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Genetic variation for corm yield and other traits in Ethiopian enset (*Ensete ventricosum* (Welw.) Cheesman)

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***Enset (Ensete ventricosum* (Welw.) Cheesman*)* is a multipurpose crop used for food, fuel, housing materials, fencing and livestock feed. The major food types obtained from enset are kocho, bulla and amicho. Kocho is fermented starch obtained from decorticated (scraped) leaf sheaths and grated corms. Bulla is obtained by squeezing out the liquid containing starch from scraped leaf sheathes and grated corm and allowing the resultant starch to concentrate into white powder. Amicho is boiled enset corm pieces that are prepared and consumed in a similar manner to other root and tuber crops. Thirty-five cultivars of *Ensete ventricosum* were grown in RCBD (two replications) to study the different quantitative morpho-agronomic characters contributing to the diversity analysis at Areka and Chichu during 2012 to 2013 cropping season. Data on 10 quantitative traits were collected and exposed to statistical analysis. Analysis of variance revealed that there was significant difference between the two locations in all tested phenotypic characters. The mean squares due to cultivars, locations and cultivars x locations interaction were highly significant (P ≤ 0.001) for all the quantitative traits. The highest corm yields were recorded for varieties Chohot, Ashakit, Bose and Gazner. Farmers in the two locations ranked cultivar Ashakit first and Kataniya took second place whereas the first best performing cultivars (*Chohot*) was among the least preferred genotypes with low score (3). Among the studied character, phenotypic and genotypic coefficient of variation was highest for corm weight (36.17 and 27.28 tha-1 y-1). High heritability was estimate for plant height (77%). The phenotypic and genotypic coefficients of correlation indicated that corm yield ha-1 y-1 was positively correlated with most of the characters. The present study indicated a considerable amount of variability for the majority of the characters of interest in *Ensete* for exploitation.**

Key words: Corm, enset, genotype-by-environment interaction, quantitative traits.

INTRODUCTION

Enset (*Ensete ventricosum*) is well-established, sustainable, and environmentally resilient plant with farming system that contributes to food security of farmers in densely populated areas of the south and south-western part of Ethiopia (Bacha and Taboge, 2003).

The major foods obtained from Enset are *kocho*, *bulla* and *amicho. Kocho* is fermented starch obtained from decorticated (scraped) leaf sheaths and grated corms. *Bulla* is a liquid that is obtained when leaf sheaths and corm are pulverized, the liquid containing starch is squeezed out from scraped leaf sheathes and grated corm and the resultant starch are allowed to concentrate into white powder. *Amicho* is boiled enset corm pieces (usually from young enset plant) that are prepared and consumed in a similar manner with other root and tuber crops (Brandt et al., 1997).

Based on quality and characteristics in terms of harvesting, softness and hardness, palatability when immature and resistance to disease and pest, enset clones can also be categorized into male and female (Yemataw et al., 2014). Female enset are seen as early maturing, more palatable (sweet or tasty in other ways, especially when the corm is boiled), more easily scraped, less fibrous and generally delicious.

The yielding ability of a genotype is the ultimate result of favorable interaction of genotype (G) with the environment (E). Environmental factors differ across years and locations, having significant influence at different developmental stages of crop growth (Bull et al., 1992). Sprague (1966) indicated that G x E interactions constitutes an important limiting factor in the estimation of variance components and in the efficiency of selection programs. The presence of significant G x E interactions for quantitative traits such as seed yield can reduce the usefulness of subsequent analysis, restrict the significance of inferences that would otherwise be valid, and seriously limit the feasibility of selecting superior genotypes (Flores et al., 1998).

Among many tools morphological characterization based on the traits are commonly used to analyze genetic diversity since they provide a simple way of quantifying genetic variation while assessing genotypic performance under normal growing environments (Revilla and Tracy, 1995).

Furthermore, the effectiveness of selection also depends on the amount of variability existing in the material, the extent to which a character is heritable and the association/correlation between traits (Pandey and Gritton, 1975). Assessment of both nature and extent of variability as well as genetic association between characters helps in identifying the most important character to be considered in the improvement program.

Thus, since a remarkable phenotypic variation among *E. ventricosum* collection have been observed (Tabogie, 1997; Tsegaye, 2002), phenotypic variability of the crop based on different use value is essential to identify and categorize different accessions grown in different area of the country to establish a bench mark for further improvement or documentation.

