

Genetic variability of F₂ foxtail millet population derived from ICERI-5 and Botok-10 cross

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Abstract. Sintia M, Suwarno WB, Ardie SW. 2023. Genetic variability of F₂ foxtail millet population derived from ICERI-5 and Botok-10 cross. *Biodiversitas* 24: 3559-3567. Foxtail millet (*Setaria italica* (L.) P. Beauv.) is a carbohydrate source with good tolerance to abiotic stress. High productivity, short stature, and early flowering time are the breeding targets of this species. This study aimed to obtain genetic variability of the F₂ foxtail millet population derived from ICERI-5 and Botok-10 cross and to predict selection response based on weighted selection index. This experiment was conducted in Bogor and consisted of 522 F₂ individuals and two parental genotypes. The results showed that the F₂ population had shorter plant height and earlier flowering time compared to Botok-10, and higher grain weight per plant compared to ICERI-5. Plant height, flowering time, and grain weight per plant showed moderate to high genotypic coefficient of variation with moderate to high broad-sense heritability. Weighted selection index using the three target traits resulted in ten F₂ individuals with higher selection index compared to both parents, with individual number I5B10-4-96 having the highest selection index. Selection responses based on a weighted selection index indicate an expected decrease in flowering time and an increase in grain weight per plant in the next generation.

Keywords: Broad sense heritability, carbohydrate source, selection response, *Setaria italica*, weighted selection index

INTRODUCTION

Foxtail millet [*Setaria italica* (L.) P. Beauv.] is an important C4 crop in arid and semi-arid areas due to its good tolerance to drought and salinity stresses (Doust and Diao 2017). The nutritional value of this underutilized crop increases the importance of this crop not only as a carbohydrate source but also as a functional food since it has a low glycemic index (Ren et al. 2015). Foxtail millet was also reported to be high in dietary fiber and protein and contain comparable antioxidants (Sharma and Niranjana 2017). Owing to its small genome (~490 MB), self-pollination nature, and genetic diversity richness (~6,000 genotypes) (Zhang et al. 2012), the species has become a remarkable model to investigate different aspects of plant biology, ranging from processes of ecological differentiation, domestication, morphological and developmental changes, genetic regulation, breeding, and genome evolution (Doust and Diao 2017).

India is the top global millet producer (40.62% of total millet production), followed by African countries (39.30%) and China (7.02%) (FAO 2021). Foxtail millet has received limited scientific interest in Indonesia and currently there is no foxtail millet superior variety has been released in the country. This species is being cultivated as a minor crop in West Sulawesi (Polewali Mandar), Buru, Sumba, and Central Java (Evizal 2020), but only a few studies of local foxtail millet genotypes have been reported. Biodiversity assessment of 10 foxtail millet genotypes using random amplified polymorphic DNA (RAPD) marker was reported

by Ardie et al. (2017) as an initial step of foxtail millet breeding. At least 26 Indonesian local genotypes of foxtail millet have been used in genotyping study using a *SiDREB2*-based SNAP marker by Widyawan et al. (2018). Six local genotypes of foxtail millet were reported to be cultivated in West Sulawesi by Ramlah et al. (2020), and two local accessions of foxtail millet were reported by Nisa and Jadid (2021). These studies implied the genetic variability richness of foxtail millet in Indonesia that needs further elaboration.

Foxtail millet is a potential catch crop in marginal areas (Lata et al. 2012), thus variety with high productivity, short stature, early flowering time, and tolerance to drought or salinity stress is the breeding target of this species. Early characterization of several Indonesian foxtail millet genotypes identified particular genotypes with desired traits. ICERI-5 genotype was reported to have low productivity (1.71 ton ha⁻¹), short stature (82.13 cm), early flowering time (35 days after planting/ DAP) (Sintia 2017), and tolerant to drought or salinity stresses (Lapuimakuni et al. 2018; Widyawan et al. 2018). In contrast, Botok-10 genotype was reported to have high productivity (2.61 ton ha⁻¹), with tall stature (193.26 cm), late-flowering time (60 DAP) (Zahroh 2017), and was predicted to be sensitive to drought or salinity stresses (Widyawan et al. 2018). Crossing of ICERI-5 x Botok-10 is expected to generate progenies with high productivity, short stature, early flowering, and tolerant to drought or salinity stresses.

