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Research Article

Diversity analysis of moringa (*Moringa oleifera* Lam.) genotypes using DUS descriptors

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Abstract
Moringa is a crop with wide variability and is known as the miracle tree due to its application in various fields as food, medicine, plant growth stimulator and animal feed because of its nutritional, pharmacological, biotechnological potential. Understanding the genetic potential of the crop helps in the exploitation of existing variability in breeding programmes. Tamil Nadu, being the state pioneering in moringa cultivation with diverse genotypes, a total of 32 genotypes collected from different regions of the state were selected to study the variability in order to identify best performing genotypes. Principal component analysis for the selected 25 morphological characters elucidated that nine principal components with eigenvalue more than one accounted for 83.59 per cent of the total variability and also revealed that maximum variation was exhibited by the number of pods per cluster, the number of pods per branch, anthocyanin colouration in petiole, pod shape, pod length and plant growth habit whereas remaining traits exhibited low variability. Based on Agglomerative Hierarchical Clustering and PCA the genotypes CBE MO 1, CBE MO 13, CBE MO 19, CBE MO 28 and CBE MO 29 were identified as the most diverse genotypes.

Key words: Moringa, PCA, cluster, variability, DUS, descriptors

INTRODUCTION

Moringa oleifera. Lam. is a softwood tree that is widely recognized as a valuable vegetable around the world. Moringa is known by a variety of titles, one of which is "Miracle tree" because of its numerous applications and medical properties. Moringa plant parts and products are used as antioxidants, anticancerous, antiulcer, antidiabetic and antimicrobial agents. In addition to its therapeutic uses, it is also cultivated for animal feed and is a natural plant growth stimulator (Masih *et al*., 2019). In the year 2015, India exported moringa leaves worth around fourteen crore rupees. Every year, the moringa export market grows at the rate of 30 per cent (APEDA, 2016). Descriptors provided by Protection of Plant Variety and Farmers Right Authority helps in the identification of varieties, assessing the varietal purity and differentiating the distinctness of the new novel variety. Score points resulted in DUS descriptors helps in finding the highly variable genotypes. In moringa wide variability is observed for plant characters due to cross-pollination and the existence of annual and perennial types. Understanding the variability pave the way for the successful exploitation of available genotypes in breeding programmes and to release a variety for commercial exploitation (Balaguru *et al*., 2020). Hence, this study was undertaken to assess the variability of 32 moringa genotypes collected from different parts of Tamil Nadu. DUS testing, univariate and multivariate analyses were done to identify the better performing genotypes.

MATERIALS AND METHODS

The 32 genotypes of *Moringa oleifera.* Lam. were newly raised in Randomized Block Design with two replications at the Orchard, Department of Vegetable Science, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore, India during 2020-2021. The recommended package of practices was followed for the maintenance of genotypes. Data on 25 qualitative characters were recorded as per the DUS general guidelines proposed by PPV and FRA, 2001 at the appropriate phase of development of the plant.

Assessment of distinctiveness and stability were made by accounting for 30 plant parts taken from six plants except for the observation on pod characters. The number of pods per cluster, the number of pods per branch, pod surface colour, pod length, pod girth, seed colour and hundred seed weight were recorded for 20 pods per replication. For the assessment of uniformity, the population standard of 5% with an acceptable probability of at least 95% was adopted.

Scores from one to nine were given to all the 32 genotypes for 25 characters. A single observation for a group of plants or parts of plants (VG) was done by visual assessment for the characters such as plant growth habit, basal shape of first leaf blade, apex shape of the first leaf blade, the colour of leaf, colour of the petiole, anthocyanin coloration of the petiole, leaf axis and branches and petiole, the number of branches per inflorescence, skin colour of pods, shape of pods, seed size and seed colour. A single observation of individual plants or parts of plants (VS) was done by visual assessment for bud colour alone. Measurement by a single observation of a group of plants or parts of plants (MG) was done for the length of the petiole (cm), length and width of primary branches axis, the number of primary branches, days for flower initiation, inflorescence length (cm), flower size, pod length (cm), pod girth (mm), the texture of pod surface, the number of pods per cluster, the number of pods per branch, days taken for pod maturity and hundred seed weight (g).

