

## Genetic Diversity Studies for Quality Traits Using Principal Component Analysis in Indigenous Moringa (*Moringaoleifera*L.) Germplasm Lines

**Comment [M1]:** Too large and not so informative.

### Abstract:

**Comment [M2]:** Should be re-written.

To know the genetic diversity among quality traits in different Moringa accessions, an investigation was carried out at Department of Vegetable Crops, Horticultural College and Research Institute (HC&RI), Tamil Nadu Agricultural University, Periyakulam during 2016 - 2017 with twenty genotypes through principal component analysis. In this study, out of five principal components (PC), only three components exhibited eigenvalues greater than 0.5 and accounted for 84.12% variability. The PC1 accounted for the highest variability (52.53% of) the total variability, followed by PC2 (17.66%) then PC3 with 13.93%. Thus the results of the principal component analysis revealed, wide genetic variability exists in this Moringa genotype accessions.

**Comment [M3]:** No need in abstract section. It's materials and methods.

**Key words:** Moringa (*Moringaoleifera*L.), Genotypes, PCA analysis, Eigen Values,

UNDER PEER REVIEW

## Introduction:

Moringa (*Moringaoleifera*L.) is a highly useful vegetable crop and native of India. *Moringaoleifera* is a valuable food that has drawn attention as it is the 'natural nutrition' of the tropical countries in the world. Both the businesses as well as scientific communities have shown interest in this crop owing to its economic and nutritional potential. The fruit, leaves, flowers and immature pods of this tree are highly nutritious and used as vegetable in many parts of the world, especially in Africa, India, Pakistan, Philippines, and Hawaii (Anwar and Bhanger, 2003). Various types of antioxidants such as ascorbic acid, flavonoids, phenolics and carotenoids present in moringa leaves offer several medicinal benefits to the human beings as mentioned by Dillard and German,(2000); Siddhuraju and Becker, (2003). In Philippines, it is used to increase woman's milk production and is sometimes prescribed for treating anaemia. Therefore, moringa is called as 'mothers' best friend' (Siddhuraju and Becker 2003). *Moringaoleifera*, native of the western and subHimalayan tracts, India, Pakistan, Asia Minor, Africa and Arabia (Somali *et al.*, 1984; Mughal *et al.*, 1999) is now distributed in the Philippines, Cambodia, Central America, North and South America and the Caribbean Islands (Morton, 1991). In some parts of the world *M. oleifera* is referred to as the 'drumstick tree' or the 'horse radish tree', whereas in some countries it is known as the kelor tree (Anwar and Bhanger, 2003), while in the Nile valley, the name of the tree is 'Shagara al Rauwaq', which means 'tree for purifying' (Von Maydell, 1986). PCA involves a mathematical procedure that transforms a number of (possibly) correlated variables into a number of uncorrelated variables called principal component (Chatfield and Collis, 1980). PCA is an important statistical method through which we can easily identify important polygenic characters which are of great importance in a plant breeding programme. PCA provides an idea for how to reduce a complex data set to a lower dimension to reveal the sometimes hidden, simplified structures that often underlie it (Ian T. Jolliffe and Jorge Cadima, 2016). Principal component analysis (PCA) is an important multivariate method in modern data analysis because it is a simple, non-parametric method for extracting relevant information from confusing data sets and it was applied for assessment of genetic diversity within moringa genotypes. The eigenvalue of a particular principal component depicts the amount of variation present in traits and explained by that principal component which is very useful for the further breeding programme. To know the genetic diversity among quality traits in different Moringa accessions, an investigation was carried out at Department of Vegetable

Crops, Horticultural College and Research Institute (HC&RI), Tamil Nadu Agricultural University, Periyakulam during 2016 -2017 with twenty genotypes.

### Materials and Methods:

The present investigation was carried out to know the variability through principal component analysis in Moringa (*Moringaoleifera*L.) Germplasm cultivated in Telangana State at Department of Vegetable Crops, Horticultural College and Research Institute (HC&RI), Tamil Nadu Agricultural University, Periyakulam (PKM) during 2016 -2017. Twenty Moringa accessions were collected from different regions of Telangana and the details of the plant materials used in the present study are listed in Table 1. Observations on biochemical characters viz., chlorophyll a (mg g<sup>-1</sup>), chlorophyll b (mg g<sup>-1</sup>), leaf soluble protein (mg/100g), crude protein (%), and ascorbic acid (mg/g). The accessions exhibited wide variability for morphological characters revealed that most of the accessions possessed phenotypic variations among them.