Selection is one of the main activities in plant breeding programs. Selection in the F₂ generation can be done as the

genetic variability in this generation is high (Hussain et al. 2021). The desired response to selection in the next generation can be expected for traits with high heritability values and with a broad genetic variance (Yahaya and Shimelis 2021). Information about correlation analysis is also important in the selection process. Correlation analysis allows for studying the relationship between two quantities (Lindley 1990). Furthermore, the selection of multiple traits can be performed using a selection index formulated from targeted traits (Yerima and Dako 2020). Genotypes with a high selection index are potential genotypes that can be continued to the next generation (Syukur et al. 2012). This study aimed to obtain genetic variability of F₂ foxtail millet population derived from ICERI-5 and Botok-10 cross and to predict selection response based on a weighted selection index.

MATERIALS AND METHODS

Plant materials

The plant materials used in this study were ICERI-5 (obtained from Indonesian Cereals Research Institute, ICERI) and Botok-10 (collected from East Nusa Tenggara, Indonesia), and 522 F₂ individuals derived from ICERI-5 and Botok-10 cross. The male sterile ICERI-5 was obtained by warm water treatment as described by Nugroho (2020) prior to hybridization, and the F₁ progenies were confirmed by *SiDREB2*-based SNAP marker developed by Widyawan et al. (2018). The habitus, panicle, and seed appearance of the parental genotypes are shown in Figure 1A, 1B, and 1C, respectively. Figure 1D shows 300 bp bands for both A and G alleles in the F₁ line, while the parent genotype ICERI-5 shows only the band specific for A allele and the parent genotype Botok-10 shows only the band for G allele.

Procedures

The research was conducted in the Cikabayan Bawah Experimental Station of IPB University, Bogor, Indonesia (6°33'24.23"S, 106°43'33.4"E) at an altitude of 240 m asl. from February to August 2021. Post-harvest observations were conducted at the Post Harvest Laboratory, Department of Agronomy and Horticulture, IPB University from September to December 2021. The average agro-climates condition during this study (February - July 2021) consisted of an average temperature of 26.02°C with an average humidity of 84.78% and an average rainfall of 279.60 mm per month (BMKG 2021).

Seeds were sown in seedling trays with a media mixture of compost and manure (1:1, v/v). The seedlings with five to six leaves were then transplanted to the field. This experiment consisted of three blocks, each 45 x 4.6 m in size. Each block consisted of three plots, each 45 x 1 m in

size. Each plot consisted of 38 rows of F₂ individuals and two rows of parental genotypes. Eight plants per row were planted with a planting distance of 75 x 10 cm. The fertilizers rates were 300, 150, and 75 kg ha⁻¹ of Urea, SP-36, and KCl, respectively. The Urea, SP-36, and KCl fertilizers were applied 2 weeks after planting (WAP) with a half rate of Urea and the full rate of SP-36 and KCl. The other half rate of Urea was applied at 6 WAP. Insecticide containing pymetrozine 50% with concentration 1 g L⁻¹ has been applied to control rice ear bug (*Leptocorisa oratorius*) at the grain filling stage. Plant net was installed at 2 WAP to protect the plants from birds and other pests in the field.

Agronomic performance was observed for 11 quantitative characters according to the UPOV descriptor (UPOV 2010) on the following characters: plant height (cm), number of leaves, stem diameter (mm), length and width (cm) of flag leaf, the number of productive tillers and panicle per plant, flowering time (day after planting/ DAP), harvesting time (DAP), panicle weight (g), and grain weight per plant (g).

Data analysis

Mean and standard deviation were calculated for each character observed. The high variance between blocks was observed and thus the obtained data were subjected to adjusted mean calculation prior to further analysis. The adjusted mean calculation was performed based on Petersen (1994) with the following formula:

$$\hat{y}_{ij} = y_{ij} - a_j, a_j = \bar{x}_{\cdot j} - \bar{\bar{x}}$$

Where: \hat{y}_{ij} : the adjusted mean of the i -th selection in the j -th block; y_{ij} : value of each character in the i -th selection in the j -th block; a_j : the adjustment factor for the j -th block; $\bar{x}_{\cdot j}$: mean value of all checks in j -th block; $\bar{\bar{x}}$: general mean

The adjusted mean value was used for further analyses, including components of variance estimation (Mahmud and Kramer 1951), phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) (Burton 1952), broad-sense heritability estimation (Allard 1960), correlation coefficients (Walpole 1992), selection differential, and predicted selection response (Sobir and Syukur 2015). Equations used in this study are summarized in Table 1. PCV and GCV were categorized according to Sivasubramanian and Madhavamenon (1973) as low (0-10%), moderate (10-20%), and high (>20%). Broad-sense heritability was categorized according to Stanfield (1983) as high ($50\% \leq h^2_{bs} < 100\%$), moderate ($20\% \leq h^2_{bs} < 50\%$), and low ($0 \leq h^2_{bs} < 20\%$). The software used in this study was Microsoft Excel 2019 and Minitab 16 for correlation analysis.

