Original Research Article

Analyzing the Variability and Correlation in M_4 mutants of Kodo Millet (*Paspaulumscrobiculatum*L.)grownin Southern India

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Abstract

Kodo millet is one of the underutilized lost crops that has renowned nutritional and climate resilient traits. ~~This being~~As a gluten free nutricereal, itis 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 evaluatedduring Kharif 2019on 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. T~~Further, t~~he prospects of effective selection with high heritability ~~cum-~~andgenetic 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.

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Keywords: cereal crop, nutritional security, heritability, genetic advance, environmental variance, agro-morphological trait~~Variability, M4, Correlation and kodo millet.~~

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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 isalso 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].

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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

Site characteristics

The experimental trail was conducted in Millet Breeding Station in the Department of Millets, Tamil Nadu Agricultural University, Coimbatore, during Kharif 2019.

Climate and soil type description, GPS coordinates ???

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Crop material tested and agro-morphological traits investigated

Planting date, which periods for observations depending on traits, date of harvest ???

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The current experiment was conducted with a set of 635 M_4 mutants from 88 families of M_3 generation.~~ The experimental trail was conducted in Millet Breeding Station in the Department of Millets, Tamil Nadu Agricultural University, Coimbatore, during Kharif 2019.~~The observations were recordedon thirteen traits in all the mutant plants of each family. Thirteen traits ~~were~~ recorded ~~and they~~ includewere ~~*viz.*,~~ Days to flowering ~~(days)~~, plant height ~~(cm)~~, number of tillers ~~(nos)~~, panicle length ~~(cm)~~, number of panicle branches ~~(nos)~~,

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leaf length~~ (cm)~~, leaf width~~ (cm)~~, number of seeds/panicle~~ (nos)~~, stomatal length~~ (µm)~~, stomatal number~~ (nos)~~, chlorophyll index, culm thickness~~(mm)~~ and single plant yield~~ (g)~~.

Experimental design

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Variability analyses of millet mutants

The variability analysis including mean, range, standard deviation, variance and coefficient of variationwere 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 per cent~~as percent of mean (GAM), was estimated by the formula suggested by Johnson *et al*[9]. T~~Inclusively, t~~he correlation to dissect the major yield contributing traits in kodo millet,putforth by Pearson[10], was analyzed statistically (Table 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.

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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

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Agro-morphological performances of millet mutants

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Variability parameters

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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 of the variability parameters from this studyare presented in the table 3. The results showcases that thePCV 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].

ModeratePCVwereobserved in the traits namely, days to first flowering, stomatal length and stomatal number. However, high PCVwasobservedin 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 ~~yielded~~ showeda 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, theGCVwas 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 byRagini*et al*[13].

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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~~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,

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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 designatedthe 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~~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].

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Selection for dependent traits like yield reinstates the importance of contributing ~~characters which~~characters, whichis estimated by the correlation [17]. 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].

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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 (Figure 1). Therefore, a focus on higher chlorophyll index and seeds per panicle in future would develop photosynthetic efficient lines in kodo millet [19,20].

Table 3: Variability ~~Parameters~~ parameters for thirteen morphological traits in kodo milletmutants

Traits	PV	GV	PCV (%)	GCV (%)	E~~C~~V	~~H2~~ H^2 (%)	GA	GAM (%)
DFF	114.25	110.20	16.80	17.10	3.16	96.46	21.24	34.59
PH	370.45	327.09	21.58	20.43	7.38	88.30	35.01	39.54
NT	20.02	6.94	30.24	15.06	21.10	34.69	3.20	26.13
PL	12.48	9.36	22.22	20.11	11.10	75.04	5.46	35.89
PB	0.72	0.49	25.23	18.90	14.34	67.70	1.19	32.04
FLL	50.66	35.56	26.43	21.26	14.43	70.19	10.29	36.70
FLB	0.05	0.04	22.09	18.84	9.53	81.39	0.36	35.01
SPP	1475.72	1302.79	24.25	23.08	8.30	88.28	69.86	44.67
STL	0.77	0.72	10.82	9.55	2.96	92.52	1.68	18.93
STN	90.48	79.58	16.21	16.02	5.63	87.95	17.23	30.94
CI	49.35	32.75	21.74	17.94	12.61	66.36	9.60	30.10
CT	0.48	0.44	40.42	26.80	8.82	92.26	1.31	55.19
YLD	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

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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 0.01level * 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

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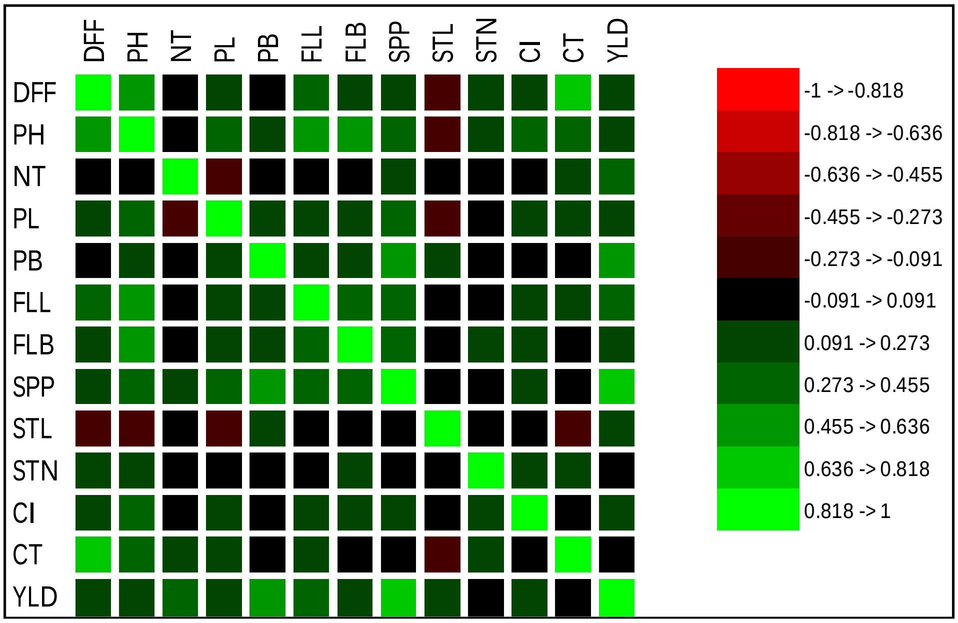
Figure 1: Correlation matrix of the traits with seed yield per plant