Principal components (PCs) with an eigenvalue of more than one were selected (Jeffers, 1967) and standardized values of the PAST 3 application were used to conduct the PCA. The components contributing a major share in the total variation were visually assessed by the scree plot. Using PAST 3 application correlation matrix were computed for all the 25 characters. Agglomerative hierarchical clustering (AHC) was accomplished by using the scores of dissimilarity matrix in the computer software Microsoft Excel along with XLSTAT by Addinsoft.

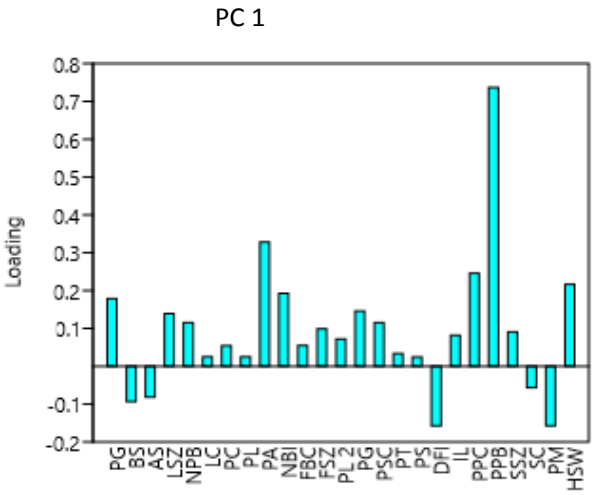
RESULTS AND DISCUSSION

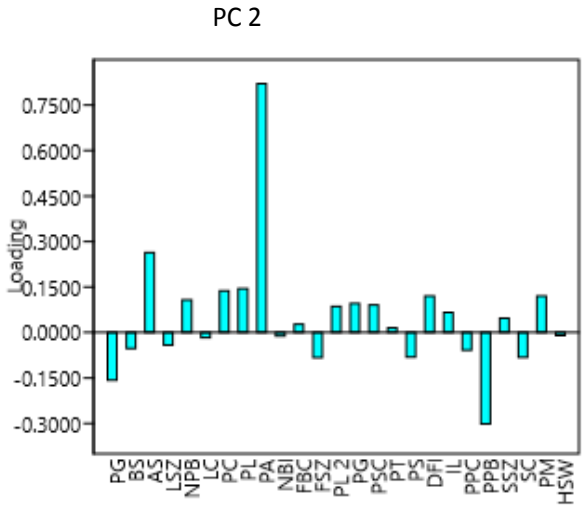
Among the multivariate statistics, Principal Component Analysis (PCA) is a potent tool that helps to find the least number of components, which helps in the explanation of maximum variability out of total variability (Morrison., 1982). PCA helps with the added advantage of ranking the genotypes based on PC scores. Therefore to assess the variability of moringa genotypes, principal component analysis was performed for 32 genotypes collected from different parts of Tamil Nadu. The analysis of 25 characters (DUS) resulted in nine principal components with an eigenvalue of more than one (**Table 1**). The total variability exhibited by the nine PCs was 83.59 per cent. Among the nine PCs, only three PCs expressed more variability of 49.50 per cent. Whereas, the experiment conducted by Kurian *et al.* (2021) accounted for 88.1 per cent of the total variance and three PCs with more than one eigenvalue in moringa.

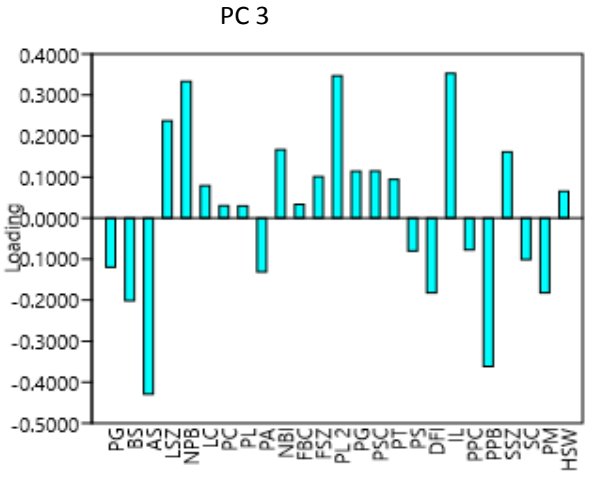
A Scree plot graph was constructed with the component number on the X-axis and eigenvalues on the Y-axis (**Fig. 1**). It explains the percentage of variance associated with each principal component. PC1 recorded maximum variability of 26.87 per cent with an eigenvalue of 6.71, which then gradually declined as observed by Gour *et al.* (2017). After the 13th PCs, semi curve line was obtained and then the semi curve turned straight with minor variations. Therefore selection of PC1, PC2 and PC3 will result in maximum variability among the 32 moringa genotypes. Interpretation for the traits having more than 0.5 in each PCs was given in **Table 2**.

