Analyzing the Variability and Correlation in M$_4$ Mutants of Kodo Millet (*Paspalum scrobiculatum* L.) Grown in Southern India

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Original Research Article

ABSTRACT

Kodo millet is one of the underutilized lost crops that has renowned nutritional and climate resilient traits. As a gluten free nutricereal, it is now being consumed in a fashion similar to that of rice and several value-added products are arising in the market day by day. Hence, this study on variability and correlation in M$_4$ mutants have been carried out to favor superior varietal development in kodo millet to sustain nutritional security in future. In this study, around 635 M$_4$ mutants were evaluated during Kharif 2019 on Millet Breeding Station of Coimbatore (India), with thirteen morphological traits. Among all, high PCV and GCV values were observed in plant height, panicle length, flag leaf length, seeds per panicle, culm thickness and seed yield. This presented the presence of genetic variability in the population. The prospects of effective selection with high heritability and genetic

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advance were exhibited by plant height, panicle length, panicle branches, flag leaf length, flag leaf breadth, seeds per panicle, stomatal number, chlorophyll index, culm thickness and seed yield per plant due to the presence of additive gene action. The correlation among traits towards yield revealed the significant positive correlation of all traits except for culm thickness and the highest associated traits were seeds per panicle and number of panicle branches. Therefore, these traits could be further focused for selection and improvement of yield in successive generations and other kodo breeding programs.

1. INTRODUCTION

Kodo millet (*Paspaulum Scrobiculatum* L.) is an allotetraploid with a chromosome number of 2n=4x=36. This is one among the small millets belonging to the *Poaceae* family and is known for its viability in longer storage conditions [1]. Kodo millet, commonly known as varagu in Tamil, is cultivated predominantly by the rainfed farmers as a cover crop. This also has a higher forage value and its leaves are used as a palatable feed for goats and sheep. Among the eleven species in small millets, kodo millet has the highest phosphorous, phenol and radical scavenging activity in its grains. Hence, consuming kodo millet reduces the risk of cardiovascular diseases. The leaves of kodo millet are rich in lecithin, a neurotransmitter and its grains are being used to manufacture processed biscuits, noodles and laddu which are gaining vast importance in the food outlets [2].

Since, the small millet foods are free of gluten with a higher fiber content, the diabetic and celiac patients completely rely on these grains for their caloric intake [3]. In addition to all these nutritional strengths, this crop has also been known to possess an inherent ability to withstand harsh climatic conditions. The roots of kodo millet are fibrous in nature with an ability to penetrate the Rocky Mountains and this crop is also called as ditch weed could survive in waterlogged conditions. Hence, the demand for kodo millet as a smart food in future will create a huge thrust on developing high yielding varieties for a sustainable production [4].

Being a self-pollinated crop owing to its cleistogamous flowers, breeding in kodo millet relies on selection and introduction [5]. Thus, mutation breeding is an alternate strategy to induce the genetic variability for developing elite mutants in small millets. In this study, the variety CO 3 in kodo millet was subjected to physical and chemical mutagenic treatments for inducing genetic variation [6]. Further, the desirable mutant families from M_2 were forwarded to M_4 generation to develop an elite line towards varietal development. Therefore, as an initiative, in this study the variability and correlation analysis for the segregants were performed to carry out an effective selection for major yield attributing traits in the upcoming generations.

2. MATERIALS AND METHODS

The experimental trail was conducted in Millet Breeding Station in the Department of Millets, Tamil Nadu Agricultural University, Coimbatore, during Kharif 2019 which lies between 11° N and 77°E. This region falls under Tropical climatic conditions. The current experiment was conducted with a set of 635 M_4 mutants from 88 families of M_3 generation. The observations were recorded on thirteen traits in all the mutant plants of each family. Thirteen traits recorded include Days to flowering, plant height, number of tillers, panicle length, number of panicle branches, leaf length, leaf width, number of seeds/panicle, stomatal length, stomatal number, chlorophyll index, culm thickness and single plant yield. Mutants were raised in ear by row method along with wild variety CO 3.