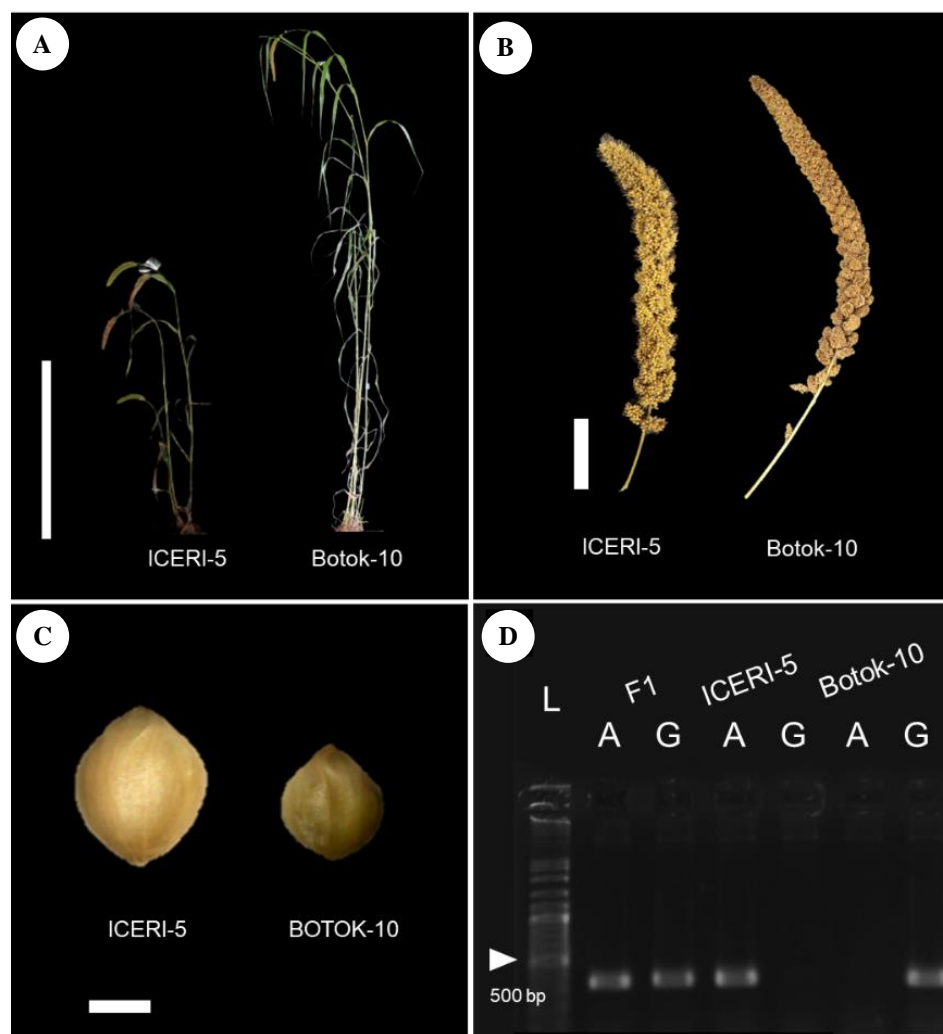


Figure 1. Characteristics of the foxtail millet parental genotypes used in this study. A. Plant habitus; bar: 50 cm. B: Panicles morphology; bar: 5 cm. C. Seed appearance; bar 1 mm of ICERI-5 and Botok-10 genotypes. D. Hybrid confirmation of ICERI-5 x Botok-10 cross in comparison to the parent genotypes using SiDREB2-based SNAP marker performed by Nugroho (2020)

RESULTS AND DISCUSSION

Mean value and standard deviation of ICERI-5, Botok-10, and *F₂* population from ICERI-5 and Botok-10 cross

