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Assessment of Iron Content and Morphological Diversity in Different Populations of Drumsticks (*Moringa oleifera*) Trees Inhabiting Rajasthan, India

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ABSTRACT

The present study was aimed at studying the variations in morphological and biochemical properties of *Moringa oleifera* Lam. plants growing in different agro-climatic regions of Rajasthan, India. Morphological variations were recorded among all accessions, and none of the traits was region or zone-specific. Irrespective of the agro-climatic zones, brown to dark brown coloured bark with rough texture and narrow spread canopy were most common. Maximum number of main branches (2.2) and sub-branches (9.6) was recorded in accessions of Alwar and Jaipur, respectively. However, no such pattern was recorded for canopy density and leaf size. These findings present first report on comprehensive diversity profiling of *M. oleifera* Lam. at phenotypic and biochemical levels. The highest iron (Fe) content (0.9 ± 0.004 mg/g) was found in accessions from Udaipur, followed by Kota, Alwar and Jaipur. This study marks the first comprehensive report on the iron content profiling of *M. oleifera*. These findings provide a foundation for further exploration into the impact of climatic conditions on the plant's biochemical characteristics. Moreover, this information can be utilized for the identification of varieties exhibiting enhanced Fe yield capacity.

Key words: Sehjan, *Moringa oleifera*, Fe content, AAS, morphological diversity

INTRODUCTION

Moringa oleifera Lam., commonly known as drumstick tree, is not only known for its uses in cuisines worldwide, but it has also been one of the important sources of the rapeutics used in Indian medicine system. The plant has been rendered as "miracle tree" due to its potential to treat more than 300 diseases, which in turn has been attributed to its rich and diverse metabolite profile (Gupta *et al.*, 2018).

Among all elements that are essentially required for normal functioning of human body, Fe is one of the most fundamental elements which has role in various biological mechanisms including antioxidant protein function, mitochondrial function maintenance, ATP generation, O_2 transport, respiration, DNA synthesis and damage repair, etc. (Liu *et al.*, 2022). Fe is also known to be a constituent of various enzymes in the form of a prosthetic group or cofactor and is thus critical for functioning and activation of such enzymes, and thereby critical for proceeding of metabolic pathways in which such enzymes are involved (Rout and Sahoo, 2015). Other than being constituent of haemoproteins, Fe is also an important constituent of ferritin, hemosiderin and myoglobin, thereby highlighting the significance of maintaining appropriate Fe levels for normal functioning of human body and other living beings.

As mentioned earlier, leaves of drumstick tree contain three times more Fe than spinach, and thus it can serve as a good Fe supplement. Being already used in various food preparations and consumed worldwide with easy availability further make it an excellent natural food source for Fe fortification. The leaves are also rich in other minerals and vitamins, thus consuming its leaves would not only aid in managing Fe deficiencies, but could also serve as supplement for various other such vital nutrients. However, effect of environmental conditions on plant population in terms of changes in morphology, genotype and metabolite content has been well documented (Csurhes and Navie, 2016).

Molecular markers are powerful tool of biotechnology which has been extensively used

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to study the genetic variability among different plants of same species with an aim to distinguish among genetically discrete varieties and to identify superior varieties. Further, combination of molecular markers along with biochemical markers and morphological descriptors have emerged as a popular tool to study the biodiversity and to understand the inter and intra specific phylogenetic relationships (Goyal *et al.,* 2015). Therefore, based on the above facts, this study aimed at studying the morphological and genetic diversity among plants of drumstick tree growing in different agro-climatic zones of Rajasthan (India). Further, to study the the variations in biochemical properties in terms of Fe content among *M. oleifera* plants growing in same and different agro-climatic zones.

MATERIALS AND METHODS

Leaf samples of *M. oleifera* were collected from four different agro-climatic zones (Jaipur, Alwar, Kota and Udaipur) of Rajasthan, India. Minimum five and maximum 10 locations in each zone (depending upon the abundance of the plant in the zone) were selected for sample collection (Table 1). All the samples were collected in triplicates. The leaves were washed under tap water to remove dirt and were then used for isolation of genomic DNA for diversity analysis and for Fe content determination.