Table 1. Eigenvalues, variance and cumulative variability of moringa genotypes

Principal component	Eigenvalue	Per cent variance	Cumulative variance
1	6.71947	26.878	26.878
2	3.2912	13.165	40.043
3	2.36591	9.4636	49.5066
4	2.03156	8.1262	57.6328
5	1.64203	6.5681	64.2009
6	1.45885	5.8354	70.0363
7	1.32599	5.304	75.3403
8	1.05454	4.2182	79.5585
9	1.01036	4.0414	83.5999







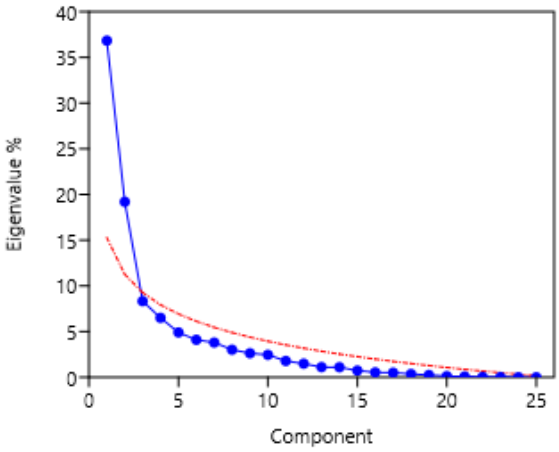


Fig. 2. Scree plot based on Eigen values

PCA analysis on 32 genotypes of moringa led to the estimation of characters based on the principal component scores (PC scores). The scores can be used for precise selection indices by the explanation of each PC by which the intensity can be understood. Genotype with maximum PC score in a particular PC signifies the maximum values for the variables in that particular genotype. Genotypes were selected based on PC score in each component having positive values and more than >1.0 in each PC. Rotated component matrix explained that PC1 accounted for yield characters such as plant growth habit, the number of primary branches, the number of pods per branch, the number of pods per cluster, pod girth and hundred seed weight (**Table 3 and Fig. 2**). PC 2 accounted for characters such as the colour of petiole and anthocyanin pigmentation in the petiole axis. Considering PC 2, it explains that among all parameters, colour of petiole and anthocyanin pigmentation in the petiole axis helps in differentiating the genotypes. Anthocyanin pigmentation helps in identifying genotypes with pink tinged buds and pink or red streaked moringa pods. PC 3 accounted for characters like pod length, inflorescence length and the number of primary branches. Inflorescence with maximum length helps in identifying long pods. (**Fig. 2**).

Scatter plots incorporating PC1 and PC2 indicated a clear pattern of clustering among the 32 moringa genotypes (**Fig. 3a**). The genotypes which occupied the convex of the hull are CBE MO 04, CBE MO 06, CBE MO 08, CBE MO 13, CBE MO 15, CBE MO 17, CBE MO 19, CBE MO 28, CBE MO 29 and CBE MO31. By the results of principal components, scatterplot for PC1 and PC2, the maximum variation was observed for pods per branch, anthocyanin colouration in petiole and plant growth habit. For PC2 and PC3, the scatter plot showed maximum variation for the number of primary branches, inflorescence length and

Fig. 1. Graphical representation of rotated component matrix for different traits in PC 1, PC2 and PC3

Table 2. Interpretation of PCA for the traits having values > 0.5 in each PCs.