Our results showed that the average plant height and flowering time of the *F₂* population from ICERI-5 and Botok-10 cross were lower than male parent Botok-10 (Table 2), indicating that *F₂* population has shorter plant height and faster flowering time compared to male parent. Moreover, grain weight per plant of the *F₂* population was higher than the female parent ICERI-5, indicating that there are potential *F₂* segregants with higher productivity. Meanwhile, the average number of productive tillers, number of panicles per plant, and number of leaves of the *F₂* population were higher than both parents. The average length and width of the flag leaf of the *F₂* population were lower than both parents and the average *F₂* population from

the character of stem diameter, harvesting time, and panicle weight per plant were lower than the male parent Botok-10.

Foxtail millet variety with high productivity, short stature, and early flowering time is the target of foxtail millet breeding program. Compared to our results, the lower mean value in plant height and flowering time, and higher mean value in grain weight per plant compared to check genotypes were also reported in the earlier study using 71 foxtail millet genotypes (Srilatha et al. 2020). Foxtail millet with short stature is desirable since tall plants were reported to be prone to lodging, thus reducing yield and quality (Tian et al. 2010). Zhang et al. (2017) reported that plant height trait was controlled by six QTLs that were distributed on chromosomes 1, 4, 5, 6, 7, and 9 of foxtail millet. He et al. (2020) further identified *Seita.1G242300*, a gene that encodes gibberellin 2-oxidase-8, as an important candidate gene controlling plant height in foxtail millet.

Table 1. Parameters and equations used to analyze the F₂ population derived from ICERI-5 and Botok-10 cross

Parameters	Equation	Reference
Phenotypic variance (σ^2_p)	$\sigma^2_{F_2}$	Mahmud and Kramer (1951)
Environmental variance (σ^2_e)	$\frac{P_1 + P_2}{2}$	
Genotypic variance (σ^2_g)	$\sigma^2_p - \sigma^2_e$	Burton (1952)
Phenotypic coefficient of variation (PCV)	$\frac{\sqrt{\sigma^2_p}}{\bar{x}} \times 100\%$	
Genotypic coefficient of variation (GCV)	$\frac{\sqrt{\sigma^2_g}}{\bar{x}} \times 100\%$	Allard (1960)
Broad-sense heritability (h^2_{bs})	$\frac{\sigma^2_g}{\sigma^2_p} \times 100\%$	
Correlation coefficients (r)	$\frac{\sum xy - \frac{\sum x \sum y}{n}}{\sqrt{(\sum x^2 - \frac{(\sum x)^2}{n})(\sum y^2 - \frac{(\sum y)^2}{n})}}$	Walpole (1992)
Weighted selection index (SI)	$b_1X_1 + b_2X_2 + b_3X_3 + \dots + b_nX_n$	Sandhu et al. (2019)
Standardization character (X_n)	$\frac{x_{ij} - \bar{x}_i}{S_i}$	
Differential selection (S)	$(\bar{x}_s - \bar{x}_o)$	Sobir and Syukur (2015)
Predicted response selection (ΔG)	$S \times h^2_{(bs)}$	

Note: P₁: parent 1, P₂: parent 2, \bar{x} : mean, n: number of observation, x and y: variables, b_n: the weight of the variable to n, X_{ij}: means of each genotype, \bar{x}_i : means of the variable, S_i: standard deviation of the variable, \bar{x}_s : mean selected population, \bar{x}_o : mean population before selection

Table 2. Mean value and standard deviation of ICERI-5, Botok-10, and F₂ population from ICERI-5 and Botok-10 cross

Traits	Mean and standard deviation		
	ICERI-5	Botok-10	F ₂
Plant height (cm)	78.01±21.58	174.50±21.34	68.95±24.34
Number of leaves	12.90±6.69	17.07±5.54	21.12±9.78
Stem diameter (mm)	2.91±1.01	4.84±0.79	3.127±0.97
Length of flag leaf (cm)	31.04±5.97	38.66±7.13	28.01±7.18
Width of flag leaf (cm)	2.02±0.38	2.27±0.41	1.916±0.51
Number of productive tillers	1.89±1.63	0.35±0.57	3.349±2.36
Number of panicles per plant	2.89±1.63	1.37±0.58	4.348±2.36
Flowering time (DAP)	28.33±2.10	53.41±3.54	27.06±3.99
Harvesting time (DAP)	41.63±1.13	106.30±4.02	62.12±3.378
Panicle weight per plant (g)	4.80±3.06	10.87±5.16	9.106±6.22
Grain weight per plant (g)	4.00±2.64	9.08±4.34	7.563±5.49