All harvested leaf samples underwent a thorough cleaning with distilled water before being subjected to drying in a hot air oven at 120°C until a consistent dry weight was achieved. Subsequently, the dried leaves were meticulously ground into a fine powder using a mortar and pestle for the estimation of Fe content. For the wet digestion method, each homogenized leaf sample was treated with concentrated HNO_3 (5 ml), following the standard procedure. The resulting mixture was covered with a watch glass and heated on a hot plate at 80-100°C for 1 h. Subsequently, the mixture underwent treatment with HNO_3 (5 ml) and 30% H_2O_2 (2 ml) through heating for an additional hour. After removing the watch glass, the mixture was heated until it reached

Table 1. Zone-wise geographical locations of different regions identified for collection of *M. oliefera* leaf

Sample No.	Accessions	Agroclimatic zone	Region of collection	Geographical coordinates
1.	TR	Jaipur	Tonk Road	26°52′ 16.9″ N 75°47′ 49.6″ E
2.	JP	Jaipur	Jaisinghpura	26°50′ 49.4″ N 75°41′ 51.3″ E
3.	KS	Jaipur	Kukas	27°02′ 17.2″ N 75°53′ 27.9″ E
4.	AB	Jaipur	Aspura Bhabru	27°87′ 0.13″ N 76°02′ 36.8″ E
5.	KTP	Jaipur	Kotputli	27°42′ 12.6″ N 76°13′ 49.9″ E
6.	MA	Alwar	Motidungri	27°33′ 08.9″ N 76°36′ 38.6″ E
7.	NA	Alwar	Narayanpura	27°31′ 48.9″ N 76°17′ 10.1″ E
8.	DA	Alwar	Dharampura	27°26′ 22.0″ N 76°31′ 16.3″ E
9.	BA	Alwar	Bansur	27°40′ 37.8″ N 76°21′ 10.7″ E
10.	SA	Alwar	Sarishka	27°23′ 12.7″ N 76°19′ 40.6″ E
11.	RM	Kota	Ramganjmandi	24°38′ 8.56″ N 75°56′ 36.24″ E
12.	DA	Kota	Dara	24°50′ 5.64″ N 76°00′ 23.4″ E
13.	MD	Kota	Mandana	24°56′ 44.88″ N 75°56′ 36.24″ E
14.	CG	Kota	Chambel Garden	25°09′ 45.36″ N 75°49′ 25.32″ E
15.	BK	Kota	Borkheda	25°10′ 41.54″ N 75°53′ 7.24″ E
16.	RR	Kota	Ravtha Road	24°56′ 0.95″ N 75°57′ 30.23" E
17.	CC	Kota	Chechat	24°45′ 55.08″ N 75°53′ 17.16″ E
18.	GY	Kota	Goyanda	24°39′ 1.08″ N 75°51′ 33.12″ E
19.	KT	Kota	Kethuli	24°37′ 36.84″ N 75°49′ 37.92″ E
20.	BH	Udaipur	Bhuwana	24°37′ 17.1″ N 73°42′ 29.4″ E
21.	UR	Udaipur	Udaipur Road	24°34′ 16.57″ N 73°41′ 29.55″ E
22.	PH	Udaipur	Pahada	24°6′ 06.14″ N 74°26′ 30.97″ E
23.	RS	Udaipur	Rupsagar	24°36′ 57.42″ N 73°42′ 33.06″ E
24.	GN	Udaipur	Ganesh Nagar	24°35′ 18.89″ N 73°43′ 20.62″ E
25.	DNR	Udaipur	Durga Nursery Road	24°34′ 54.27″ N 73°42′ 34.54″ E
26.	HM	Udaipur	Hiran Magri	24°32′ 48.12″ N 73°41′ 59.28″ E
27.	BL	Udaipur	Balicha	24°21′ 15.4″ N 74°14′ 20.4″ E
28.	GD	Udaipur	Gogunda	24°44′ 59.99″ N 73°31′ 59.99″ E

a semi-dried state. To this semi-dried mixture, 2 N HNO_3 (5 ml) was added and heated for 2 min. After cooling, the mixture was diluted with 2 N HNO_3, filtered using Whatman Filter Paper No. 42, and collected in a volumetric flask. The filtrate was then brought to a volume of 25 ml with deionised water.

