

**GENETIC DIVERSITY IN WILD POPULATIONS OF
Moringa oleifera Lam. (DRUMSTICK TREE) IN
NORTH-WEST INDIA**

Thesis

by

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(F-2019-12-D)**

submitted to



**Dr. YASHWANT SINGH PARMAR UNIVERSITY
OF HORTICULTURE AND FORESTRY
SOLAN (NAUNI) HP - 173 230 INDIA**

in

partial fulfilment of the requirements for the degree

of

**DOCTOR OF PHILOSOPHY
(FORESTRY)
FOREST GENETIC RESOURCES**

**DEPARTMENT OF TREE IMPROVEMENT AND GENETIC
RESOURCES
COLLEGE OF FORESTRY**

2023

FRONTISPIECE



Species: *Moringa oleifera* Lam.
Family: Moringaceae



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CERTIFICATE-I

This is certify that the thesis entitled “**Genetic diversity in wild populations of *Moringa oleifera* Lam. (Drumstick tree) in north-west India**”, submitted in partial fulfilment of the requirements for the award of degree of **DOCTOR OF PHILOSOPHY (FORESTRY)** in the discipline of **FOREST GENETIC RESOURCES** to Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan (HP) is record of bonafide research work carried out by **Mr. MOHIT KUNDAL (F-2019-12-D)** son of **Sh. Hans Raj** under the guidance and supervision of Dr. Atul Gupta, Associate Director (R&E), RHR&TS, Jachh with the overall technical and administrative supervision and control of the undersigned. No part of this thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of investigations have been fully acknowledged.

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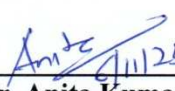
CERTIFICATE-II

This is to certify that the thesis entitled “**Genetic diversity in wild populations of *Moringa oleifera* Lam. (Drumstick tree) in north-west India**”, submitted by **Mr. MOHIT KUNDAL (F-2019-12-D)** son of **Sh. Hans Raj** to Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan (HP) in partial fulfilment of the requirements for the award of degree of **DOCTOR OF PHILOSOPHY (FORESTRY)** in the discipline of **FOREST GENETIC RESOURCES** has been approved by the Advisory Committee after an oral examination of the student in collaboration with the external examiner.


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ACKNOWLEDGEMENTS

Pride, praise and perfection belong to the “**Waheguru**” alone so I would like to bow down before the Supreme for the mental strength he bestowed on me to accomplish this painstaking venture and enabling me to see today “The joy of a task done”.

I feel privileged to express my deep sense of gratitude and heartfelt thanks to my Major Advisor **Dr. Atul Gupta**, Retired Associate Director (R&E), Regional Horticultural Research & Training Station, Jachh for his expert, valuable and tireless guidance, personal attention with sustained interest, innovative ideas, deep scientific vision and incessant encouragement during the tenure of these investigations. I emphatically owe my sincere thanks and obligations towards the members of my Advisory Committee, **Dr. Anita Kumari**, (Assistant Professor) Department of Tree Improvement and Genetic Resources, **Dr. Shikha Thakur**, (Assistant Professor) Department of Tree Improvement and Genetic Resources and **Dr. Parul Sharma**, (Assistant Professor) Department of Biotechnology for providing me necessary facilities, ideological contribution and indistinctive help in scrutinizing the manuscript.

I will ever remain indebted to the Professor and Head **Dr. Hari Paul Sankhyan** for trusting me and for the support, guidance and providing field and lab facilities timely during the entire journey of this manuscript along with academic staff and field staff.

Nevertheless, my successful accomplishment of the tasks at hand also owes much to the cooperation, personal inspiration, unfailing encouragement and moral support extended by **Dr. Sanjeev Thakur**, Retd. Dean, College of Forestry for his cooperation and guidance.

Inspite of all these, I can never forget my affectionate family; Father **Sh. Hans Raj**, Mother **Smt. Parvesh Kanta**, Brother Munish, Sister Rimpi, Brother-in-law Rajesh and Sister-in-law Preeti who encouraged, supported me at every better and worse conditions of life to undergo higher achievements. Their selfless persuasion, sacrifice, heartfelt blessings and constant inspiration have made this manuscript a little remuneration to translate their dreams into reality.

I extend my sincere thanks to working staff members of Department of Tree Improvement and Genetic Resources, viz., Sh. Des Raj, Sh. Vidya Nand and S. Makhan Singh for their kind and patience help during my thesis work. I would like to mention my sincere thanks and co-operation to the field workers at RHR&TS, Jachh for their support for establishing and handling nursery.

On a personal note, I wish to place on record my sincere thanks to the Range Forest Officers, Forest Guards and Local farmers for guiding me and helping me in survey at field level in the difficult times of COVID-19.

I would like to mention my sincere thanks and co-operation to my beloved seniors, viz., Divya, Parminder, Gitika, Aditi and Anmol and my juniors, viz., Shweta, Akshiptika and Gauri. I profoundly thank my friends Jyoti, Gurjot, Ashan, Amna, Hardil, Puneet, Jaspal, Aman, Jagmeet, Mukesh, Devender, Ishita, Vivek, Madhur, Manjot, Navdeep and Vinod for whose inputs have helped me to complete this research work successfully. The help and timely suggestion, everlasting patience received from Aman and Jagmeet during statistical analysis is gratefully acknowledged.

Ending is inevitable journey for any kind of work. Though acknowledging is an endless task, I would finally like to express my sincere gratitude and thanks to all those who I may have inadvertently failed to mention here, but have helped and supported me directly or indirectly during the course of my studies.

Finally, the words of special appreciation go to Ajay for sincere efforts in bringing this manuscript into its final form.

Needless to say, errors and omissions are mine.