	PC 1	PC 2	PC 3
Traits	Plant growth habit	Petiole anthocyanin colouration	Number of primary branches
	Hundred seed weight	Colour of petiole	Pod length
	Number of Branches per Inflorescence	Apex shape of first leaf blade	Inflorescence length
	Number of pods per cluster		
	Pod girth		
	Petiole anthocyanin colouration		

Table 3. Selection of genotypes on the basis of PC score in each component having positive values & more than >1.0 in each PCs

PC1	PC2	PC3
CBE MO 01 (3.9853)	CBE MO 04 (4.6412)	CBE MO 13 (2.9199)
CBE MO 04 (8.9893)	CBE MO 17 (7.2881)	CBE MO 15 (3.1678)
CBE MO 05 (3.0094)	CBE MO 19 (4.0762)	CBE MO 24 (3.623)
CBE MO 06 (4.1005)	CBE MO 20 (4.4938)	CBE MO 29 (3.9832)
CBE MO 07 (3.0941)	CBE MO 21 (4.1683)	CBE MO 30 (3.8643)
CBE MO 08 (3.3319)	CBE MO 25 (5.9967)	
CBE MO 13 (5.7988)	CBE MO 28 (5.2205)	
CBE MO 15 (5.8343)	CBE MO 04 (7.0337)	
CBE MO 19 (7.9051)		
CBE MO 20 (6.8155)		
CBE MO 21 (4.7527)		
CBE MO 28 (7.2038)		
CBE MO 29 (6.7320)		

Note: ( ) = PC scores

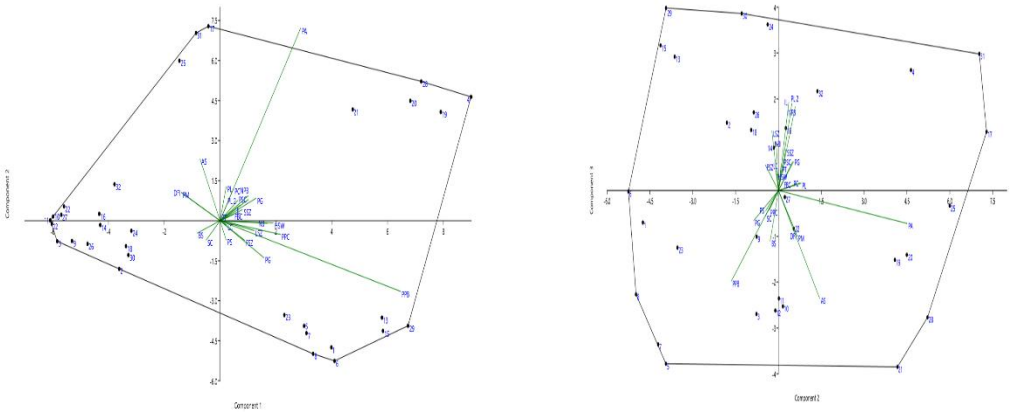


Fig. 3a **Fig.3b**

Fig. 3. Genotype scatter plot illustrating the relationship between PC1 and PC2

pod length (**Fig. 3b**). The remaining DUS characteristics displayed a low level of diversity. The cumulative variation shown in the scatter plot was 40.04 per cent (PC1 and PC2). Similarly, Chan *et al.* (2018) observed 40.33 per cent of the cumulative variation in scatter plots drawn for moringa genotypes.

Correlation between the characters helps in designing a hybridization programme (Popoola *et al.*, 2016). Correlation between the morphological traits in the parental individuals will be conserved in hybrid individuals. The diagnostic feature of hybrids was considered to be this character coherence (Anderson *et al.*, 1949). In this study, the correlation plot showed that plant growth habit was positively correlated with the number of pods per branch. A positive correlation existed between the