The absorbance of digested leaf samples was recorded using Model A Analyst 300 AAS (Perkin Elmer) as per standard operating procedure. The concentration of Fe was determined using calibration curve obtained by measurement of absorbance of series of standard solutions of known Fe concentration (prepared from its 1000 ppm standard stock solution) HNO_3 was used as blank and all the readings were taken in triplicates.

Morpho-variants of *M. oleifera* were identified from the population located in different agro-climatic zones of Rajasthan, India, and were characterized on the basis of morphological plant descriptors: (i) canopy spread and shape, (ii) bark colour and texture, (iii) stem morphology, and (iv) branching habit and pattern.

RESULTS AND DISCUSSION

Significant variations were observed in branching habit and pattern, bark colour and texture, canopy size, shape and spread, stem morphology, tree height, leaf morphology (size and shape) and number of primary branches (Table 2). However, no significant morphological variations were recorded among plants belonging to different agro-climatic zones, than compared to those belonging to same region.

Among all the 28 samples, 21% of them had whitish grey bark with smooth texture and ~31% had rough textured, brown to dark brown bark, while less than 10% of them having either greyish, whitish or brownish bark with rough or smooth texture (Table 2). 28%, ~18% and 14% of the total accessions had narrow, wide and medium spread canopy, respectively. Among different types of canopy shape, umbrella and dome shaped canopy was most common among the plants studied. Further, no definite pattern in terms of density of the canopy was recorded.

The number of primary branches among accessions from different regions varied from 1 - 4, and that of sub-branches ranged from 4-15. The region/zone-wise average number of main branches was maximum (2.2) among accessions of Alwar, while that of sub-branches was maximum (9.6) among accessions of Jaipur.

Analysis of morphological diversity revealed significant variations in the morphological plant descriptors of the trees growing in different regions. However, the variations in morphological traits were irrespective of the agro-climatic zones and based on the findings of this study it could be said that morphological traits vary not only due to agro-climatic conditions, but are also affected by the soil characteristics and its immediate surrounding environment. Presence of intra specific morphological and biochemical variations signified the adaptive behaviour of plants which can be attributed to the agro-ecological factors and environmental conditions (Okello *et al.*, 2018).

Variations were also recorded in size and shape of the pods produced among the same and different trees of *M. oleifera*, respectively. Variations in pod length of the tree were prominently observed within a single tree, while the pod shape varied plant to plant. Similar type of variations in terms of fruit length, fruit colour and shape has also been reported in *Citrullus colocynthis* (Verma *et al.*, 2017), *Sclerocarya birrea* (Mkwezalamba *et al.*, 2015), *Pithecellobium dulce* (Macário *et al.*, 2020) and *Tamaraindus indicus* (Araújo *et al.*, 2019). Diversity in fruit morphology can serve as key factor to identify superior germplasms which could further lead to development of high quality fruit based products (Juhaimi *et al.*, 2017).

Significant differences were recorded in Fe content of leaves collected from different agro-climatic zones. Highest content was recorded in leaves collected from Udaipur (0.89 ± 0.0036 mg/g) followed by Kota (0.851 ± 0.029 mg/g), Alwar (0.826 ± 0.006 mg/g) and Jaipur (0.728 ± 0.012 mg/g). The leaf size was, however, comparable among all accessions (Table 3) and no significant influence of leaf size was recorded on their Fe content.