Hence, information on the extent and pattern of G x E interactions on enset is scarce. Therefore, this study was proposed with the following objectives: (i) to determine the magnitude of G x E interaction for corm yield of enset cultivars under Ethiopian conditions, (ii) to determine the value and magnitude of genetic variability among 35 enset cultivars from morphological and agronomic variables that might guide the choice of parents for future breeding works in enset.

MATERIALS AND METHODS

Description of the research area

The experiment was conducted in two locations of Southern Ethiopia at Areka and Chichu in 2012-2013 enset cropping season. Areka is located at 7° 09′ N and 37° 47′ E and at an elevation ranging from 1,750 to 1,800 m above sea level (m.a.sl). Areka has an average rainfall of 1,539 mm and a minimum and maximum mean temperature of 14.5 and 25.8°C, respectively. The soil is silty loam with a pH of 4.8 to 5.6 and low to medium organic matter content (2.65-5.67%) (Esayas, 2003). Chichu is one of the kebele in Dilla Zuria woreda of the Gedeo Zone, Southern Nations Nationalities and People’s Regional State (SNNPRS), Ethiopia. It is situated at 6° 21’-6°24’ N latitude and 38° 17’-38° 20’ E longitude. It is warm humid temperate with an altitude of 1600 m.a.sl. and annual temperature ranging from 22-29°C. The soil is sandy clay loam.

Treatments and design

A total of 35 cultivars collected from different parts of the country by Areka Agricultural Research Centre were included for this experiment (Table 1). The experiment was laid out in a randomized complete block design and each accession was replicated two times. The respective spacing between plants and rows were 1.5m and 2m (a plot size of 12 m2).

Crop management and data collection

Equal sized suckers were directly planted in holes on the prepared experimental units on May 12, 2012 at Areka and on May 15, 202012 at Chichu. The experiment was conducted under rain-fed condition. Four plants per plot were considered for characters measured on individual plant basis. The whole plant was harvested two years after transplanting to the main plot. A total of 10 quantitative characters were recorded for evaluation. These are Plant Height, Pseudo stem Height, Pseudo stem Circumference, Leaf length, Leaf Width, Leaf Number, Corm Yield Per Hectare Per Year, Corm circumference, Corm length, Corm Yield Per Hectare Per Year.

$$\text{Corm yield (tons ha-1 yr-1)} = \frac{\text{Corm Yield per plant x10000m}^2}{No\ of\ years\ to\ maturity x\ plot\ area\ (m^2)}$$

Statistical analysis

The analysis of corm yield and other quantitative traits was performed using SAS computer software packages (SAS, 2002). Corm yield data was subjected to analysis of variance separately

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Table 1. List of enset cultivars, with their collection site.

S/N	Cultivar name	Locality/origin of collection	S/N	Cultivar name	Locality/origin of collection
1	Sebera	Kembata-Tembaro	19	Astara	Gurage
2	Switea	Kembata-Tembaro	20	Chohot	Gurage
3	Tessa	Kembata-Tembaro	21	Qibnar	Gurage
4	Qoyina	Kembata-Tembaro	22	Ashakit	Gurage
5	Guariye	Kembata-Tembaro	23	Gazner	Gurage
6	Bose	Kembata-Tembaro	24	Fenqo	Gurage
7	Leqaqa	Kembata-Tembaro	25	Agade	Gurage
8	Bino	Kembata-Tembaro	26	Diqa	Dawro
9	Sirareia	Wolaita	27	Musula	Dawro
10	Neqaqa	Wolaita	28	Bukuniya	Dawro
11	Shelequmia	Wolaita	29	Neqaqa	Dawro
12	Silqantia	Wolaiyta	30	Switeia	Dawro
13	Haleko	GamoGoffa	31	Argema	Dawro
14	Matiya	GamoGoffa	32	Arkiya	Dawro
15	Keteniya	GamoGoffa	33	Niffo	Gededo
16	Gena	GamoGoffa	34	Addo	Sidama
17	Tuffa	GamoGoffa	35	Gedeme	Sidama
18	Zinka	GamoGoffa			

Where, Y_{ijk} = observed value of cultivars i in block k of environment (location) j, ì = grand mean, Gi = effect of cultivar i, Ej = environment or location effect, GE_{ij} = the interaction effect of cultivar i with location (environment) j, Bk(j) = the effect of block k in location (environment) j, ε_{ijk} = error (residual) effect of cultivar i in block k of location (environment) j.