Note: DAP: days after planting

Flowering time is another important trait in plant adaptation to environments, and it has been identified as one of the drought escape mechanisms in plants (Shavrukov et al. 2017). A detailed comparison of QTL for flowering in *Setaria*, sorghum, and maize indicates that *CONSTANS* (*CO*), a gene controlling flowering time in a photoperiod pathway, is conserved across grasses (Mauro-Herrera et al. 2013). The *CO*-ortholog was identified in rice as *HEADING DATE 1* (*HDI*) in rice (Yano et al. 2000). Although the *HDI* gene is colocalized with a QTL for flowering time in foxtail millet (Mauro-Herrera et al. 2013), a study conducted by Fukunaga et al. (2015) suggested the complexity regulation of the trait, thus the

effect of a single gene on flowering time might be masked by the effects of other genes influencing the trait. Grain yield is one of the most important traits in cereals. A QTL analysis using RIL population of foxtail millet conducted by Wei et al. (2022) identified a potential yield-related gene, *Si035172m*. The *Si035172m* homologous gene in rice, *OsTE*, is a co-activator of APC/C. The APC/C-TE complex has been reported to be involved in the lateral branch and tillering regulation, which is an important factor to determine the plant type and grain yield (Ermawati and Wibisono 2017). The information of key gene(s)/ QTL related to the targeted traits in foxtail millet can be further utilized for selecting potential progenies.

The standard deviation of the *F₂* population in this study was higher for most characters than the two parents. A high standard deviation in the *F₂* population was also reported in rice by Mustikarini et al. (2019). The population at *F₂* generation has the highest variation due to segregation (Cheng et al. 2010), which is required for selection to be conducted. Variance component and heritability need to be estimated to provide information on the extent of genetic response to selection.

Variance component and broad-sense heritability of *F₂* population of ICERI-5 and Botok-10 cross

The variance components, phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), and broad-sense heritability of *F₂* population of ICERI-5 and Botok-10 cross are shown in Table 3. The variance components, phenotypic and genotypic coefficient of variation, and heritability are important parameters to study the extent of genetic variability more precisely (Patel et al. 2020). In this study, PCV ranged from 5.44 to 72.69%. Meanwhile, GCV ranged from 2.62 to 60.21%. Higher PCV compared to GCV were recorded for all characters. Previous studies in rice (Sadimantara et al. 2021) and maize (Alam et al. 2022) also reported higher PCV than GCV. High PCV and GCV (>20%) were recorded for the number of leaves, number of productive tillers, number of panicles per plant, panicle weight per plant, and grain weight per plant. High PCV and GCV for the number of productive tillers, and grain weight per plant were previously reported in foxtail millet (Toppo et al. 2023) and in other cereals, such as rice (Priyanka et al. 2019; Beena et al. 2021) and sorghum (Subhashini and Selvi 2019). Moderate estimates (10-20%) of PCV and GCV were observed for flowering time (PCV=14.75 and GCV=10.09) in this study, as was previously reported in another study in foxtail millet (Srilatha et al. 2020). Meanwhile, low estimates (<10%) of PCV and GCV were observed for harvesting time (PCV=5.44 and GCV= 2.62). The degree of the differences between the PCV and GCV indicates the influence of environmental and genetic factors on a particular trait, where large differences reflect a large environmental effect, whereas small differences reveal a high genetic influence (Patel et al. 2020; Tripathy and Mallikarjunarao 2020). The minimum differences between PCV and GCV were recorded in one targeted trait, flowering time, indicating high genetic influence in this trait. Further individual selection should consider the combination of GCV and heritability estimates in targeted traits.

Heritability plays a pivotal role in foxtail millet breeding programs because it can influence the choice of selection procedures, predict selection response, and determine the genetic effects (Anuradha and Patro 2020). In this study, the broad-sense heritability ranged from 10.89 to 73.05%.