### Quality traits estimation:

#### Chlorophyll content:

One gram of fresh leaf sample was collected and macerated with 10 ml of 80 per cent acetone and centrifuged at 3000 rpm for 10 minutes. After centrifugation, supernatant was collected and made up into 25 ml by using 80 per cent acetone. The color intensity was observed as OD values at 645 nm, 652 nm and 663 nm using a Spectrophotometer. The contents of chlorophyll 'a', chlorophyll 'b' and total chlorophyll were estimated and expressed as mg g<sup>-1</sup> (Yoshida *et al.*, 1971).

#### Leaf soluble protein:

The soluble protein content was estimated at 660 nm using Foline Ciocalteu reagent by following the procedure described by Lowry *et al.* (1951) and expressed in mg g<sup>-1</sup> of fresh weight of leaves. The soluble protein was extracted from the leaf samples with phosphate buffer. One ml of the extract was reacted with 5 ml of alkali copper reagent for 30 min, followed by the addition of 0.5 ml of phenol reagent and the colour intensity was measured at 660 nm. From the standard graph, the amount of soluble protein present in leaf sample was calculated and expressed as mg albumin equivalent to soluble g<sup>-1</sup> on fresh weight basis. Crude protein was estimated through Kjeldahl method by A.O.A.C (1975). Ascorbic acid was estimated according to the procedure given by A.O.A.C. (1975).

**Comment [M4]:** Same line in abstract. I think the authors are not interested to published the paper. How they write same line in abstract and introduction?? Where is the objectives of the work??

**Comment [M5]:** Reference is too old, not updated.

**Comment [M6]:** Reference is too old, not updated.

**Comment [M7]:** See previous comments.

**Table .1. List of Moringa accessions employed in the study**

S.No.	Name of Accession	Description of Accession	Place of collection & District	Latitude & Longitude
1.	MO 1	Long poded perennial type	Warangal, Warangal	18 <sup>0</sup> 38.60N, 79 <sup>0</sup> 36'0 .10 E
2.	MO 2	Long poded perennial type	Malyal, Warangal	18 <sup>0</sup> 21' 48.80 N, 80 <sup>0</sup> 18' 23.66 E
3.	MO 3	Medium poded perennial type	Ghanpur, Warangal	17 <sup>0</sup> 49' 58.89 N, 78 <sup>0</sup> 59' 57.35 E
4.	MO 4	Short poded perennial type	Regonda, Warangal	18 <sup>0</sup> 23' 77.70 N, 79 <sup>0</sup> 77' 50.80 E
5.	MO 5	Long podedperennial type	Jagithyala, Karimnagar	18 <sup>0</sup> 46'0.66 N, 78 <sup>0</sup> 54'42 .83 E
6.	MO 6	Short poded perennial type	Peddapally, Karimnagar	18 <sup>0</sup> 37' 24.72 N, 79 <sup>0</sup> 22' 47.59 E
7.	MO 7	Short poded perennial type	Armor, Nizamabad	18 <sup>0</sup> 48' 37.14 N, 78 <sup>0</sup> 17' 7.00 E
8.	MO 8	Short poded perennial type	Nandipeta, Nizamabad	18 <sup>0</sup> 52' 34.06 N, 78 <sup>0</sup> 31' 14.68 E
9.	MO 9	MediumPoded perennial type	Rudrur, Nizamabad	18 <sup>0</sup> 34' 45.48 N, 77 <sup>0</sup> 52' 31.27 E
10.	MO 10	Short poded perennial type	Satyanarayanapuram,Nizamabad	18 <sup>0</sup> 32' 40.61 N, 77 <sup>0</sup> 53' 31.39 E
11.	MO 11	Medium poded perennial type	Basara, Nirmal	18 <sup>0</sup> 52' 40.63 N, 77 <sup>0</sup> 56' 57.01 E
12.	MO 12	Short poded perennial type	Mudhol, Nirmal	18 <sup>0</sup> 98' 26.81 N, 77 <sup>0</sup> 92' 05.10 E
13.	MO 13	Short poded perennial type	Ichoda, Adilabad	19 <sup>0</sup> 26' 1.02 N, 78 <sup>0</sup> 27' 14.82 E
14.	MO 14	Short poded perennial type	Adilabad, Adilabad	19 <sup>0</sup> 38' 53.14 N, 78 <sup>0</sup> 31' 14.68 E
15.	MO 15	Medium poded perennial type	Amaravathi,Manchiriyal	18 <sup>0</sup> 54' 15.05 N, 79 <sup>0</sup> 28' 58.30 E
16.	MO 16	Short poded perennial type	Doragaripalli, Manchiriyal	18 <sup>0</sup> 53' 59.5 N, 79 <sup>0</sup> 27' 41.2 E
17.	MO 17	Medium poded perennial type	Kyathanpalli, Manchiriyal	18 <sup>0</sup> 55'18.8 N, 79 <sup>0</sup> 28' 13.4 E
18.	MO 18	Short poded perennial type	Suryapeta, Nalgonda	17 <sup>0</sup> 14' 8.70 N, 79 <sup>0</sup> 36' 34.07 E
19.	MO 19	Medium poded perennial type	Gollapally, Nalgonda	17 <sup>0</sup> 31' 23.59 N, 80 <sup>0</sup> 52' 19.91 E
20.	MO 20	Short poded perennial type	Narayanapuram,Nalgonda	17 <sup>0</sup> 10' 36.74 N, 80 <sup>0</sup> 52' 19.91 E