Mean separation was conducted using least significant difference (LSD) test to discriminate the genotypes and identify superior ones based on the trait of interest.

Estimation of phenotypic and genotypic variances

The genotypic and phenotypic coefficients of variation (GCV and PCV) for each trait were calculated using the following formula:

Genotypic coefficients of variation (GCV) = (σ^2g/grand mean of character) x 100

Phenotypic coefficients of variation (PCV) = (σ^2p /grand mean of character) x 100

Estimation of heritability and expected genetic advance

Broad sense heritability and expected genetic advance (gain) with one cycle of selection were estimated for each character using variance components as described by Allard (1960):

$$\text{Heritability, } H^2 = \frac{\sigma^2 g}{\sigma^2 p} \times 100$$

Genetic advance as percent of mean, GAM = (GA/Ȳ) × 100, where GA = genetic advance and Ȳ = mean of the trait for all cultivars.

Analysis of phenotypic and genotypic correlation coefficients

Genotypic and phenotypic correlation coefficients for corm yield and its components were estimated by calculating the variance and covariance at phenotypic and genotypic levels by using the formula suggested by Singh and Chaudhury (1985).

Farmers' preferences

In this study, group discussion was used for evaluation and data collection with farmers. Through focus group discussions with key informants in the two locations, a total of 4 (color, test, texture, overall acceptance) different criteria were used for selection purpose. After harvested, the cultivars are tested by farmers and gave its ranking of selected cultivars based on the evaluation criteria. The pair-wise ranking (Russell, 1997) method was used to analyze the position of each of cultivar in tested areas by farmers evaluation criteria. A matrix table of cultivars in the two locations was constructed. Farmers were asked to compare each cultivar to the other ones with regards to the values of each criteria and the priority each farmer gives to the cultivar. Each cultivar was compared in turn with each of the other cultivars.

RESULTS

The combined analysis of variance over locations showed ensete corm yield was significantly (P<0.001) affected by location, which is a proxy for environment (E), cultivar, which is a proxy for genotypes (G) and cultivar x location interactions (Table 2). The significance of the interaction indicated the best cultivars in one locations (and hence environment) are not necessarily, the best in another.

The highly significant genotype x environment (G x E) interaction may be either a crossover G x E interaction or a non-crossover nature. In crossover nature, the GXE interaction a significant change in ranks occurs from one environment to another. In non-cross over, GXE

Table 2. Pooled analysis of variance, coefficient of variation (CV), and coefficient of determination (R^2) for 10 traits assessed in 35 Enset cultivars across three environments.

Source of variation	Mean Squares									
	DF	PH	PSH	PSC	LL	LW	LN	COM CI	COMLEN	CORTON
Treatment	34	3.59***	0.24***	0.35***	2.15***	0.07***	28.52***	0.04***	0.01**	187.34***
Location	1	109.32***	0.55***	1.72***	53.82***	0.93***	10.76NS	1.29***	0.002 NS	6349.17***
Treatment x Location	34	1.48***	0.10***	0.15***	0.87***	0.03***	16.49***	0.03***	0.013**	123.77***
Error	210	0.58***	0.16***	0.20***	0.45***	0.09***	2.16***	0.12***	0.08***	5.15***
CV		20.88	24.84	23.15	23.76	16.34	17.64	18.47	31.95	43.24
R^2		0.79	0.70	0.68	0.76	0.75	0.61	0.57	0.39	0.75

***, **, *, ns= significant at 0.1, 1, 5%, and non-significant, respectively. CV= coefficient of variation, R^2 = Coefficient of determination, ***, **, *, ns= significant at 0.1, 1, 5%, and non-significant, respectively. SV= source of variation, CV= coefficient of variation, R^2 = Coefficient of determination, PH=Plant Height, PSH=Pseudo stem Height, PSC=Pseudo stem Circumference, LL=Leaf length, LW=Leaf Width, LN=Leaf Number, CORTON = corm Yield Per Hectare Per Year, COMCI= Corm circumference, COMLEN= corm length, CORTON = corm Yield Per Hectare Per Year.

interaction, ranking of genotypes remains constant across environments and the interaction is significant because of changes in the magnitude of response (Matus et al., 1997).