The heritability of one of the major targeted traits, grain weight per plant, was categorized as high (>50%). High estimates of heritability for grain weight per plant were previously reported in foxtail millet (Toppo et al. 2023) and in other cereals, including rice (Roy and Shil 2020;

Limbongan et al. 2021; Sadimantara et al. 2021), barley (Sootrakar et al. 2020), wheat (Laghari et al. 2021), and maize (Hasan et al. 2021). High heritability was also observed in the yield components, namely the number of leaves, the number of productive tillers, the number of panicles per plant, and panicle weight per plant. High heritability in the number of productive tillers was reported in rice (Beena et al. 2021), while high heritability for the number of leaves was also reported in the *F₂* population of maize (Hasan et al. 2021). High estimates of heritability for certain traits suggest that they are under genetic control (Patel et al. 2020). Meanwhile, the heritability estimates for other traits, namely plant height, the width of flag leaf, flowering time, and harvesting time were categorized as moderate (20-50%). Moderate heritability for plant height was previously reported in wheat (Nuraeni et al. 2021) and for flowering time in rice (Tirtana et al. 2021). The stem diameter and the length of the flag leaf had low (<20%) heritability.

In this study, the targeted traits such as plant height, flowering time, and grain weight per plant showed moderate to high GCV with moderate to high heritability, indicating that selection based on these traits is potential to be performed. According to Singh et al. (2022), heritability is useful in forecasting the predicted level of improvement that would be attained by the selection and combined with the genotypic coefficient of variation (GCV). However, additional information that is unavailable in this study, including additive genetic variance, narrow-sense heritability, and type of gene action are required to determine whether the selection is promising to be conducted in early generation. The GCV and heritability category obtained in this study indicate that plant height, flowering time, and grain weight per plant could be considered for multiple-trait selection using a weighted selection index.

Correlations analysis of the *F₂* population of ICERI-5 and Botok-10 cross

The aim of correlation studies is primarily to identify secondary traits that are suitable for indirect selection since the correlation coefficient estimates mostly indicate the inter-relationships of the traits (Boyhan 2013). The correlation analysis between all traits in this study is shown in Table 4. The grain weight per plant is considered an essential component of productivity and it is one of the ultimate goals of the foxtail millet breeding program. Yield (grain weight per plant) is a complex trait that is largely influenced by many associated traits. The information on the strength and direction of correlation among traits to yield (grain weight per plant) would be useful in designing foxtail millet breeding program in particular for yield improvement (Pallavi et al. 2020). The correlation analysis showed that a significant and positive correlation in the yield components was observed in the number of productive tillers ($r = 0.30^{**}$), number of panicles per plant ($r = 0.30^{**}$), and panicle weight per plant ($r = 0.99^{**}$). The number of productive tillers seems to be an important trait associated with yield in rice, as a significant positive correlation between this trait and rice yield was reported in

many other studies (Saha et al. 2019; Prasannakumari et al. 2020; Prathiksha et al. 2022). Furthermore, the high correlation value ($r = 0.99^{**}$) close to 1 in panicle weight per plant in this study indicates that this trait will influence foxtail millet yield greatly. Panicle weight per plant also has been reported to have a significant positive correlation with yield in rice (Prasannakumari et al. 2020). A significant and positive correlation value between these traits with yield (grain weight per plant) indicates that an increase in the value of these traits will be followed by an increase in yield (grain weight per plant). Hence, traits that positively correlated with grain weight per plant can be used as reliable selection criteria to improve foxtail millet production. This is also indicated that the simultaneous selection of all these traits is important for yield improvement.

A significant positive correlation between plant height ($r=0.57^{**}$) and flowering time (0.12^{**}) with grain weight per plant in this present study is not desirable as higher yield will be followed by taller plants and longer flowering time. A significant positive correlation between plant

height and flowering time with yield was also reported in previous studies in foxtail millet (Pallavi et al. 2020) and rice (Asante et al. 2019; Saha et al. 2019; Sadimantara et al. 2021; Prathiksha et al. 2022). Therefore, multiple-traits selection using a weighted selection index is necessary to perform proper selection based on desired traits and to achieve the breeding objective.

Selection index and predicted selection response of F_2 population of ICERI-5 and Botok-10 cross

The multiple-traits selection in this study was carried out using a weighted selection index based on the three priority traits of foxtail millet breeding. A selection index is a single score that reflects the merits and demerits of various traits and selection among genotypes is based on the relative values of the index scores (Baker 1986). The model of selection index (SI) used was $SI = - \text{plant height} - \text{flowering time} + (3 \times \text{grain weight per plant})$. Shorter plant stature and earlier flowering time are desired traits, thus negative sign was used for these traits in the model.