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

Please indicate on Fig 1 what correlation coefficients are above and below the diagonal

4. Conclusions

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References

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1.Burton, G. W. (1940). A CYTOLOGICAL STUDY OF SOME SPECIES IN THE. *Journal of Agricultural Research*, *60*, 193.

2.Deshpande, S. S., Mohapatra, D., Tripathi, M. K., &Sadvatha, R. H. (2015). Kodo millet-nutritional value and utilization in Indian foods. *Journal of grain processing and storage*, *2*(2), 16-23.

3.Sao, A., Singh, P., Kumar, P., & Pradhan, A. (2017). Estimates of genetic parameters for yield and contributing traits in kodo millet (*Paspalumscrobiculatum* L.). *Research Journal of Agricultural Sciences*, *8*(1), 120-122.

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4.Hariprasanna, K. (2017). Kodo millet, *Paspalumscrobiculatum* L. *Millets and sorghum: biology and genetic improvement*, 199-225.

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5.Nirubana, V., Ganesamurthy, K., Ravikesavan, R., &Chitdeshwari, T. (2017). Genetic variability studies for yield and yield components in kodo millet (*Paspalumscrobiculatum* L.). *Electronic Journal of Plant Breeding*, *8*(2), 704-707.

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6.Jency JP., Ravikesavan R., Sumathi P., Raveendran M., (2016). Determination of lethal dose and effect of physical mutagen on germination percentage and seedling parameters in kodo millet variety CO 3. Electron J Plant Breed. 7(4),1122–1126

7.Burton, G. W. (1952). Quantitative inheritance in grasses. *Pro VI IntGrassl Cong*, *1952*, 277-283.

8.Lush, J. L. (1949). Heritability of quantitative characters in farm animals. *Heritability of quantitative characters in farm animals.*

9.Johnson, H. W., Robinson, H. F., & Comstock, R. E. (1955). Estimates of genetic and environmental variability in soybeans 1. *Agronomy journal*, *47*(7), 314-318.

10.Pearson, K. (1902). V. on the methematical theory of errors of judgement, with special reference to the personal equation. *Philosophical Transactions of the Royal Society of London*.

11.Sivasubramanian, S., &Madhavamenon, P. (1973). Genotypic and phenotypic variability in rice. *Madras Agric. J*, *60*(9-13), 1093-1096.

12.Searle, S. R. (1965). The value of indirect selection: I. Mass selection. *Biometrics*, 682-707.

13.Ragini, S., Subramanian, A., Nirmalakumari, A., &Bapu, J. K. (2015). Genetic diversity in kodo millet (*Paspalumscrobiculatum*L) relative to culm strength and yield. *Journal of Crop Improvement*, *29*(4), 420-431.

14. Subramanian, A., Nirmalakumari, A., &Veerabadhiran, P. (2010). Trait based selection of superior Kodo millet (*Paspalumscrobiculatum* L.) genotypes. *Electron. J. Plant Breed*, *1*, 852-855.

15.Thakur, J., Kanwar, R. R., &Shyam, K. K. (2020). Studies of correlation between yield and yield attributing characters of Kodo millet (*Paspalumscrobiculatum* L.). *Journal of Pharmacognosy and Phytochemistry*, *9*(6), 1919-1922.

16.Yadav, Y., Lavanya, G. R., Pandey, S., Verma, M., Ram, C., & Arya, L. (2016). Neutral and functional marker based genetic diversity in kodo millet (*Paspalumscrobiculatum* L.). *Actaphysiologiaeplantarum*, *38*(3), 1-12.

17.Vetriventhan, M., &Upadhyaya, H. D. (2019). Variability for Productivity and Nutritional Traits in Germplasm of Kodo Millet, an Underutilized Nutrient□Rich Climate Smart Crop. *Crop Science*, *59*(3), 1095-1106.

18.Sreeja, R., Balaji, S., Arul, L., NirmalaKumari, A., KannanBapu, J. R., &Subramanian, A. (2016). Association of lignin and FLEXIBLE CULM 1 (FC1) ortholog in imparting culm strength and lodging resistance in kodo millet (*Paspalumscrobiculatum* L.). *Molecular breeding*, *36*(11), 1-9.

19.Nirubana, V., Ravikesavan, R., &Ganesamurthy, K. (2021). Evaluation of underutilized kodo millet (*Paspalumscrobiculatum* L.) accessions using morphological and quality traits. *Indian Journal of Agricultural Research*, *55*(3), 303-309.

20.Anuradha, N., Patro, T. S. S. K., Triveni, U., Rao, P. J., Rani, Y. S., &Priya, P. K. (2020). Assessment of genetic variability estimates in Kodo millet *Paspalumscrobiculatum*L~~(L.)~~. *IJCS*, *8*(5), 1907-1909.

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