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LIST OF ABBREVIATIONS

AD	:	Anno Domini
BC	:	Before Christ
BCE	:	Before Common Era
bp	:	Base pair
CD	:	Critical difference
cm	:	Centimeter
cm ²	:	Square centimeter
CPTs	:	Candidate plus trees
CV	:	Coefficient of variation
DBH	:	Diameter at breast height
g	:	Gram
ha	:	Hectare
HP	:	Himachal Pradesh
i.e.	:	That is
I.U.	:	International Unit
kg	:	Kilogram
m	:	Meter
M	:	Molar
m ²	:	Square meter
mA	:	Milliampere
mg	:	Milligram
mg/100g	:	Milligram per 100 gram
mg/g	:	Milligram per gram
mg/kg	:	Milligram per kilogram
mg/L	:	Milligram per liter
mL	:	Milliliter
mL/min	:	Milliliter per minute
mm	:	Millimeter
msl	:	Mean Sea level

N	:	Normal
nm	:	Nanometer
OD	:	Optical Density
PCR	:	Polymerase chain reaction
pH	:	Potential of hydrogen
PIC	:	Polymorphism information content
ppm	:	Parts per million
rpm	:	Rotations per minute
UT	:	Union Territory
V	:	Volt
Viz	:	Videlicet (namely)
μl	:	Microlitre
μl/mL	:	Microlitre per millilitre
⁰ C	:	Degree centigrade
⁰ E	:	Degree East
⁰ N	:	Degree North
&	:	and
%	:	Per cent

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Chapter-1

INTRODUCTION

Plants have been the major source of drugs in Indian system of medicine and other ancient systems in the world. The earliest description of the curative properties of medicinal plants is found in the Rig-Veda. Charaka Samhita and Sushrasha Samhita give an extensive description on various medicinal herbs (Kirtikar and Basu, 1934). These features have been identified in the pre-historic times and the worldwide use of herbal therapies and health care preparations that are prescribed in ancient books like Vedas and the Bibles given a way for discovering of natural products with medicinal values.

The primary health care of over 80 per cent of the world's population still depends on plant based traditional medicines. There are about 45,000 plant species (nearly 20 per cent of the global species) found in Indian subcontinent. Of these, about 3,500 species of both higher and lower plant groups are of medicinal values. Of around 500 medicinal plant species used by the contemporary Ayurvedic industry, around 80 per cent are procured from wild areas, mostly notified as forest land (Niraj *et al.*, 2002). Millions of rural households use medicinal plants in selfhelp mode. Demand for medicinal plants is increasing in both developing as well as developed countries due to growing recognition of natural products, being non-narcotic, having no side effects, easily available at affordable process and sometimes the only source of health care available to the poor. Medicinal plant sector has traditionally occupied an important position in the socio-cultural, spiritual and medicinal arena of rural and tribal lives of India (Akshay *et al.*, 2014).

Threats to genetic diversity as well as species survival has been increased due to these medicinal plant collection practices from wild associated with habitat destruction, over-exploitation, land use changes and other pressures (Engels and Arora, 1993). Moreover, success in developing and maintaining food security and adequate nutritional levels will require a multifaceted approach with a continued focus on developing best practice production strategies for indigenous or naturalized drought-tolerant species. These species should have a wide range of adaptation, multiple uses, rapid growth, propagate readily, and social acceptance for use (Nautiyal and Venkataraman, 1987). Once identified, simple and specific cultural practices appropriate for local growing conditions need to be identified to ensure sustainable levels of production.

Moringa oleifera Lam. is most widely distributed and naturalized species of the monogeneric family Moringaceae (Ramachandran *et al.*, 1980). The genus *Moringa* has more than 13 species (Verdcourt, 1985) of which two species, viz., *Moringa oleifera* Lam. and *Moringa concanensis* occur in India (Pandey *et al.*, 2011). *Moringa oleifera* (drumstick tree, horse radish tree or West Indian Ben) is a fast growing and medium sized tree having tripinnate leaves. It is a drought resistant species and is found widely in the sub-Himalayan tracts of northern India (Balakumbahan *et al.*, 2020). The tree itself is rather slender with drooping branches and height ranges from 5 to 10 m. It can grow well in the humid tropics or hot drylands and survives in destitute soils (Morton, 1991). It tolerates a wide range of rainfalls (250 to 3000 mm) and soil pH (5.0-9.0) (Palada and Changl, 2003).

The use of *Moringa oleifera* as a medicinal herb was first described around 2000 BCE in India. *Moringa* has a long history of use in traditional Indian and African cuisine and medicine and was considered a panacea that was used to treat more than 300 conditions. (Gopalakrishnan *et al.*, 2016).

Drumstick is an important food commodity which has received enormous attention as the 'natural nutrition' of the tropics. *Moringa* has many medicinal properties. Almost all parts, viz., root, bark, gum, leaf, pod, flower, seed and seed oil have been used for treatment of various inflammation and infectious diseases along with cardiovascular, gastrointestinal, haematological and hepatorenal disorders (Siddhuraju and Becker, 2003). Leaves of *Moringa* represent an important source of nutrients for rural populations (Lockett *et al.*, 2000). Many reports indicate that *Moringa* leaves are rich in protein and present an amino acid composition, which is suitable for human and animal nutrition (Freiberger *et al.*, 1998). It is referred to as a nutrient dense food source because of its high nutritional value of leaves, pods and seeds. In general, 100 g of *Moringa* leaves contain four times more vitamin A than the same quantity of carrots, four times more calcium than 100 ml of milk, four times more iron than 100 g of spinach, seven times more vitamin C than 100 g of oranges and three times more potassium than 100 g of bananas. The protein quality of drumstick leaves also rivals that of milk and eggs (Fahey, 2005).

Leaves contain 9.1 per cent carbohydrates, 2.1 per cent dietary fiber, 1.7 per cent fat and 8.1 per cent protein. Flowers are used as stimulant, tonic and diuretic. They are useful in increasing the flow of bile (Abbas *et al.*, 2018). The seeds of *Moringa* are considered to be antipyretic, acrid, bitter (Oliveira *et al.*, 1999) and reported to show antimicrobial activity (Sastri, 1962). The seeds of *Moringa* are used as water purifier. The oil extracted from the

seed, known as “Ben”, is used as lubricant in watches, for edible purposes and in cosmetics. The kernel of the seed is rich in crude protein, fatty oil and fiber.

India is the prevalent prime producer of Moringa crop, with an annual production of 2.2 million tonnes of tender fruits from an area of 38,000 ha. Among the states, Andhra Pradesh leads in both area and production followed by Karnataka and Tamil Nadu. Tamil Nadu is the pioneering state as it has broad genetic diversity (annual and perennial Moringa) from diversified geographical areas, as well as few introductions from Sri Lanka (Balakumbahan *et al.*, 2020). Genetic improvement of *Moringa oleifera* can boost the tropical and sub-tropical breeding programmes.