Mean, range, variability analysis and correlation coefficient were analyzed. The variability analysis including mean, range, standard deviation, variance and coefficient of variation were carried out. The phenotypic variance in this program is the variation occurring in the segregating population and the variation in the wild type (Control) explains the environmental variation. The genotypic variance is the difference in the phenotypic variance to the environmental variance. The coefficient of variation explains the phenotypic coefficient of variance (PCV), genotypic coefficient of variance (GCV). PCV and GCV were calculated by the method given by Burton [7]. Heritability in broad sense was calculated by using formula given by [8] and the

genetic advance as percent of mean (GAM), was estimated by the formula suggested by Johnson et al. [9]. The correlation to dissect the major yield contributing traits in kodo millet, put forth by Pearson [10], was analyzed statistically (Tables 1 & 2). The variability and the correlation analysis for the mutants were performed in Microsoft Excel and XL stat version 2019 and the results were tabulated.

Please indicate calculation of phenotypic and genotypic correlation coefficients. ??

Table 1. Categories of PCV and GCV [11]

S. No.	Range of CV	Category
1.	<10%	Low
2.	10-20%	Moderate
3.	>20%	High

Table 2. Categories of heritability and genetic advance [12]

S.No.	Range of h2	Category	Range of GA as % of mean	Category
1.	<40%	Low	<10%	Low
2.	40-60%	Moderate	10-20%	Moderate
3.	60-80%	High	>20%	High
4.	>80%	Very high		

3. RESULTS AND DISCUSSION

The experimental material consist of M4 mutants which were raised in the ear to row method along with the wild CO 3. The results of mean, range and variability parameters from this study are presented in the Table 3. Variability measures the extent of genetic variance which is explained by the genetic factors such as PCV, GCV, heritability and genetic advance as per cent of mean. The results showcases that the PCV was higher than the GCV for all traits observed. This implies that the variation in the population was not only due to genotypes but also by means of the influence in the environment. Hence, selection on these traits needs to be emphasized based on their heritability and genetic advance [3].

Moderate PCV were observed in the traits namely, days to first flowering, stomatal length and stomatal number. However, high PCV was observed in the rest of the traits that comprised of plant height, number of tillers, panicle length, panicle branches, flag leaf length, flag leaf breadth, seeds per panicle, chlorophyll index, culm length and seed yield. Subsequently, these traits *viz.*, plant height, panicle length, flag leaf length, seeds per panicle, culm thickness and seed yield that showed a higher PCV also recorded a higher GCV. Hence the variability for the above traits in the mutants were accountable due to the genetic effects and selection for these traits would be reliable. Despite this, the GCV was found to be low in stomatal length and moderate in traits such as days to first flowering,

number of tillers, panicle branches, flag leaf breadth, stomatal number and chlorophyll index. Thus, selection for these traits would be misleading and similar results for low PCV and GCV for these traits were reported by Ragini et al. [13].

Heritability and Genetic advance are the important parameters that plays a major role in effective selection from the variance. Owing to this a very high heritability was observed in days to first flowering, plant height, flag leaf breadth, seeds per panicle, stomatal length, stomatal number and culm thickness. Successively the traits, panicle length, panicle branches, flag leaf length, chlorophyll index and seed yield showed a higher heritability. However, moderate heritability was observed only in number of tillers per plant. A higher heritability cum genetic advance proposes the role of additive genes and such expression were seen in the traits namely, plant height, panicle length, panicle branches, flag leaf length, flag leaf breadth, seeds per panicle, stomatal number, chlorophyll index, culm thickness and seed yield per plant. Therefore, selection for these traits would be effective and these traits possess the scope for further improvement by selection (Table 3). Among all, the stomatal length showed a high heritability with moderate genetic advance as per cent of mean. This designated the role of non-additive gene action. Since, the high heritability in this trait was due to the influence of environment, selection for this trait might not be effective [14]. However, moderate heritability with high genetic advance was attained in number of tillers per

plant, which implies that this trait is governed by additive genes and the moderate heritability is put forth by the high environmental effect [15]. Thus, the selection for this trait will be effective in breeding programs. Therefore, to conclude the traits namely plant height, panicle length, panicle branches, flag leaf length, flag leaf breadth, seeds per panicle, stomatal number, chlorophyll index, culm thickness, number of tillers per plant and seed yield per plant could be considered for effective selection and improvement by breeding programs of kodo millet in near future [16].