Genotypes differ significantly in their mean yield performance. The G x E interaction of genotypes in this study was of crossover nature. Cultivars' performances across the two locations differ significantly in their mean yield performances (Table 3). The genotypes 'Chohot, Ashakit, Bose and Gazner had the highest values for corm yield ha^{-1} $year^{-1}$ (Table 3). Hence, cultivars which were grouped in high and medium yielding were found to be promising for corm yield. These highest yielding cultivars should be released as varieties for wide adaptation.

Sensory evaluation

For selection, good performance is not sufficient; the cultivar must also have desirable sensory and utilization characteristics. *Ashakit* and *kataniya* were the most preferred cultivars with all test attributes having good scores (2.0), whereas the first best performing cultivar (*Chohot*) was among the least preferred genotypes with low score (5) (Table 4).

Variability components

A wide variation was observed between maximum and minimum values for most of the characters (Table 5). The phenotypic coefficient of variation was higher than the genotypic coefficient of variation for all the assessed traits (Table 5).
The PCV ranged from 12.74 for corm circumference to 36.17 for corm weight tha^{-1} y^{-1} and the genotypic coefficient of variation ranged from 5.54 for corm circumference to 27.28 for corm weight tha^{-1} y^{-1}.

The heritability estimates obtained for the traits studied ranged between 19 (corm circumference) and 77% (plant height) (Table 6). High to medium broad sense heritability was observed for plant height, leaf width, pseudostem height, leaf length, leaf number, pseudostem circumference, corm weight in tone per hectares per year and corm weight per plot. The low broad sense heritability observed for corm length (39%) and corm circumference (19%). High to medium heritability and genetic advance as percent of the mean were recorded for corm weight in tone per hectares per year, corm weight per plot, plant height, pseudostem height and leaf length. High heritability estimates with low genetic advance observed for leaf number, leaf width and pseudostem circumference.

Genotypic coefficients of correlation, in general, were higher than the corresponding phenotypic coefficients of correlation (Table 5, above and below diagonal, respectively) indicating relatively little influence of environment on any inherent association among the traits studied. The phenotypic and genotypic coefficients of correlation indicated that corm yield ha^{-1} y^{-1} was positively correlated with most of the characters. This suggests that selection for corm yield can be done through selection of those traits with which it is strongly correlated (Table 6).

DISCUSSION

Information on phenotypic variation and its geographical distribution is important for genetic

Table 3. Average values for plant growth and yield traits of highly performing enset cultivars evaluated across two locations.

Cultivar name	PH	PSH	PSC	LL	LW	LN	COM CI	COMLEN	CORTON
Chohot	4.07	1	1.21	2.97	0.61	15.37	0.71	0.34	23.29
Ashakit	3.1	0.71	1.17	2.26	0.58	15.87	0.75	0.32	20.13
Bose	3.78	0.85	1.22	2.79	0.67	12.37	0.74	0.27	19.66
Gazner	3.04	0.7	0.96	2.21	0.56	12.87	0.69	0.25	18.53
Neqaqa	4.17	0.9	1.32	3.12	0.68	10.5	0.64	0.22	17.29
Sebera	2.84	0.74	1.2	2.12	0.62	13.25	0.6	0.31	16.92
Musula	2.83	0.74	1.01	2.37	0.5	13.12	0.66	0.26	16.6
Fenqo	3.14	0.68	0.98	2.41	0.5	12.62	0.72	0.24	16.55
Keteniya	3.38	0.83	1.01	2.56	0.61	13.12	0.77	0.26	15.94
Tessa	3.23	0.7	1.07	3.06	0.52	14.62	0.66	0.21	15.42

PH=Plant Height, PSH=Pseudo stem Height, PSC=Pseudo stem Circumference, LL=Leaf length, LW=Leaf Width, LN=Leaf Number, CORTON = corm Yield per Hectare per Year, COMCI= Corm circumference, COMLEN= corm length, CORTON = corm Yield Per Hectare Per Year.

Table 4. Over all preference ranking of high yielder cultivars.