Like morphological traits, no significant variations were recorded in either leaf size or in Fe content of the accessions of *M. oleifera* belonging to different agro-climatic regions. However, one of the recent studies has reported variations in leaf size (Aditama

Table 2. Morphological characteristics of *M. oleifera* accessions collected from different agro-climatic zones of Rajasthan (India)

Geographical coordinates	Agroclimatic zone	Bark texture and colour	Canopy size, shape and density	No. of main and sub-branches
26°52′ 16.9″ N 75°47′ 49.6″ E	Jaipur	Greyish black and rough	Medium spread and dome-Umbrella shaped	2 and 8
26°50′ 49.4″ N 75°41′ 51.3″ E	Jaipur	Brown and Smooth with lines	Medium spread and dense	1 and 10
27°02′ 17.2″ N 75°53′ 27.9″ E	Jaipur	Dark brown and rough	Narrow spread and dome shaped	1 and 6
27°87′ 0.13″ N 76°02′ 36.8″ E	Jaipur	Whitish Gray and Smooth	Wide spread and intermediary scattered shaped	2 and 13
27°42′ 12.6″ N 76°13′ 49.9″ E	Jaipur	Slightly grey and Smooth	Wide spread and dense	2 and 11
27°33′ 08.9″ N 76°36′ 38.6″ E	Alwar	Dark brown and Rough	Narrow spread and scattered	1 and 7
27°31′ 48.9″ N 76°17′ 10.1″ E	Alwar	Greyish and smooth	Medium and scattered	3 and 7
27°26′ 22.0″ N 76°31′ 16.3″ E	Alwar	Whitish grey and Smooth lines	Narrow and scattered	1 and 6
27°40′ 37.8″ N 76°21′ 10.7″ E	Alwar	Slightly grey and rough	Wide spread and scattered	4 and 15
27°23′ 12.7″ N 76°19′ 40.6″ E	Alwar	Brown and Rough	Medium spread and scattered	2 and 10
24°38′ 8.56″ N 75°56′ 36.24″ E	Kota	Dark brown and Rough	scattered and triangle	4 and 8
24°50′ 5.64″ N 76°00′ 23.4″ E	Kota	Slightly brown and smooth	Umbrella shaped and scattered	1 and 8
24°56′ 44.88″ N 75°56′ 36.24″ E	Kota	Brown and rough	Narrow and scattered	2 and 5
25°09′ 45.36″ N 75°49′ 25.32″ E	Kota	Grey and Rough	Narrow and scattered	1 and 7
25°10′ 41.54″ N 75°53′ 7.24″ E	Kota	Whitish grey and smooth	Narrow and dome shaped	2 and 4
24°56′ 0.95″ N 75°57′ 30.23″ E	Kota	Brown and rough	Narrow umbrella shaped	3 and 5
24°45′ 55.08″ N 75°53′ 17.16″ E	Kota	Gray and rough	Narrow and scattered	1 and 5
24°39′ 1.08″ N 75°51′ 33.12″ E	Kota	Whitish grey and smooth	Narrow scattered	1 and 4
24°37′ 36.84″ N 75°49′ 37.92″ E	Kota	Dark brown and Rough	Narrow spread	2 and 4
24°37′ 0.12″ N 73°42′ 20.16″ E	Udaipur	White and smooth	Narrow	1 and 4
24°34′ 16.57″ N 73°41′ 29.55″ E	Udaipur	Greyish brown and rough	Medium and scattered	1 and 8
24°6′ 06.14″ N 74°26′ 30.97″ E	Udaipur	Whitish grey and Smooth lines	Narrow and scattered	1 and 4
24°36′ 57.42″ N 73°42′ 33.06″ E	Udaipur	Greyish brown and Rough	Medium spread and scattered	2 and 8
24°35′ 18.89″ N 73°43′ 20.62″ E	Udaipur	Whitish grey and Smooth	Narrow	1 and 4
24°34' 54.27″ N 73°42′ 34.54″ E	Udaipur	Dark grey and Rough	Wide spread	3 and 10
24°32' 48.12″ N 73°41′ 59.28″ E	Udaipur	Gray and rough	Medium and scattered	1 and 11
24°21' 15.4″ N 74°14′ 20.4″ E	Udaipur	Brown and Rough	Wide spread and umbrella shaped	3 and 13
24°44' 59.99″ N 73°31′ 59.99″ E	Udaipur	Dark brown and Rough	Wide spread and scattered	2 and 11

