± 9.72	6.84 ± 4.05	1.23	6.50
A/G	B10I6-15-237	75.69 ± 4.17	115.14 ± 27.86	4.19 ± 1.77	1.07	11.52
A/G	B10I6-15-57	66.48 ± 7.73	125.04 ± 26.21	3.37 ± 1.94	0.84	12.23
G/G	Botok-10	95.69 ± 6.27	211.04 ± 25.62	7.49 ± 2.96	-0.31	11.62
A/A	ICERI6	64.90 ± 2.51	116.36 ± 20.81	1.47 ± 0.42	-1.45	7.91
G/G	Mauliru-2	83.25 ± 3.98	135.96 ± 11.10	6.74 ± 2.37	3.03	5.82
G/G	NTB-1	78.27 ± 4.87	126.06 ± 18.91	3.57 ± 1.93	-0.62	8.57
G/G	Toraja	69.72 ± 1.57	97.02 ± 15.62	2.74 ± 1.43	0.41	6.20

Note: DAP: days after planting; Mean SD = mean of the standard deviation of the three target traits

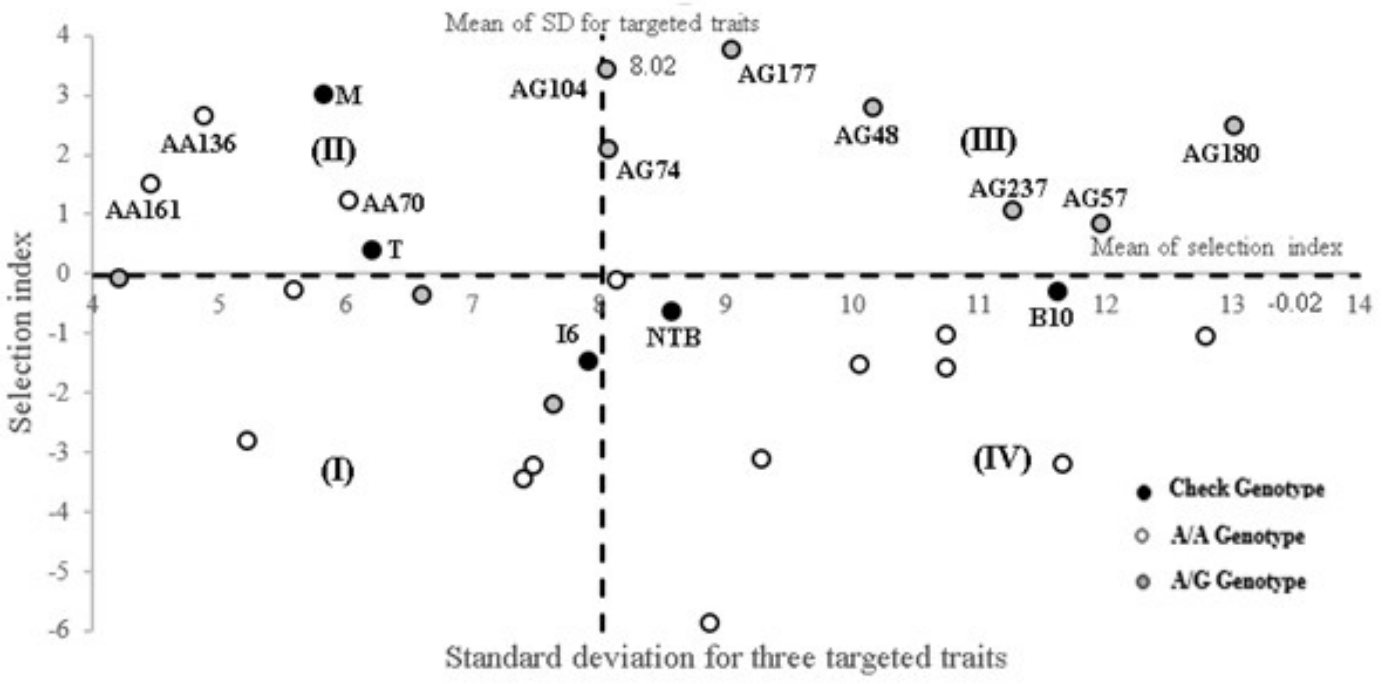

Figure 2. Scatter plot of 25 F_3 foxtail millet family numbers derived from Botok-10xICERI-6 cross. B10 = Botok-10; I6 = ICERI-6; M = Mauliru-2, T= Toraja.

deviation of the check genotypes. Moreover, these three F_3 families have A/A genotypes that indicate their potential tolerance to drought/salinity stress. The Mauliru-2 genotype is also located in quadrant (II), confirming its potential as a superior foxtail millet variety. Although it is located in quadrant (III), the pedigree number B10I6-15-177 with A/G genotype showed the highest selection index. Therefore, F_4 families with A/A genotype generated from F_3 individuals in this pedigree also potential to be evaluated further.

CONCLUSIONS

Multiple-traits selection using *SiDREB2*-SNAP marker combined with weighted selection index on F_3 families of foxtail millet from the cross of Botok-10xICERI-6 identified 10 potential F_3 families with the highest selection index. Three F_3 families with A/A genotypes (pedigree numbers B10I6-15-136, B10I6-15-161, and B10I6-15-70) are considered putative transgressive segregants and are recommended to be continued to the next generation for further breeding process.

AUTHOR CONTRIBUTION

L.K.S.B. performed the genotyping and phenotyping experiments at the F_3 generation, analyzed the data, and wrote the manuscript; D.D.S. conducted experiments on the F_2 generation; W.B.S. and S.W.A. designed research, supervised the research and data analysis, script writing and editing.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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