Cultivar	Scores				
	Color	Texture	Test	Over all acceptance	Pair-wise rank
Bose	3	3	2	3	2
Chohot	5	5	5	5	3
Keteniya	2	2	2	2	1
Ashakit	2*	2	2	2	1

*Note: 1=Excellent, 2=Very good, 3=good, 4=not bad, 5=bad

conservation, plant breeding and efficient utilization of plant genetic resources (Bekele, 1996).

Highly significant difference between the (cultivars) of enset under study may be due to differences in their genetic background and diverse nature of origin. The highly significant differences between locations indicated an existence of variation in the prevailing environment during the growth and developmental stages of the experiments. The highly significant genotype x environment (G x E) interaction may be either a crossover G x E interaction or a non-cross over nature.

The G x E interaction of genotypes in this study was of crossover nature. In crossover, significant change in ranks occurs from one environment to another (Matus et al., 1997). Genotypes (cultivars) differ significantly in their mean yield performance. Tabogie (1997), Tsegaye (2002) and Yemataw et al. (2014) reported a wide phenotypic variation among enset cultivars across a broad set of agro-ecological zones in southern Ethiopia.

Cultivar *Chohot*, *Ashakit Bose* and *Gazner* had superior corm yields. For selection, good performance is not sufficient; the cultivar must also have desirable sensory and utilization characteristics. Cultivars *Ashakit* and *katania,* which had the second and the ninth corm yield, were moderately liked by taste panelists. This implies that taste attributes may be as important as agronomic traits when farmers are making decision on which cultivars to adopt or reject. This is in agreement with Kapinga et al. (2009) who reported that sweet potato varieties adoption highly dependent on farmers' main criteria such as high yield, early maturity, disease and pest tolerance, sweetness, root firmness, low fiber content and extended ground storability. Moreover, Faye (2002) reported similar results on cowpea in Senegal and found that buyers are willing to pay a premium for grain size and white skin color but discount price for other color and number of bruchid holes on the grain.

The wide variation in observed traits may point to opportunities for selecting enset cultivars with desirable characters. The wide range in each of the traits studied offers broad opportunities for selecting parents of interest in breeding programs to develop varieties suitable for different agro-ecologies of the country. Similar results were obtained by Yemataw et al. (2012), who studied the variability of 240 ensete cultivars for *kocho* yield.

The authors reported a wide variability in *kocho* yield ranging from 1.29 to 25.32 t ha^{-1} y^{-1}. Nevertheless, there was a close relationship between phenotypic and genotypic coefficients of variation for all traits. Closeness of the two coefficients of variation indicates the

Table 5. Estimates of ranges, means, PCV, GCV, heritability (%) in broad sense (h^2b) and genetic advance as percent of the mean (GAM) for 10 quantitative morphological characters in 35 Enset cultivars.

Character	Mean	Range		GCV	PCV	h^2b	GAM
	± SE	Minimum	Maximum				
PH(m)	2.79±0.07	0.37	5.12	19.51	28.64	77	32.94
PSH(m)	0.63±0.01	0.07	1.40	21.53	32.84	74	35.64
PSC(m)	0.65±0.02	0.10	1.81	19.17	29.02	65	27.43
LL (m)	2.06±0.05	0.36	7.96	20.10	29.00	72	32.1
LW (m)	0.53±0.01	0.14	1.30	13.76	20.89	75	22.51
LN	12.27±0.18	3.00	20.00	15.07	20.14	69	21.83
Comci (m)	0.65±0.01	0.23	1.10	10.21	12.74	19	4.96
ComLe (m)	0.26±0.005	0.10	0.44	11.51	14.95	39	12.26
ComW (Kg)	5.77±0.24	0.20	18.5	32.78	35.04	54	39.09
CORTON ($tha^{-1}yr^{-1)}$	11.91±0.54	0.43	41.71	26.39	36.17	57	42.39

***, **, *, ns= significant at 0.1, 1, 5%, and non-significant, respectively. SV= source of variation, CV= coefficient of variation, R^2 = Cofficient of determination, PH=plant height, PSH=pseudostem height, PSC=pseudostem circumference, LL=leaf length, LW=leaf width, LN=leaf number, Comci= Corm circumferance, Comle= corm length, ComW= corm yield per plant, CORTON= corm yield per hectare per year.

Table 6. Genotypic (above diagonal) and phenotypic (below diagonal) correlation coefficient for selected traits of enset cultivars for Amicho trial at Areka.