Nevertheless, there is a deficient understanding of its detailed gene flow pattern and population genetic structure, which causes uncertainty in designing, planning and managing Moringa seed orchards breeding programmes. Despite limited knowledge of the levels on genetic diversity and relatedness of introduced populations, their utilization as a source of seed material for planting is widespread. Knowledge of population genetic diversity is one of the prerequisites for development of any plant species conservation strategies. Regarding the wild species that represent genetic resources for cultivated plants, an understanding of their genetic diversity is very much essential as they are the prime source of genetic material that are used to improve elite cultivars.

The genetic diversity pattern, genetic makeup and nutritional variation need to be studied for successful breeding and improvement, domestication and large scale cultivation. The knowledge of genetic diversity of tree crop is very important for rational planning of conventional, modern breeding and improvement programme for the purpose of improving the yield and its quality of produce (Rafii *et al.*, 2012). The genetic diversity can be assessed based on morphological markers but it is difficult to classify the accessions solely based on their morphological characters therefore the use of molecular markers, such as Random Amplified Polymorphic DNA (RAPD), Inter-Simple Sequence Repeat (ISSR), Amplified Fragment Length Polymorphism (AFLP) and Simple Sequence Repeat (SSR) has gained popularity as a genetic diversity assessment methods of tree crops (Muluvi *et al.*, 1999).

The last few decades have witnessed the emergence of widespread concern to solve the major problem of the erosion of genetic resources. The only realistic solution of the continuing loss of plant germplasm is the genetic conservation; that is the collection and

systematic preservation of germplasm in gene resource centres with a wide representation of genetic diversity of the species as practicable.

With the advent of PCR technique, a revolution came into the molecular studies by its multifarious applications. (Mullis and Faloona, 1987). PCR based systems such as RAPD, ISSR, AFLP and SSR are fast and allow maximum automation and are thus being used extensively for DNA based diversity studies. DNA fingerprinting will help to characterize populations, estimate variability, within and among populations and monitor diversity and structure of populations, generated, maintained and manipulated in the breeding process. The screening and maintenance of identifies germplasm will improve the overall reliability. Identification could open market for and prevent theft of proprietary genotypes.

Application of SSR markers at population level provides the opportunity to explore the extent and distribution of genetic variation at a wide range of loci within the species.

For the genetic improvement through selection of superior phenotypes and provenance trial, it is desirable to evaluate the naturally available genetic variation and to utilize the best material for its diverse medicinal properties and for future tree improvement programme. So far, limited systematic work has been reported. Hence, the present study entitled “Genetic diversity in wild populations of *Moringa oleifera* Lam. (Drumstick tree) in north-west India” was planned with the following objectives:

Objectives

The study will fulfil following objectives:

1. To survey and select *Moringa oleifera* genotypes from wild populations on the basis of morphometric characters
2. To study the morphological variations in selected half-sib progenies of *Moringa oleifera* at nursery stage
3. To identify superior accessions of *Moringa oleifera* having higher nutritional values
4. To assess the genetic diversity of *Moringa oleifera* genotypes using DNA based molecular markers

Chapter-2

REVIEW OF LITERATURE

A successful breeding programme is associated with the existence of diversity among the genotypes within a reasonable range. More the diversity, better are the chances of improving the economic traits under consideration in the resulting crosses (Singh, 1982). The basic aim of any plant breeding programme is the efficient management of genetic variability to develop superior varieties and hybrids that can combine both productivity and quality under favourable as well as stress environments. However, certain specific gene combinations can be pooled in a single plant or varieties depending upon the system through which the genes present in the plant material are mobilized. The basic principle underlying the development of new varieties of any crop is the improvement in their genotypic variation.

The available literatures relevant to the present investigation pertaining to *Moringa oleifera* Lam. and most relevant in some other species too has been presented under the following heads:

2.1 Occurrence of *Moringa oleifera*

The indigenous knowledge and use of Moringa is referenced in more than 80 countries including Pakistan and known in over 200 local languages. Moringa has been used by various societies (Roman, Greek, Egyptian, and Indian to mention a few) for thousands of years with writings dating as far back as 150 A.D. The history of Moringa dates back to 150 B.C. Historical proofs reveal that ancient kings and queens used Moringa leaves and fruit in their diet to maintain mental alertness and healthy skin. Ancient Maurian warriors of India were fed with Moringa leaf extract in the warfront. The elixir drink was believed to add them extra energy and relieve them of the stress and pain incurred during war. These brave soldiers were the ones who defeated Alexander the Great (Fuglie, 2001).

Swati *et al.*, (2020) reported populations of *Moringa oleifera* Lam. in Bilaspur and Mandi districts of Himachal Pradesh, Dehra Dun and Nainital districts of Uttarakhand and Latur and Pune districts of Maharashtra.

Considerable genetic variability is available in the tarai tracts of Uttarakhand and Uttar Pradesh, India (Ramachandran *et al.*, 1980; Grubben and Denton, 2004). In the areas of natural distribution, it occurs as a component of the mixed broad leaved/conifer forest along

forest of sub-Himalayan tracts, often associated with *Shorea robusta* and *Acacia catechu* forest or nearby crop fields and river side (Gaur 1999; Singh *et al.*, 2000). In Bhabar area of Kumaon, Uttarakhand rare types of Moringa with beautiful pink-white flowers were recorded at an altitude of 1,000-2,000 m (Strachey, 1974). In neighbouring regions in the Nepal Himalaya, it occurs as a common forest species (Bhattarai and Baral, 2006). They also found pink flowered population in Panchkula district of the foothills of Himachal Pradesh in the western Himalaya. This species occurs under protected habitats in the tribal dominated tracts of central India (parts of Bihar, Chhattisgarh and Simlipal region of Orissa) evidently exhibiting trends of protection/semi-domestication (Duthie, 1960). Moringa is commonly cultivated as perennial in home gardens in South India. In several areas, it is also seen to run wild near areas of cultivation in states of Kerala, Rajasthan, etc. (Shetty and Singh, 1987; Murthy and Venu, 2005). Due to high value of fruits and seed production, this species has rapidly spread to many tropical countries of the world in over last century.