Table 3. Variance component, phenotypic coefficient of variation, genotypic coefficient of variation, and broad-sense heritability of F_2 population of ICERI-5 and Botok-10 cross

Traits	F_2 (ICERI-5 X Botok- 10)						Category
	σ_p^2	σ_e^2	σ_g^2	PCV (%)	GCV (%)	h_{bs}^2 (%)	
Plant height (cm)	592.32	460.47	131.85	35.30	16.65	22.26	Moderate
Number of leaves	95.64	37.77	57.87	46.30	36.02	60.51	High
Stem diameter (mm)	0.93	0.83	0.10	30.88	10.19	10.89	Low
Length of flag leaf (cm)	51.65	43.21	8.44	25.66	10.37	16.33	Low
Width of flag leaf (cm)	0.26	0.16	0.10	26.55	16.74	39.73	Moderate
Number of productive tillers	5.57	1.50	4.07	70.44	60.21	73.05	High
Number of panicles per plant	5.57	1.51	4.06	54.27	46.34	72.91	High
Flowering time (DAP)	15.93	8.47	7.45	14.75	10.09	46.79	Moderate
Harvesting time (DAP)	11.40	8.76	2.65	5.44	2.62	23.21	Moderate
Panicle weight per plant (g)	38.65	18.00	20.65	68.27	49.90	53.42	High
Grain weight per plant (g)	30.22	12.93	17.29	72.69	54.98	57.21	High

Note: σ_p^2 : phenotypic variance, σ_e^2 : environmental variance, σ_g^2 : genotypic variance, PCV: phenotypic coefficient of variation, GCV: genotypic coefficient of variation, h_{bs}^2 : broad-sense heritability, DAP: days after planting

Table 4. The correlation coefficient among different agronomic traits in F_2 population of ICERI-5 x Botok-10 cross

	PH	NL	SD	LF	WF	NT	NPP	FT	HT	PWP
NL	-0.14 ^{**}									
SD	0.70 ^{**}	-0.04 ^{ns}								
LF	0.65 ^{**}	0.02 ^{ns}	0.55 ^{**}							
WF	0.48 ^{**}	0.15 ^{**}	0.55 ^{**}	0.67 ^{**}						
NT	-0.25 ^{**}	0.82 ^{**}	-0.11 [*]	-0.06 ^{ns}	0.08 ^{ns}					
NPP	-0.26 ^{**}	0.82 ^{**}	-0.11 [*]	-0.06 ^{ns}	0.08 ^{ns}	1.00 ^{**}				
FT	0.50 ^{**}	-0.23 ^{**}	0.31 ^{**}	0.26 ^{**}	0.15 ^{**}	-0.37 ^{**}	-0.37 ^{**}			
HT	0.62 ^{**}	-0.39 ^{**}	0.49 ^{**}	0.27 ^{**}	0.21 ^{**}	-0.42 ^{**}	-0.42 ^{**}	0.52 ^{**}		
PWP	0.54 ^{**}	0.41 ^{**}	0.50 ^{**}	0.44 ^{**}	0.51 ^{**}	0.36 ^{**}	0.36 ^{**}	0.10 [*]	0.24 ^{**}	
GWP	0.57 ^{**}	0.35 ^{**}	0.52 ^{**}	0.46 ^{**}	0.52 ^{**}	0.30 ^{**}	0.30 ^{**}	0.12 ^{**}	0.29 ^{**}	0.99 ^{**}

Note: PH: plant height (cm), NL: number of leaves, SD: stem diameter (mm), LF: length of flag leaf (cm), WF: width of flag leaf (cm), NT: number of productive tillers, NPP: number of panicles per plant, FT: flowering time (DAP), HT: harvesting time (DAP), PWP: panicle weight per plant (g), GWP: grain weight per plant (g), ^{**}: Significant at α 0.01, ^{*}: Significant at α 0.05, ns= not significant.

Meanwhile, the weighting of grain weight per plant was made three times greater, because of its importance over the other traits in foxtail millet breeding program. The resulting index values were then ranked and ten *F₂* individuals with the highest index value were selected and presented in Table 5 in comparison with their parental genotypes. The selection index of the top ten individuals ranged from 6.23 to 16.90, while the parental genotypes showed a selection index of less than zero. Individual number I5B10-4-96 had the highest index value (16.90) compared to the other *F₂* individuals and the parental genotypes. The *F₂* individuals with high index scores were then selected to be continued in the next generation. Weighted selection index was also used to select promising rice lines as reported by Anshori et al. (2021), Akbar et al. (2021), and Gunarsih et al. (2022). It was also used to select promising barley lines (Zali and Barati 2020). Furthermore, selection differential and predicted response to selection could also be calculated from index selection (Haris 1964).