Twenty moringa genotypes were evaluated by using International Plant Genetic Recourses Institute (IPGRI) minimal descriptors. The recommended agronomic practices were followed. Observations were recorded for five biochemical characters. Data were recorded on five different traits *viz.* chlorophyll a (mg g<sup>-1</sup>), chlorophyll b (mg g<sup>-1</sup>), leaf soluble protein (mg/100g), crude protein (%), and ascorbic acid (mg/g) using leaf material. The PCA analysis reduces the dimensions of a multivariate data to a few principal axes, generates an eigenvector for each axis and produces component scores for the characters (W. F. Massay, 1965).

### Results and Discussion:

The results of PCA explained the genetic variation among the genotypes for quality characters under study. Data were considered in each component with eigen values more than 0.5, which determines as a minimum 10% of the variation. Superior Eigen values are considered as best attributes in principal components. In our study, three components exhibited eigen values of greater than 0.5 and showed cumulative variation of 84.12%. It indicates that the identified characters within these components exhibited immense influence on the phenotype of the genotypes. Table 2 presents principal components, Eigen values and percentage contribution of each component to the total variation in the moringa germplasm. The three components *viz.* PC1, PC2, and PC3 showed 52.53%, 17.66% and 13.93% of variations among the characters. Only well loaded characters in each component values within 10% of the highest factor loading were retained for further explanation. Results revealed by rotated component matrix showed that the PC1 which accounted for the maximum variability (52.53%) and highly loaded with characters such as chlorophyll a content (0.478), ascorbic acid (0.455), chlorophyll b (0.448), leaf soluble protein (0.444) and crude protein (0.406), it clearly indicated that the variation in PC1 is mainly contributed by quality characters. PC2 accounted 17.66% of the total variation and loaded with the traits *viz.* crude protein (0.612), chlorophyll b (0.492), contributed in positive direction, whereas chlorophyll a (-0.442), ascorbic acid (-0.397) and leaf soluble protein (-0.173) contributed in negative direction. PC3 had the contribution from the characters like ascorbic acid (0.538), crude protein (0.445), which accounted for 13.93% of the total variation. Quality characters like leaf soluble protein (0.607) and crude protein (0.303) had contributed 9.53% of the total variation in PC 4. PC5 is accounted for 6.35% of total variation and it was loaded by the traits such as ascorbic acid (0.563), chlorophyll b (0.449) respectively.

**Comment [M8]:** ???

**Comment [M9]:** In the Result & Discussion, related Literature is very poor and should be rewritten and improved. I can't see the related work section? Thus, please provide several solid literature reviews (discussion and argument). Prefer to be updated with recent studies.