Moringa trees though native in the sub-Himalayan tracts, it is widely cultivated in Africa, Central and South America, Sri Lanka, India, Mexico, Malaysia, Indonesia and the Philippines. According to Muluvi *et al.*, (1999), the Moringa tree wide natural spread in the world and introduced to Africa from India where it was used as a health supplement and it was originally an ornamental tree in the Sudan, planted during British rule in the alleys along the Nile, public parks, and the gardens of foreigners.

Moreover, a study in the Virudhunagar district of Tamil Nadu, India reported Moringa among the species utilized by traditional Siddha healers (Mutheeswaran *et al.*, 2011). The leaves of Moringa were harvested from a village in Cuddalore district, Tamil Nadu, India and authenticated by a senior Botanist. (Sudha *et al.*, 2020).

Ganesan *et al.*, (2014) reported populations of *Moringa oleifera* Lam. in Valayapatti, Palamedu, Tiruchirappalli, Karur, Theni, Varusanadu, Kandamanoor, Periyakulam, Erode and Kovilpatti districts of Tamil Nadu and Kangra district of Himachal Pradesh. Tak and Maurya (2015) reported *Moringa oleifera* Lam. populations in Ajmer, Jhalawar, Kota and Udaipur districts of Rajasthan through a survey made during 2012. Trees selected were growing naturally on road side or planed in open field/boundary wall or grown in kitchen garden.

The tree is wild in the Sub-Himalayan tracts from Chenab to Oudh. It grows at elevations from sea level to 1400 m. It is very commonly cultivated near houses in Assam,

Bengal and peninsular India (Gupta, 2012). It is also cultivated in north-eastern Pakistan, north-eastern Bangladesh, Sri Lanka, West Asia, the Arabian peninsula, East and West Africa, throughout the West Indies and southern Florida, in Central and South America from Mexico to Peru, as well as in Brazil and Paraguay (Roloff *et al.*, 2009).

2.2 Plus tree selection

Paray *et al.*, (2017) selected hundred CPTs of *Salix alba* based on the comparison tree method from twenty places across the two selected districts of Kashmir valley. The tree characteristics used were height, bole height, DBH, and crown diameter. Furthermore, the mean values recorded for these dimensions were 12.50 m for height, 28.70 cm for DBH, 5.40 m for bole height, and 3.40 m for crown diameter, respectively.

Chauhan and Gera (2012) developed a procedure and criteria for selecting the CPTs of six fast growing agroforestry species in state of Punjab. The superiority of growth rate of the selected CPTs was compared with the average of surrounding check trees and scores were assigned to each selected CPTs for various silvicultural parameters indicating higher productivity and good health. A total of 19, 16, 15, 14, 8 and 11 CPTs were identified for *Eucalyptus* spp., *Dalbergia sissoo*, *Albizia procera*, *Acacia catechu*, *Azadirachta indica* and *Melia composita*, respectively. The superiority index of all the selected species was calculated, which is expected to further help in identifying the best individuals for the progeny trails and multi-locational clonal trials.

Eighty one CPTs of *Dalbergia sissoo* were selected by Yadav *et al.*, (2005) based on the intensive survey in the states of Haryana, Utter Pradesh and Himachal Pradesh. They reported wide variation in plant height, clear bole height, straightness, 100 seed weight and germination percent among selected trees. Kumar *et al.*, (2005) conducted a study on forty five CPTs of *Dalbergia sissoo* which were selected at the different locations of Himachal Pradesh and reported considerable variability among individual trees for a particular trait.

Sidhu (1997) selected sixteen CPTs of *Dalbergia sissoo* and recorded the observations on tree height, clear bole height, diameter, fork diameter and number of crooks. From them, twelve plus trees of *Dalbergia sissoo* were selected by mean values for total height, clear bole height and apical dominance which were significantly better than baseline trees.

2.3 Variability in growth, biomass and nutritional parameters

Treatment [Soil (50%) + Vermiculite (25%) + Perlite (25%)] was found best in respect to the germination parameters, growth parameters and physiological parameters like days to germination (7.0), germination percentage (87 %), seed vigour index (3.03), plant height (140.03 cm), number of leaves per plant (380.43), stem girth (2.05 cm), number of nodes (34.17), length of inter node (20.15 cm), fresh weight of shoot (21.48 g), dry weight of shoot (10.50 g), fresh weight of root (8.54 g), dry weight of root (3.90 g) and root: shoot ratio (0.40) of annual drumstick (*Moringa oleifera* Lam.) cv. PKM-1 (Shreehari *et al.*, 2022).

Significant variation was observed in all different characteristics, viz., tree height, DBH, crown spread, 100 seed weight, germination percentage and seed colour among the samples of *Toona ciliata* collected from different provenances (Singh *et al.*, 2021).

Progeny performance of the genotypes of *Moringa oleifera* Lam. provided significant germination percent (%), collar diameter (cm), number of leaves, shoot length (cm), root length (cm), total plant height (cm), fresh weight of seedlings (g) and dry weight of seedlings (g). Wide range of variability was observed in tree growth, DBH and crown spread. For pod parameters, G-11 found to be superior whereas P-15 and P-22 were superior at nursery stage. Similarly higher significant results were observed for moisture content, fat, crude protein, crude fibre, ash content (%), total sugar content (g/100g) in genotypes G-21, G-20, G-5, G-22, G-12 and G-11, respectively (Redhu, 2021).

Gupta *et al.*, (2020) found significant results for seedling height, collar diameter, internodal length, fresh shoot weight, fresh root weight, dry shoot weight, dry root weight, root: shoot ratio and seedling biomass for *Acacia nilotica*.

Leaves of *Morus alba* Lam., *Leucaena leucocephala* Lam De Wit. and *Moringa oleifera* Lam. were evaluated based on their chemical composition. All tree fodder forages had similar dry matter content, but crude protein was higher in *Moringa oleifera* Lam. (28.6 %) followed by *Leucaena leucocephala* Lam De Wit. (24.5 %) and *Morus alba* Lam. (24.1 %). Ash content was highest in *Morus alba* Lam. followed by *Moringa oleifera* Lam. with *Leucaena leucocephala* Lam De Wit. having lowest amounts. Polyphenols ranged from 2.72 to 3.64 %, with *Leucaena leucocephala* Lam De Wit. having highest and mulberry lowest amounts. Dietary fibre were highest in *Morus alba* Lam. and *Moringa oleifera* Lam. In vitro gas production was higher in *Moringa oleifera* Lam. followed by *Morus alba* Lam. with *Leucaena leucocephala* Lam De Wit. having lowest amounts (Simbaya *et al.*, 2020).