Table 3. Fe content in *M. oleifera* leaves collected from four different agroclimatic regions of Rajasthan (India)

Sample	Leaf size±SD (cm)	Fe±SD (mg/g)
TR (Tonk road)	10.3±0.2	0.686±0.0075
JP (Jaisinghpura)	10.36±0.15	0.728±0.012
KS (Kukas)	10.26±0.15	0.709±0.021
AB (Aspura Bhabru)	10.53±0.2	0.723±0.013
KTP (Kotputli)	10.46±0.32	0.32±0.011
MA (Motidungri)	10.3±0.2	0.75±0.009
NA (Narayanpura)	10.36±0.15	0.751±0.012
DA (Dharampura)	10.26±0.15	0.734±0.015
BA (Bansur)	10.53±0.2	0.762±0.027
SA (Sarishka)	10.46±0.32	0.826±0.006
RM (Ramganjmandi)	10.43±0.32	0.833± 0.016
DA (Dara)	10.46±0.15	0.84±0.007
MD (Mandana)	10.36±0.11	0.851±0.029
CG (Chambel garden)	10.53±0.25	0.84±0.006
BK (Borkheda)	10.43±0.2	0.846±0.017
RR (Ravtha road)	10.26±0.15	0.837±0.014
CC (Chechat)	10.36±0.37	0.847±0.008
GY (Goyanda)	10.63±0.3	0.834±0.007
KT (Kethuli)	10.4±0.26	0.844±0.01
BH (Bhuwana)	10.43±0.15	0.89±0.0036
UR (Udaipur road)	10.6±0.3	0.88±0.014
PH (Pahada)	10.23±0.15	0.875±0.015
RS (Rupsagar)	10.33±0.15	0.86±0.014
GN (Ganesh Nagar)	10.4±0.26	0.85±0.01
DNR (Durga nursery road)	10.33±0.25	0.88±0.0047
HM(Hiran Magri)	10.6±0.3	0.851±0.007
BL (Balicha)	10.53±0.2	0.849±0.0047
GD (Gogunda)	10.26±0.15	0.858±0.017

et al., 2021), and the same has also been associated with increased absorption of radiation even under lesser (15%) light intensity, which in turn enhances photosynthetic rate along with reducing harmful effects on plant metabolism, due to luminosity. The *M. oleifera* tree is an important perennial tree that is naturally grown and cultivated by farmers for its nutritional, medicinal, and industrial importance in tropical and sub-tropical agro-ecologies around the world in general and in the Indian subcontinent in particular (Saini *et al.*, 2016).

In the current study, maximum Fe content (0.8 - 0.9 mg/g) was recorded among accessions of Alwar, Kota and Udaipur region. However, based on the literature available, variation in its levels due to agro-climatic conditions is still unclear and needs to be studied further thoroughly to deduce any conclusive facts.

CONCLUSION

This study provided a first comprehensive report on morphological variations in *M. oleifera* growing in different agro-climatic zones of Rajasthan (India). Variations in various morphological traits viz., bark texture and colour, canopy size, shape and density, number of primary and secondary branches were recorded not only among the accessions growing in different agro-climatic zones, but also among those growing in different regions of same agro-climatic zone. This study, therefore, provides preliminary findings, based on which a detailed study to determine the factors leading to changes in morphological and biochemical properties could be designed. Further, molecular markers leading to these variations could either be identified or developed for rapid identification of morpho/ geno types with maximum Fe content. This study represented the comprehensive analysis of biochemical variations, specifically focusing on Fe content, within *M. oleifera* plants cultivated in diverse agro-climatic zones across Rajasthan (India).

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