Characters	PH (m)	PSH (m)	PSC (m)	LL (m)	LW (m)	LN	Comci (m)	ComLe (m)	ComW (Kg)	CORTON ($tha^{-1}yr^{-1)}$
PH (m)	1.0	0.95***	0.81***	0.94***	0.79***	0.33	0.76***	0.33	0.82***	0.69**
PSH (m)	0.92***	1.0	0.79***	0.92***	0.77***	0.38	0.64	0.34	0.76***	0.65
PSC (m)	0.78	0.75	1.0	0.89***	0.62***	0.52**	0.77***	0.28	0.91***	0.89***
LL (m)	0.93***	0.86***	0.79***	1.0	0.75***	0.40	0.81***	0.18	0.87***	0.74**
LW (m)	0.74	0.69**	0.62	0.68**	1.0	0.27	0.85***	0.53	0.63**	0.53
LN	0.40	0.41	0.59**	0.43	0.34	1.0	0.06	0.44	0.69**	0.74***
Comci (m)	0.54	0.52	0.47	0.55**	0.40	0.50	1.0	0.24	0.86***	0.86***
ComLe (m)	0.26	0.26	0.18	0.16 ns	0.22	0.25 ns	0.42	1.0	0.25 ns	0.21 ns
ComW (Kg)	0.68	0.69**	0.76**	0.71**	0.49	0.53	0.72	0.28 ns	1.0	1.00
CORTON ($tha^{-1}yr^{-1)}$	0.60	0.62**	0.72	0.64	0.41	0.54	0.71**	0.26	0.95***	1.0

***, **, *, ns= significant at 0.1, 1, 5%, and non-significant, respectively. SV= source of variation, CV= coefficient of variation, R^2 = Cofficient of determination, PH=plant height, PSH=pseudostem height, PSC=pseudostem circumference, LL=leaf length, LW=Leaf Width, LN=leaf number, Comci= Corm circumferance, Comle= corm length, ComW= corm yield per plant, CORTON= corm yield per hectare per year.

importance of the genotype/genetic makeup in determining the phenotypic traits. In general, enset cultivars used in this study were phenotypically as well as genotypically diverse, which points to the existence of a large diversity in enset for quantitative characters.

The broad sense heritability is the relative magnitude of genotypic and phenotypic variances for the traits and it is used as a predictive role in selection procedures (Allard, 1960). This gives an idea of the total variation ascribable to genotypic effects, which are exploitable portion of variation. The low broad sense heritability observed for corm length and corm circumference indicates the influence of the environment on these traits. The low heritability recorded for these traits indicates that direct selection for these traits will be ineffective. Since high heritability does not always indicate high genetic gain, heritability with genetic advance considered together should be used in predicting the ultimate effect for selecting superior varieties (Ali et al., 2002). High to medium heritability and genetic advance as percent of the mean suggests that these traits are primarily under genetic control and selection for them can be achieved through their phenotypic performance. High heritability estimates with low genetic advance for those traits indicates non additive type of gene action and that G x E interaction plays a significant role in the expression of the traits.

Correlations between characters are of interest to determine whether selection for one trait will have an effect on another (De Araujo and Columan, 2002). The association between vegetative traits such as plant height, pseudostem height, pseudostem circumference, leaf length and corm weight has positive correlation (Taboge et al., 1996). This is in agreement with Yemataw et al. (2012) who reported that *kocho* yield was positively and significantly correlated with plant height, pseudostem circumference, leaf sheath number and leaf sheath weight. Therefore, it is logical to examine the correlation between various yield components and measure the intensity of the association. These relationships may reveal the yield components or agronomic traits that are useful indicators of ensete corm yield.

Conclusion

Cultivars *Chohot*, *Ashakit, Bose* and *Gazner* had superior corm yield. For selection, possession of good performance was not good enough; it must also have desirable sensory and utilization characteristics. Cultivars *Ashakit* and *katania,* were moderately liked by taste panelists. Taste attributes may be as important as agronomic traits when farmers are making decision on which cultivars to adopt or reject. The broad sense heritability, genetic advance as percent of the mean and correlation analysis of the study revealed that plant height, pseudostem height, leaf width and corm weight were the most important yield components. Therefore, the results suggest that these four traits are important yield contributing traits and selection based on these traits would be most effective.

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Conflict of interests

The authors declare that they have no competing interest.

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