Thangjam *et al.*, (2020) studied variations on different traits of *Parkia timoriana* (D.C.) Merr. in twelve provenances and found significant variations for tree height, DBH, crown spread, pod and seed traits and germination parameters.

Thirty six plus trees of *Toona ciliata* were selected from twelve different seed sources following baseline method which varied significantly for tree height, clear bole height, girth and straightness score. Seed sources, viz., S1 (Talwara), S2 (Kamahi Devi) and S6 (Chabutra) exhibited higher values for tree characteristics. Considerable variations were observed in seed length (1.20-1.56 cm), 100 seed weight (704.63-846.62 mg), germination percent (27.00 - 67.25 %), survival percent (62.50-87.97 %), seedling height (8.69-28.65 cm), number of leaves (27.42- 66.25), dry shoot weight (0.99-1.93 g) and dry root weight (0.58-1.67 g) among seed sources. Progenies obtained from seed sources S1 (Talwara), S2 (Kamahi Devi), and S3 (Ludhiana) exhibited outstanding performance for growth and biomass traits (Deepanjli, 2018).

Shaltout *et al.*, (2017) studied morphological properties of *Moringa oleifera* Lam. indicating some variations in the features of species attributes (trees, leaves, pods and seeds) at 8 sites in Egypt. They concluded that those variations were not only related to environmental conditions (particularly soil properties) but also due to genetic differences with in the species.

Ananias *et al.*, (2016) conducted an experiment to analyse the mineral compositions of *Moringa ovalifolia* and *Moringa oleifera* Lam. Significant differences between the concentration of different elements such as Ca, K, Fe, Ni, Cu, Mn, Co and Cd in seeds of both the species was found among the sites. The contents of these metals varied depending on geographical sites. Both *Moringa ovalifolia* and *Moringa oleifera* Lam. were good source of important minerals and need to be explored further.

A study was conducted to compare the performance of *Moringa ovalifolia* and *Moringa oleifera* Lam. seed germination and seedlings establishment. ANOVA revealed that *Moringa oleifera* Lam. and *Moringa ovalifolia* mean germination rates and germination days were significantly different. *Moringa ovalifolia* had higher seed germination rate compared to *Moringa oleifera* Lam. Also, *Moringa ovalifolia* germinated faster than *Moringa oleifera* Lam. Conversely, *Moringa oleifera* Lam. had faster seedlings establishment compared to *Moringa ovalifolia* which had slower growth rate over time (Korsor *et al.*, 2016).

Morphological characterization of *Moringa oleifera* Lam. based on measurements taken on the tree, leaves, leaflets and fruits, and ethno-botanical survey on cultivation techniques and management of plantation of the species were performed by Agoyi *et al.*, (2015). The morphological analysis showed significant variation between populations of *M. oleifera* considered in relation to tree height, leaf length, petiole diameter, length and width of leaflets; length, median diameter and fresh weight of pods.

Banyal and Sharma (2015) revealed significant variation for seedling height, collar diameter and survival among progenies of plus trees of *Cedrus deodara*. Further, higher extent of genotypic variation for germination (63.39 %) and height (61.44 %) and low for collar diameter (23.90 %) was found.

Significant variations between provenances for morphological characteristics and biomass production were observed. Two-month old *Moringa oleifera* Lam. exhibited significant differences between provenances. The average height ranged from 40-107 cm, number of branches from 8-15, number of pinnae per leaf from 5-12, leaf length from 16-44 cm and leaves width from 34-105 cm. The aboveground biomass productions ranged from 1-14 g, underground biomass production from 1-7 g, ratio of under: aboveground from 0.4-0.9 and total dry weight from 2-21 g (Dao and Kabore, 2015).

Fredrick *et al.*, (2015) carried out a study on provenance variation in seed morphological characteristics and germination behaviour among the provenances of *Faidherbia albida* at the World Agroforestry Centre in Nairobi and observed significant variations for seed length, seed thickness, seed weight and seed germination.

Thirty-six genetically diverse genotypes of drumstick were collected from different places of Rajasthan (Ajmer, Jhalawar, Kota, Udaipur) and significant difference in characters like pod weight (26.37-66.43 g), pod length (24.43 to 59.47 cm), pod girth (7.33 to 23.67 mm), number of leaflets/leaf (8.00 to 13.33), and leaf length (28.63 to 66.37 cm) was observed. Similarly, a wide variation was recorded for nutrients in *Moringa oleifera* Lam. leaves. It was 22.07 to 37.47 % for dry matter; 5.33 to 7.87 % for TSS; 0.72 to 1.43 mg/g for chlorophyll (Tak and Maurya, 2015).

The proximate analysis of dried leaf of *Moringa oleifera* Lam. showed that it is highly rich in protein (28.00±0.33 %), fat (3.88 %), ash (9.88 %), crude fibre (12.57 %) and carbohydrate (37.87 %). The leaf was found to be rich in minerals and vitamins. It contained high concentrations of Zn, Mg, Ca, K and P; low, but appreciable levels of iron. The rich

array of results obtained in this study displays that *M. oleifera* is a multipurpose plant that could contribute immensely towards meeting both human and livestock nutritional requirement (Okiki *et al.*, 2015).

The results on yield and yield-related traits of *Vigna unguiculata* showed that first three principal components contributed 82.23 per cent of the total variability. It revealed that number of seeds per pod, 100-seed weight, pod length, days to 50 per cent maturity, seed yield, number of leaves per plant contributed significantly to the total genetic variability while for proximate composition, four principal components accounted for 93.75 per cent of the total genetic divergence. Cluster analysis revealed that accessions were grouped not necessarily based on geographical location but genetics. Selection for high yielding accessions should be done on cluster 2 as we recommend selection and hybridization of accessions from cluster 1, 2, and 3 for optimal benefit (Udensi and Edu, 2015).

(Offor *et al.*, 2014) in his experiment showed that the mean nutritional content of the dried *Moringa oleifera* Lam. leaf were: protein (24.5 %), moisture (14.8 %), crude fibre (17.3 %), Fat (4.5 %), Ash (3.8 %) and carbohydrate (50.6 %). The metal contents in the leaves were Fe (505 mg/l), Zn (85,810.95 mg/l), Cu (1.02 mg/l), Mg (386.56 mg/l) and Mn (79.56 mg/l).