Selection for dependent traits like yield reinstates the importance of contributing characters, which is estimated by the correlation [17]. Since the M4 mutants are segregants which were raised in the ear to row method gives the estimates of phenotypic correlation coefficient. Among the thirteen traits observed, except for stomatal number and culm thickness, all the traits established a significant positive correlation to seed yield per plant. Thus, breeding for non-lodging types by increasing the culm thickness has to be reinforced in future to overcome the yield compensation [18]. The highest significant correlation with yield in kodo millet was observed in number of seeds per panicle and following this, number of panicle branches established a significant positive association with seed yield

per plant. While observing the correlation for number of seeds per panicle, the traits namely, number of branches per panicle, plant height and flag leaf length had a higher significant positive correlation. Thus, it is understood that the key selection indices for improving yield in kodo millet should include the major traits *viz.,* number of seeds per panicle, number of branches per panicle, plant height and flag leaf length [5,13].

Considering the scope of analyzing the photosynthetic efficiency with Chlorophyll Index, the traits namely, plant height, seeds per panicle, days to first flowering and yield were significantly correlated. Hence, chlorophyll index could be observed to be influenced by the above traits and these are the major parameters to be affected by the chlorophyll fluorescence. Further, the stomatal number was observed to be significantly correlated to plant height and chlorophyll index (Table 4). Thus, the photosynthetic efficiency in kodo millet significantly influences the plant height, seeds per panicle and days to flowering. Among these interrelated traits, it is also observed that the seeds per panicle and plant height are the major key indicators for high yield (Fig. 1). Therefore, a focus on higher chlorophyll index and seeds per panicle in future would develop photosynthetic efficient lines in kodo millet [19,20].
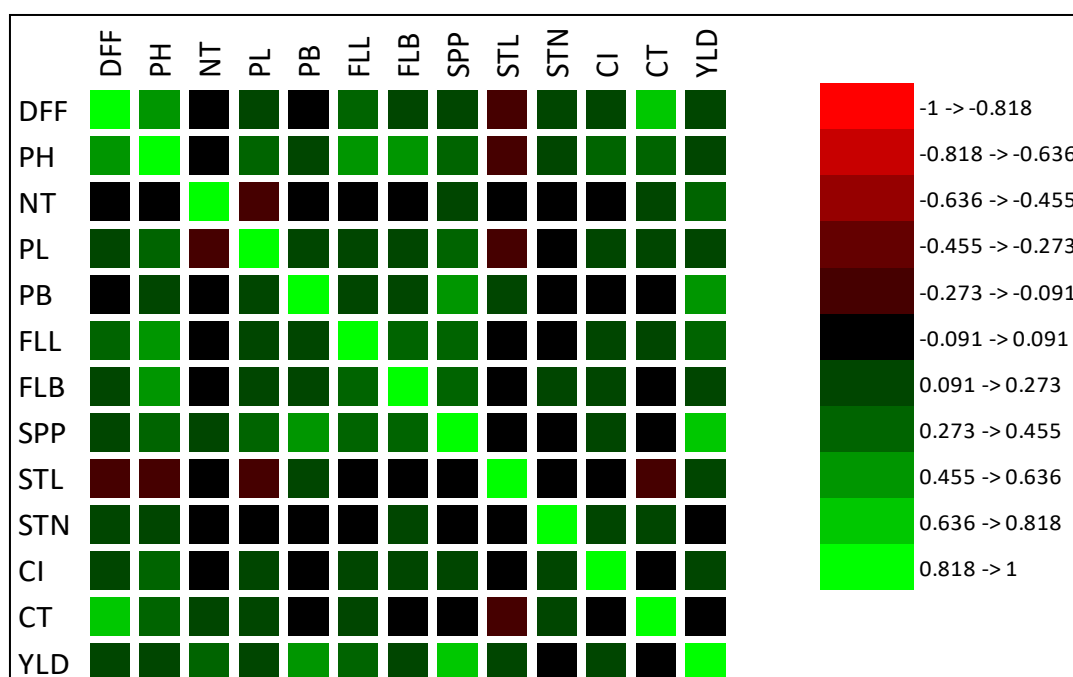

Fig. 1. Correlation matrix of the traits with seed yield per plant
DFF – Days to first flowering, PH- Plant height, NT- number of tillers, PL- Panicle length, PB- Panicle Branches, FLL- Flag leaf length, FLB – Flag leaf Breadth, SPP-Seeds per panicle, STL- Stomatal length, STN- Stomatal number, CI- Chlorophyll Index, CT- Culm Thickness, YLD- Seed yield per plant

Table 3. Variability parameters for thirteen morphological traits in kodo millet mutants