Selection differential is the difference in phenotypic means between selected *F₂* individuals and the overall population mean (Syukur et al. 2012). The selection

differential is a basic quantity in the prediction of response to selection (Hill 2013). In this study, a selection intensity of 10% was applied to 522 *F₂* individuals resulting in 52 *F₂* individuals with the highest selection index which were used further to calculate the selection differential and to predict the selection response as shown in Table 6. Early flowering and higher productivity of foxtail millet can be expected from this population as the selection response showed an increase in grain weight per plant and a decrease in flowering time. Expected increasing mean values were also observed in the yield components, namely the number of productive tillers, number of panicles per plant, and panicle weight per plant. The expected increase of grain weight per plant, number of productive tillers, and number of panicles per plant were also reported in rice (Manalu et al. 2017), while a decreased mean value of flowering time was reported in chili (Yunandra et al. 2017). The mean plant height of the *F₂* population was shorter than their parents (Table 2), but a slight increase in plant height (2.02 cm) can be expected in the next generation of this population (Table 6), indicating that the selected 52 individuals tend to have taller plant height.

Table 5. Weighted selection index of ten *F₂* individuals of ICERI-5 and Botok-10 cross and their parental genotypes

Genotypes	Mean			Standardized value			Selection index
	FT	PH	GWP	FT	PH	GWP	
I5B10-4-96	21.04	23.23	32.40	-1.46	-1.86	4.53	16.90
I5B10-4-190	27.75	111.14	28.29	0.15	1.70	3.78	9.48
I5B10-4-215	25.75	86.94	24.34	-0.33	0.72	3.06	8.78
I5B10-4-60	22.04	41.73	18.52	-1.22	-1.11	2.00	8.32
I5B10-4-191	27.75	112.34	25.82	0.15	1.75	3.33	8.08
I5B10-4-180	27.75	110.44	24.51	0.15	1.67	3.09	7.44
I5B10-4-74	26.04	43.03	17.84	-0.26	-1.06	1.87	6.93
I5B10-4-79	21.04	38.33	15.12	-1.46	-1.25	1.38	6.84
I5B10-4-218	29.75	98.84	23.31	0.64	1.20	2.87	6.77
I5B10-4-78	20.04	37.43	13.49	-1.71	-1.28	1.08	6.23
ICERI-5	28.33	78.01	4.00	0.29	0.36	-0.65	-2.60
Botok-10	53.41	174.50	9.08	6.34	4.26	0.28	-9.77

Note: FT: flowering time (DAP), PH: plant height (cm), GWP: grain weight per plant (g)

Table 6. Selection response based on weighted selection index of *F₂* population of ICERI-5 and Botok-10 cross

Traits	\bar{X}_0	\bar{X}_s	S	ΔG
Plant height (cm)	68.95	78.04	9.10	2.02
Number of leaves	21.12	29.87	8.75	5.30
Stem diameter (mm)	3.13	3.53	0.40	0.04
Length of flag leaf (cm)	28.01	30.37	2.37	0.39
Width of flag leaf (cm)	1.92	2.26	0.34	0.14
Number of productive tillers	3.35	5.45	2.11	1.54
Number of panicles per plant	4.35	6.45	2.10	1.53
Flowering time (DAP)	27.06	25.97	-1.09	-0.51
Harvesting time (DAP)	62.12	62.42	0.31	0.07
Panicle weight per plant (g)	9.11	20.55	11.44	6.11
Grain weight per plant (g)	7.56	17.76	10.19	5.83

Note: \bar{X}_0 : mean population before selection, \bar{X}_s : mean selected population, S: differential selection, ΔG : predicted selection response, DAP: day after planting

In conclusion, our study in the F₂ generation of ICERI-5 and Botok-10 has considerably high genetic diversity for the three traits of interest, namely plant height, flowering time, and grain weight per plant. Therefore, selection using the above-mentioned traits can be considered to be conducted. Selection responses based on a weighted selection index indicate an expected decrease in flowering time and an increase in grain weight per plant in the next generation.

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