Fakankun *et al.*, (2013) observed mean concentrations of the mineral elements were 26000, 643, 8210, 2980, 69.9, 169 and 15.3 mg/kg for Ca, Mg, K, Na, Mn, Fe and Zn, respectively and no significant difference was found in different parts of *Moringa oleifera* Lam.

Thirty four *Moringa oleifera* Lam. ecotypes maintained in the germplasm bank of Department of Vegetable Crops, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu and found that the trees significantly varied for plant height, leaf length, leaf area, leaf area index, trunk girth and number of flowers per inflorescence (Selvakumari, 2013).

Pod length, number of seeds per pod, 100-seed weight and percent germination after harvesting, appearance (straight or curled), colour of fresh pod (green or pale green), colour of dried pod (golden brown or brownish), colour of stem bark (grey or whitish), colour of flowers (white or creamy, with or without purple patches) and branching habit (horizontal branching or vertical branching) showed significant variations in *Moringa* accessions (Amoatey *et al.*, 2012).

Osman and Abohassan (2012) evaluated seven populations of *Moringa peregrina* Forssk. to assess phenotypic variations and determine the types and levels of major nutrients in the seeds and leaves. Pod length was negatively correlated to weight of seed/pod, whereas gross pod weight was positively correlated to number of seeds/pod, weight of seeds/pod and 100 seed weight. Oil percentage in the seed, across locations, ranged from 54.29 per cent to 57.25 per cent, whereas protein content ranged from 11.7 per cent to 14.6 per cent. Out of the 17 amino acids found in leaves and seeds, 9 were classified as essential amino acids.

Significant differences were observed by Shu *et al.*, (2012) among the 15 different provenances of *Magnolia officinalis* for various seed traits, seed germination and growth characteristics. Among provenances, seed weight showed maximum variation.

Tak (2012) surveyed and selected 36 genotypes of *Moringa oleifera* Lam. in Rajasthan in various cultivation types, viz., kitchen garden, field plantation and road Side plantation. She found significant differences for tree height, tree girth and bitterness magnitude of the trees. The per se performance of the pod and leaf characters ranged from 26.37- 66.43g for pod weight; 24.43 to 59.47 cm for pod length and 7.33 to 23.67 mm for pod girth; 8.00 to 13.33 for number of leaflets/leaf and 28.63 to 66.37 for leaf length. The genotype JWRM -31 had more no. of seed/pod whereas the genotype JWRM-32 registered for greater seed weight. Similarly, a wide variation was recorded for nutrients in drumstick leaves. It was 22.07 to 37.47 % for dry matter; 5.33 to 7.87 % for TSS; 0.72 to 1.43 mg for chlorophyll; 155.45 to 220.26 mg for vitamin C 22.50 to 26.60 % for protein; 3461.40 to 4091.77 mg for N; 126.73 to 203.46 mg for P; 654.27 to 203.46 mg for K; 1460.77 to 2044.27 mg for Ca; 14.03 to 26.80 mg for Fe and 245.40 to 350.00 mg for Mg.

The dried *Moringa oleifera* Lam. leaves had crude protein of 30.3 per cent and 19 amino acids. The dried leaves had the following mineral contents: calcium (3.65 %), phosphorus (0.3 %), magnesium (0.5 %), potassium (1.5 %), sodium (0.164 %), sulphur (0.63 %), zinc (13.03 mg/kg), copper (8.25 %), manganese (86.8 mg/kg), iron (490 mg/kg) and selenium (363 mg/kg). 17 fatty acids were observed with α -Linolenic acid (44.57 %) having the highest value followed by heneicosanoic (14.41 %), g-linolenic (0.20 %) palmitic (0.17 %) and capric acid (0.07 %). Vitamin E had the highest concentration of 77 mg/100 g than beta-carotene, which had 18.5 mg/100 g in the dried leaves. The fiber content was neutral detergent fibre (NDF) (11.4 %), acid detergent fibre (ADF) (8.49 %), acid detergent lignin (ADL) (1.8 %) and (acid detergent cellulose (ADC) (4.01 %). The condensed tannins had a

value of 3.2 %, while total polyphenols were 2.02 %. The values of amino acids (26.03 %) desirable nutritional balance (Moyo *et al.*, 2011).

Anjorin (2010) analysed the mineral composition of *Moringa oleifera* Lam. from two regions of Nigeria, viz., Sheda and Kuje and revealed that Ca, Mg, Fe and Cu from Sheda were comparatively higher than that from Kuje. Toxic elements such as Pb was absent from both of the locations confirming that these locations can be a reference point in the selection of these genotypes.

Variability studies of twenty-four candidate plus trees from naturally available *Pongamia pinnata* revealed that, genotype CPT-19 recorded maximum values for six traits viz. pod length (65.64 mm), 100-pod weight (542.35 g), 2D surface area (351.18 mm²), seed length (27.93 mm), 100-seed weight (202.89 g) and total oil content (44.33 %). However, maximum pod thickness (12.72 mm), seed length (17.49 mm), pod-seed ratio (2.89) germination capacity (94.67 %) was recorded by the genotype CPT-6 (Divakara *et al.*, 2010).

Moringa leaves from 11 different agro climatic regions distributed in Thailand were analysed and found to contain 19.15-28.80 % of protein; 2.06-2.47 % of fat; 16.30-23.89 % of fiber; 8.52-13.53 % of moisture. The calcium, potassium and iron contents of Moringa leaves (100 g dry weight) were found in the range of 1510.41-2951.13 mg, 1504.23-2054.05 mg and 20.31- 37.60 mg, respectively. The results revealed that Moringa leaves indigenous to different agro-climatic regions of Thailand contained an appreciable amount of nutrients and might be used as a good supplement for some nutrients such as protein, fiber and minerals (Jongrungruangchok *et al.*, 2010).

Hooda *et al.*, (2009) selected thirty nine plus trees of *Pongamia pinnata* and carried out a progeny trial in nursery. The results showed significant variation among progenies for germination percentage, collar diameter, shoot length, root length and seedling biomass.

The pattern of variation in seed characteristics, germination behaviour and seedling traits of *Jatropha curcas* was elucidated by Dhillon *et al.*, (2008). They revealed significant variations among all the accessions.