Traits	Mena	Range	PV	GV	PCV (%)	GCV (%)	EV	H² (%)	GA	GAM (%)
DFF	63.77	39.00-83.00	114.25	110.20	16.80	17.10	3.16	96.46	21.24	34.59
PH	89.07	42.00-135.00	370.45	327.09	21.58	20.43	7.38	88.30	35.01	39.54
NT	14.83	7.00-25.00	20.02	6.94	30.24	15.06	21.10	34.69	3.20	26.13
PL	15.87	8.00-24.62	12.48	9.36	22.22	20.11	11.10	75.04	5.46	35.89
PB	3.31	2.00-7.00	0.72	0.49	25.23	18.90	14.34	67.70	1.19	32.04
FLL	27.03	10.00-45	50.66	35.56	26.43	21.26	14.43	70.19	10.29	36.70
FLB	0.96	0.50-1.50	0.05	0.04	22.09	18.84	9.53	81.39	0.36	35.01
SPP	157.26	55.00-247.00	1475.72	1302.79	24.25	23.08	8.30	88.28	69.86	44.67
STL	8.15	5.20-9.82	0.77	0.72	10.82	9.55	2.96	92.52	1.68	18.93
STN	58.59	33.00-84.00	90.48	79.58	16.21	16.02	5.63	87.95	17.23	30.94
CI	32.17	11.40-56.70	49.35	32.75	21.74	17.94	12.61	66.36	9.60	30.10
CT	1.74	0.82-3.56	0.48	0.44	40.42	26.80	8.82	92.26	1.31	55.19
YLD	13.15	4.10-34.70	37.25	25.54	44.75	27.54	20.50	68.57	8.62	52.42

DFF – Days to first flowering, PH- Plant height, NT- number of tillers, PL- Panicle length, PB- Panicle Branches, FLL- Flag leaf length, FLB – Flag leaf Breadth, SPP-Seeds per panicle, STL- Stomatal length, STN- Stomatal number, CI- Chlorophyll Index, CT- Culm Thickness, YLD- Seed yield per plant

Table 4. Correlation coefficients of thirteen morphological traits

Variables	DFF	PH	NT	PL	PB	FLL	FLB	SPP	STL	STN	CI	CT	YLD
DFF	1.000												
PH	0.556**	1.000											
NT	0.056	0.043	1.000										
PL	0.232**	0.452**	-0.105**	1.000									
PB	-0.004	0.137**	0.049	0.118**	1.000								
FLL	0.392**	0.554**	-0.074	0.255**	0.210**	1.000							
FLB	0.233**	0.512**	0.029	0.268**	0.187**	0.386**	1.000						
SPP	0.226**	0.382**	0.114**	0.283**	0.563**	0.323**	0.287**	1.000					
STL	-0.128*	-0.124*	0.080	-0.121**	0.145**	-0.047	-0.056	0.090*	1.000				
STN	0.178**	0.227**	0.057	0.086*	-0.080*	0.038	0.217**	0.028	-0.029	1.000			
CI	0.189**	0.278**	0.086*	0.109**	0.024	0.140**	0.174**	0.242**	-0.012	0.147**	1.000		
CT	0.694**	0.294**	0.094*	0.207**	0.043	0.211**	0.090*	0.031	-0.119**	0.136**	-0.055	1.000	
YLD	0.131**	0.229**	0.290**	0.122**	0.473**	0.276**	0.174**	0.675**	0.204**	-0.022	0.164**	0.075	1.000

** significance at 0.01 level; * significance at 0.05 level

DFF – Days to first flowering, PH- Plant height, NT- number of tillers, PL- Panicle length, PB- Panicle Branches, FLL- Flag leaf length,   FLB – Flag leaf Breadth, SPP-
Seeds per panicle, STL- Stomatal length, STN- Stomatal number, CI- Chlorophyll Index, CT- Culm Thickness, YLD- Seed yield per plant

4. CONCLUSION

The present study revealed the ambient amount of variability present among the traits studied in the population. Higher genetic variability along with higher heritability and high Genetic advance as per cent of mean was observed in seed yield per plant, seeds per panicle, flag leaf length, plant height and Culm thickness. Selection based on these traits would be effective for the improvementof the population. Improvement of yield could be identified using positive significant correlation expressed with all the traits except culm thickness. These traits for the improvement of yield would be effective.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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