**Comment [M10]:** Supposition without proof. Add references and compare the value.

**Table 2: Eigen Values, Per Cent Variance and Cumulative Variance Values of Moringa Germplasm**

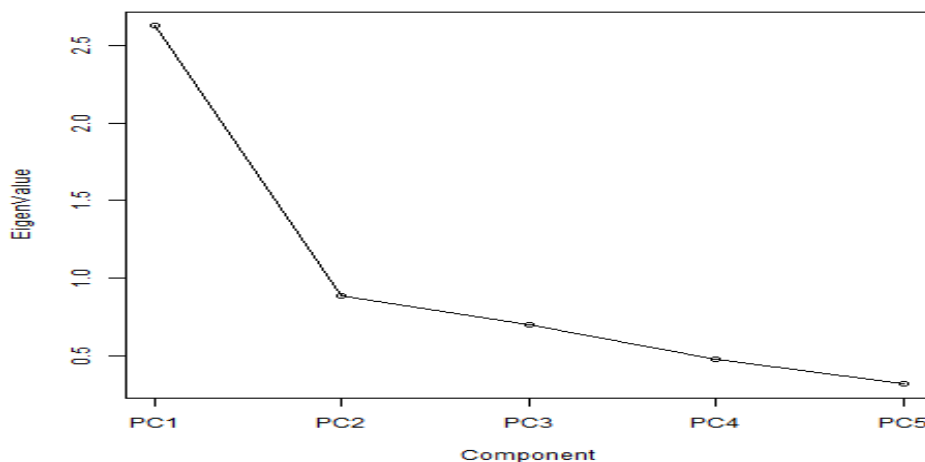
Comment [M11]: Table is not clear

	PC1	PC2	PC3	PC4	PC5
Eigen value	2.62	0.88	0.69	0.47	0.31
Total Variance	52.53	17.66	13.93	9.53	6.35
Cumulative Variance (%)	52.53	70.19	84.12	93.65	100.00
<b>Trait</b>	<b>Eigenvalues</b>				
Chlorophyll a(mg g-1)	0.478	-0.442	0.0094	-0.517	-0.554
Chlorophyll b(mg g-1)	0.448	0.492	-0.333	-0.493	0.449
Leaf soluble protein (mg/100g)	0.444	-0.173	-0.632	0.607	-0.0561
Crude protein (%)	0.406	0.612	0.445	0.303	-0.412
Ascorbic acid (mg/g)	0.455	-0.397	0.538	0.166	0.563

Comment [M12]: Not clear

Scree plot showed in the Fig.1 explained the percentage of variation associated with each principal component obtained by drawing a graph between Eigen values and principal component numbers. PC1 showed 52.53% variability with the Eigen value of 2.62. The Eigen values are gradually declined from PC1 to PC5. The Eigen values are 0.88, 0.69, 0.47, and 0.31 for PC2, PC3, PC4, and PC5 respectively. Elbow type line is obtained after PC3 tended to straight with minute difference observed in each PC and it is clearly showed that the utmost variation was observed in PC1.

**Scree Plot**



**Figure 1 Scree plot**

## Conclusion:

The phenotypic value of each trait measures the importance and contribution of each component to the total variance. The component contributed the maximum for phenological traits, Chlorophyll a, Chlorophyll b, Leaf soluble protein, Crude protein, Ascorbic acid are the chief contributors towards genetic divergence in moringa genotypes. Thus, the prominent characters coming together in different principal components and contributing towards the variability. Finally these quality traits may be kept into consideration during the utilization of these characters in the breeding program for further improvement of Moringa especially for quality traits.

## References:

- Anwar, F. and Bhangar, M.I. 2003. Analytical characterization of *Moringa oleifera* seed oil grown in temperate regions of Pakistan. *J. Agric. Food Chem.*, 51: 6558-6563.
- Dillard CJ and German JB. 2000. Phytochemicals: nutraceuticals and human health: A review. *J. Sci. Food Agric.*, 80: 1744-1756.
- Siddhuraju, P. and Becker, K. 2003. Antioxidant properties of various solvent extracts of total phenolic constituents from three different agro-climatic origins of drumstick tree (*Moringa oleifera* Lam.). *J. Agric. Food Chem.* 15: 2144-2155.
- Somali MA, Bajnedi MA, Al-Faimani SS. 1984. Chemical composition and characteristics of *Moringa peregrina* seeds and seed oil. *J Am Oil Chem Soc* 61: 85-86.
- Mughal MH, Ali G, Srivastava PS, Iqbal M. 1999. Improvement of drumstick (*Moringa pterygosperma* Gaertn.) – a unique source of food and medicine through tissue culture. *Hamdard Med* 42: 37-42.
- Morton, J.F. 1991. The horseradish tree, *Moringa pterygosperma* (Moringaceae): A boon to arid lands? *Economic Botany.* 45: 318-333.
- Von Maydell HJ. 1986. Trees and Shrubs of Sahel, Their Characterization and Uses. Deutsche Gesellschaft für Technische Zusammenarbeit, Germany: Eschborn, 334-337.
- Chatfield, C. and Collins. 1980. Introduction of Multivariate Analysis, USA by Chapman and Hall in Association with Methuen, New York, ISBN 0-412-16030-7, 48 pp.

**Comment [M13]:** What are the novel outcomes from this study?

**Comment [M14]:** Reference formatting is not consistent. Need to follow the journal guideline for formatting.

**Comment [M15]:** And is here

**Comment [M16]:** There is no And

Ian T. Jolliffe and Jorge Cadima. 2016. Principal component analysis: a review and recent developments. Philosophical transactions A. pp.: 1-16.

**Comment [M17]:** Why pp???

Yoshida, S., Forno, D. A., Cook, J. H. and Gomez, K. A. 1971. Laboratory Manual for Physiological Studies on Rice, IRRI, Manila, Philippines pp. 82

Lowry, O.H., Rosenbrought, N.T., Farr, L.A. and Randall, R.J. 1951. Protein measurement with folin phenol reagent. J. Biol. Chem., 193: 265-275.

**Comment [M18]:** There is no pp

A.O.A.C.1975.Official and tentative methods of analysis.Association of Official Agricultural Chemists, Washington D.C., U.S.A. 12th Ed.

W. F. Massay.1965. Principal components regression in exploratory statistical research.Journal of the American Statistics Association. 60: 234-246.

UNDER PEER REVIEW