Anwar and Rashid (2007) examined the physico-chemical characteristics of *M. oleifera* seeds and seed oil from a wild provenance of Pakistan. The seeds exhibited an oil yield of 34.80 per cent. Protein, fiber, moisture and ash contents were 31.65 per cent, 7.54 per cent, 8.90 per cent and 6.53 per cent, respectively. The wild *Moringa oleifera* Lam. seed oil was found to contain oleic acid up to 73.22 per cent, followed by palmitic, stearic, behenic

and arachidic acids 6.45 per cent, 5.50 per cent, 6.16 per cent and 4.08 per cent, respectively and fell in the category of high-oleic oils. The results of different quality attributes of *Moringa oleifera* Lam. oil from a wild provenance of Pakistan reveal that it could be employed for edible and commercial applications.

Forty CPTs of *Pongamia pinnata* were selected based on the morphometric traits to identify suitable seed sources with high oil content and for production of quality seedlings for mass afforestation in different forestry and agroforestry programmes. Significant genetic variability and association were recorded among 40 CPTs for pod and seed traits. Maximum 100-seed weight (186.80 g) and pod-weight (403.94 g) was recorded in CPT-33, while CPT-18 showed maximum oil content (44.07 %) (Kaushik *et al.*, 2007).

Seventy-seven accessions of *Moringa oleifera* Lam. were collected from Tamil Nadu, Karnataka and Pondicherry. All these accessions showed wide range of variability in fruit weight, fruit length and fruit girth. High coefficient of variation (CV) was observed for fruit weight followed by fruit girth and fruit length. They concluded that wide variability in leaf, flower and fruit characters can be exploited for the improvement of drumstick. Promising drumstick germplasm identified were IIHR-35, IIHR-39 and IIHR-26 with regard to their fruit characters. These accessions could either be directly used for commercial cultivation or utilized in the breeding programmes (Varalakshmi and Devaraju, 2007).

Arya and Toky (2006) evaluated the progenies of 50 plus trees of *Acacia nilotica* collected from five states at Hisar and found wide variability in seed weight, seed germination, seedling height and collar diameter.

Singh *et al.*, (2006) observed significant variations for seed traits, viz., seed length, seed breadth seed weight, seed germination, survival percent and other growth and biomass traits among 13 provenances of *Celtis australis* in Central Himalayas.

Significant differences were observed among provenances of *Cordia africana* in all studied attributes except root collar diameter after 4 months of growth (Loha *et al.*, 2006).

Dogra *et al.*, (2005) evaluated 34 progenies of *Dalbergia sissoo* for height, girth and clear bole height as measure of the overall growth performance of individual progeny with a view to remove the inferior progenies from the site and converted the progeny trial into seedling seed orchard. Analysis of data indicated that there were significant differences in height, girth at breast height and clear bole between the progenies.

Resmi *et al.*, (2005) reported that cultivars i.e. MO 13, MO 24, and MO 26 of *Moringa oleifera* Lam. from Thiruvananthapuram showed three distinct flowering peaks in a year, while others showed one or two peaks. Fruits per plant (174 to 612), fruit yield (8.94 to 70.46 kg per tree), leaf vitamin A (8108 to 13216 I.U) and fruit vitamin A (95 to 185 I.U.) also showed considerable variations. Vitamin C content in leaf and fruit was highest for MO 18 (226 mg per 100 g leaf and 129 mg per 100 g fruit). Overall ranking showed the relative superiority of MO 26 and MO 28 over others.

2.4 Genetic estimates, correlation, principle component analysis, path coefficient analysis and cluster analysis

Twenty genotypes used to study the genetic diversity for different yield attributing characters of *Moringa* by principal component analysis. In this study, out of twelve principal components, five components exhibited eigen value and revealed about 99.54 per cent variability among the traits. The PC1 accounted for the highest variability (62.20 %), followed by PC5 (28.86 %), PC6 (6.25 %), PC7 (1.01 %) and PC4 (0.75 %). The results of the Principal Component Analysis revealed that wide genetic variability exists in these *Moringa* genotypes (Karunakar *et al.*, 2022).

The estimates of PCV was marginally higher than the corresponding GCV in *Moringa* assessments. GCV was recorded to be high for marketable pod yield per plant. High heritability observed for stem thickness. High genetic advance as per cent of mean was estimated for marketable pod yield per plant. (Kumar *et al.*, 2022).

Magnitude of phenotypic coefficient of variation was higher than its genotypic level for all the traits, indicating the environmental influence on their expression in *Moringa*. Higher genotypic and phenotypic coefficient of variation was observed for all the leaf yield traits indicating that higher variability and simple selection would be effective. High heritability coupled with high genetic advance was observed for stem length, whole leaf weight/shoot and edible leaf weight/shoot which revealed that these characters are under additive gene action and showed higher responses of these trait towards selection (Mandal *et al.*, 2022).

High range of variability and high genetic variance were observed for all the traits in both locations for *Telfairia occidentalis*. Close differences between genotypic and phenotypic variances and genotypic, phenotypic and environmental coefficient of variations were observed for all the traits. Moderate broad sense heritability with moderate genetic advance

was observed which indicated the influence of environmental variance is more than genetic variance (Okon *et al.*, 2022).

Genetic parameters studies for variability, correlation and path coefficients analysis of twenty genotypes of *Moringa* for twelve yield attributes revealed that the characters leaf length, number of pod per plant, plant height, pod girth, number of branches per plant and number of seeds per pod showed high GCV and high heritability along with high genetic advance and these characters are controlled by additive gene. Seeds per pod showed positively significant correlation with pod length whereas had negatively significant correlation with pod breadth. Pod length also negatively correlated with pod breadth. The study of path analysis indicated that the direct selection of number of leaves per rachis, number of branches per plant, number of pods per plant, number of flower per inflorescence, stem girth, leaf length and plant height could be used as selection criteria for improvement. Association analysis revealed that selection criteria based on number of branches per plant, length of pod, leaf length, pod girth and number of pod per plant, pod weight and plant height can provide better result for improvement of pod yield in *Moringa* (Asati *et al.*, 2021).