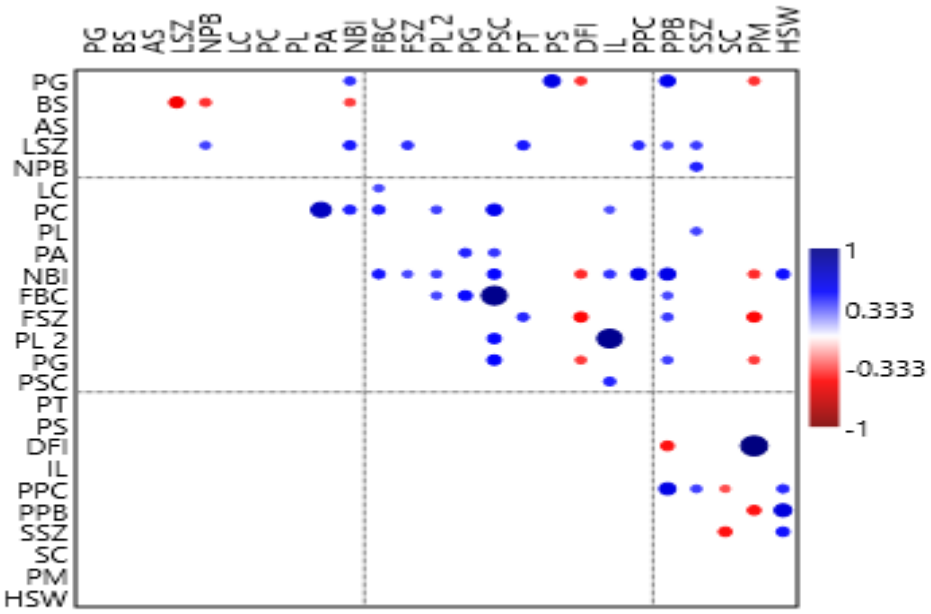


Fig. 4. Plot diagram of Correlation between different traits

characters such as pod length and inflorescence length, the number of pods per cluster and the number of pods per branch. (**Fig. 4**). A high positive correlation was observed between the colour of the petiole, flower bud colour and the skin colour of pods. Time taken for flower initiation was positively correlated with the time taken for pod maturity. According to Amoatey *et al.* (2012) characters such as frequency of flowering and the number of days to maturity helped the breeder to schedule synchronised flowering for hybridization.

Dendrogram of Agglomerative Hierarchical Cluster (AHC) analysis resulted in two clusters. Cluster A consisting of

Table 4. Shannon–Weaver diversity indices (H')

DUS DESCRIPTORS	H'
Plant growth habit	3.41
Basal shape of first leaf blade	3.421
Apex shape of first leaf blade	3.398
Size of leaf in primary branches axis for length & width	3.427
No of 1º branches	3.4
Colour of leaf	3.433
Colour of petiole	3.444
Length of petiole	3.435
Petiole anthocyanin coloration of axis & branches	2.916
No of branches in inflorescence	3.418
Flower bud colour	3.377
Flower size	3.433
Pod length (cm)	**3.43**
Pod girth (mm)	3.43
Skin colour of pods	3.414
Texture of pod surface	3.421
Shape of pods	3.444
Days to flower initiation	3.423
Inflorescence length	3.416
Pods/ cluster	3.27
Pods/branch	3.047
Seed size	3.42
Seed colour	3.43
Pod maturity	3.423
Hundred seed weight (g)	3.42

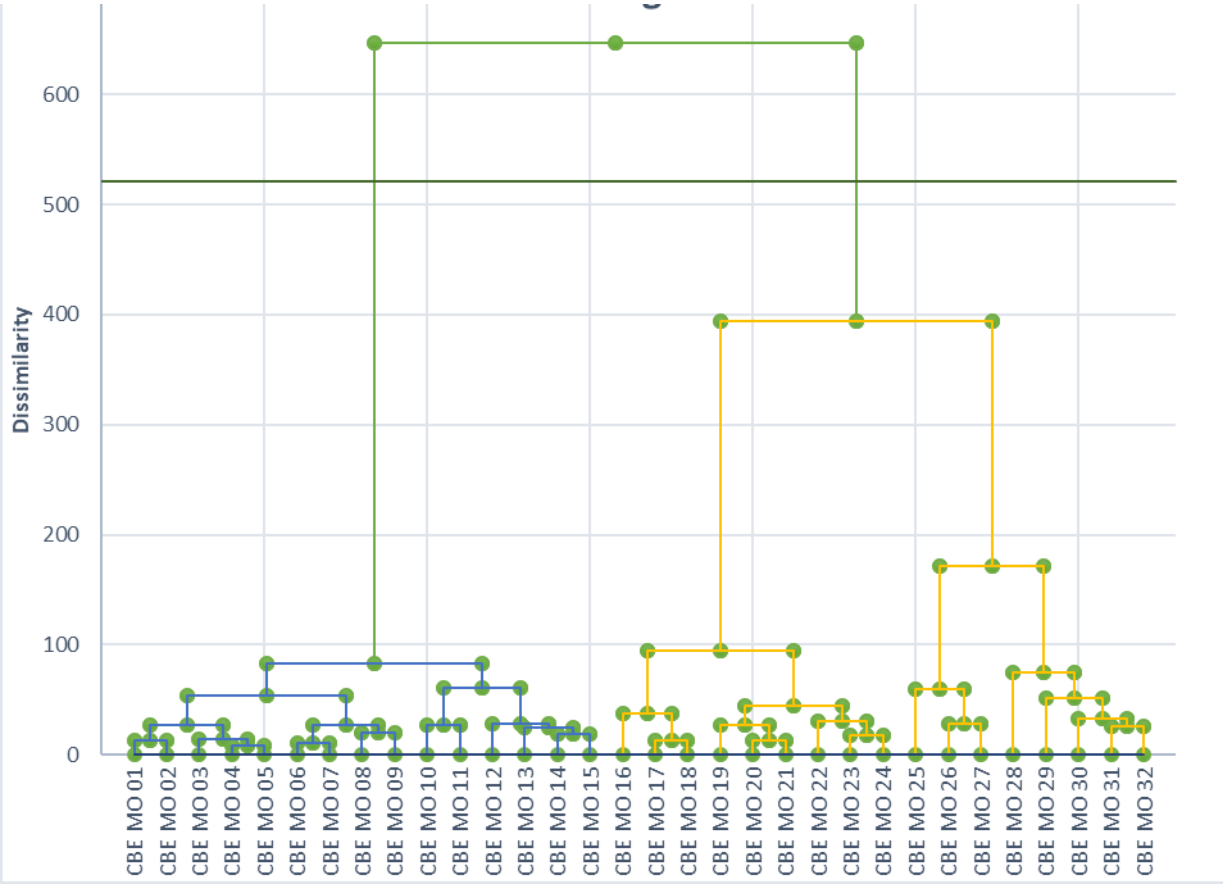


Fig. 5. Agglomerative Hierarchical Clustering pattern of moringa genotypes

13 annuals and 2 perennials and cluster B consisting of 14 annuals and 3 perennials. In cluster A, genotypes such as CBE MO 17, CBE MO 25 and CBE MO 31 having anthocyanin pigmentation in the petiole were in the same sub-cluster. Cluster B comprised of genotypes *viz*., CBE MO 01, CBE MO 13, CBE MO 19, CBE MO 28 and CBE MO 29 with more numbers of pods per branch and more numbers of pods per cluster **(Fig. 5).** Both perennial (CBE MO 29) and annual (CBE MO 13) with the highest number of pods per branch, pods per cluster and spreading branching habit were placed in a sub-cluster of cluster B. Genotypes *viz.,* CBE MO 05, CBE MO 07, and CBE MO 08 were classified in a different sub-cluster of cluster B because of their curved pod shape. In addition, the genotypes such as CBE MO 04, CBE MO 19, CBE MO 20, CBE MO 21, and CBE MO 28 which exhibited petiole anthocyanin coloration were placed in the same sub-cluster of cluster B. Annual and perennial genotypes were grouped in both A and B clusters. The reason is that moringa is a cross-pollinated crop, with natural cross pollination accounting for 10.28 per cent of fruit set (Bhattacharya and Mandal, 2004). Cross pollination is possible between annual and perennial moringa genotypes. Hence, annual and perennial moringa genotypes are grouped under the same cluster.

Shannon–Weaver diversity indices (H') explained that the petiole colour, pod shape (3.44), pod length (3.43) and pod girth (3.43) contributed to the maximum variation in the 32 moringa genotypes (**Table 4**). The variability studies (PCA, AHC, Correlation and H') for morphological characters (DUS descriptors) resulted in the identification of five genotypes *viz*., CBE NO 1, CBE MO 13, CBE MO 19, CBE MO 28 and CBE MO 29 which are highly variable in characters such as the number of pods per branch, the number of pods per cluster, pod girth and anthocyanin colouration. According to correlation and clustering analyses, morphological features such as anthocyanin pigment in the petiole, pod length, inflorescence length, plant branching habit, the colour of buds and the surface colour of pods play a vital role in explaining the diversity among genotypes.

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