Widest range of variation was observed in pod length, number of pods per plant, single pod weight and pod yield per plant in *Moringa oleifera* Lam. Maximum genotypic and phenotypic coefficient of variation was observed for number of primary branches, pod length, number of pods per cluster, single pod weight and pod yield per plant. Higher value of heritability associated with high genetic advance as percent mean was observed for pod yield per plant, single pod weight, pod length, plant height, leaf length, pod girth, number of pod per cluster and number of branches per inflorescence (Nair *et al.*, 2021).

Principal component analysis of qualitative morphological descriptors (shape, colour, presence of wings and wing colour) and quantitative (length, width, and weight) was carried out in *Moringa oleifera* Lam. The principal component analysis indicated that components 1 (70.58 %) and 2 (25.59 %) contributed 96.17 per cent of the variation, and the cluster analysis identified four groups. The evaluated populations showed qualitative variation (shape, colour, presence of wings, and colour of wings) and quantitative (length, width, and weight) (Raphael *et al.*, 2021).

Higher genotypic and phenotypic coefficients of variation were discovered for trends in crown spread, diameter at breast height (DBH), and pod weight, number of leaves, Shoot Vigour Index (SVI), shoot length, plant height, ash content and 100 seed weight in *Moringa*

oleifera Lam. Excessive heritability as a percentage of mean was observed for crown spread, 100 seed weight, crude fiber, shoot length, plant height, and diameter at breast height (DBH), number of leaves, fat content, and crude protein (Redhu, 2021).

Highest broad-sense heritability values were observed in germination traits, followed by seedling collar diameter in *Parkia timoriana* D.C. (Thangjam *et al.*, 2020).

Analysis of variance in *Moringa oleifera* Lam. revealed a significant differences for pod yield, number of pods per tree, total carotenoids, iron and pod weight indicating maximum variability among the different genotypes. High estimated of heritability coupled with high genetic advance obtained for all the traits except crude fiber content. Presence of high heritability coupled with moderate genetic advance for crude fiber revealed that the straight selection has a limited scope for further improvement of this trait (Balaguru *et al.*, 2020).

A study conducted by Islam *et al.*, (2020) on two cultivars of *Moringa oleifera* Lam. in Bangladesh revealed that nutrients including calcium and iron were significantly higher in leaves (2314-3487 ppm and 276-418 ppm, respectively) than pods (2017-2032 ppm and 61-68 ppm, respectively). There were no significant differences in potassium content of both cultivars in both location, but pod contained more than four times higher phosphorus (1.5- 1.7 %) than leaves (0.34-0.38 %). The highest amounts of magnesium (1768-1861 ppm) and sodium (496-535 ppm) were found in pods. Hence both cultivar and plant part (e.g. leaves) of *Moringa oleifera* Lam. can be considered for nutritional security.

Twenty-five drumstick accessions of *Moringa oleifera* Lam. were evaluated for morphological, qualitative and quantitative characters. Considerable variation was observed among the accessions. Tree shape varied from upright to spreading. Accession VKMo 3 recorded greatest tree height and trunk girth. Principal component analysis for the quantitative characters revealed that first three principal components accounted for 88.10 per cent of the total variation which was contributed by number of seeds per fruit, fruit girth, trunk girth and tree height. Clustering of the accessions resulted in formation of five clusters. (Kurian *et al.*, 2020).

The genotypic and phenotypic correlation and path analysis was studied in forty *Grewia optiva* families for morphometric and fodder quality parameters. In general, the magnitude of genotypic correlations was higher than phenotypic correlations. The genotypic correlation coefficients among different characters revealed that total fresh leaf biomass had

significant positive association with plant height (0.263), estimated number of leaves (0.937), leaf area (0.783), fresh weight of 100 leaves (0.896), dry weight of 100 leaves (0.819), fodder yield (0.903) and ether extract (0.3501). The highest positive direct effect on total fresh leaf biomass was imposed by dry weight of leaves (0.627) followed by estimated number of leaves (0.537), leaf dry matter content (0.195), nitrogen free extract (0.095), crude fibre (0.075), leaf area (0.063), fodder yield (0.049), plant height (0.044), fresh weight of 100 leaves (0.037), crude protein (0.035), and total ash (0.027). Diameter had negative direct effect on total fresh leaf biomass (-0.029). On the basis of this study, a higher leaf biomass would be achieved through direct selection based on fresh weight of leaves, leaf area, plant height and diameter. On the basis of fodder quality traits, a higher leaf biomass would be achieved through direct selection based on ether extract, crude protein. Therefore, the current study is important in selection of traits of economic importance based on other characters, whose direct effect is not visible (Bhagta *et al.*, 2019).

Hierarchical Cluster Analysis (HCA) resulted in three significant clusters of *Anacardium occidentale* mother trees. Discriminant stepwise analysis carried out on all 31 morphometric descriptors showed that 15 were found to be the most discriminating (5 quantitative and 10 qualitative descriptors) of the morphometric groups (N'Djolosse *et al.*, 2019).

Twenty superior trees progenies of *Melia dubia* were grouped into eight clusters on the basis of morphological and biomass traits. Cluster V exhibited the highest number of five superior tree progenies. Cluster V exhibited maximum intra cluster distance (4.79) indicating that progenies in this cluster were more diverse than the other clusters. The maximum inter cluster D2 value was obtained between cluster VI and VII (9.21). Seedling height contributed the maximum towards divergence. The study also showed that the phenotypic coefficient of variation (PCV) was higher than the corresponding genotypic coefficient of variation (GCV) for all the morphological and biomass traits which revealed that the traits were sensitive to environmental fluctuations. (Yadav *et al.*, 2019).

Chan *et al.*, (2018) identified three distinct clusters of 24 Moringa landraces from five different areas. Cluster analysis and principal component analysis showed grouping patterns that were identical. When 23 morphological characters were studied using Ward's method, there was around 92 per cent dissimilarity among these Moringa landraces. This finding showed that this species had high level of morphological variation.

In *Toona ciliata* M. Roem., the correlation matrix revealed that highly significant and positive correlation exists between seed weight and germination percentage, and survival percentage, seedling height and collar diameter, seedling height and dry shoot weight, therefore improvement in one trait is bound to make positive response in the correlated trait. The higher estimates of genotypic coefficient of variability, phenotypic coefficient of variability and heritability (broad sense) were found for seed traits suggesting additive gene control in the inheritance of these traits (Deepanjli, 2018).

Analysis of variance indicated that there is an existence of significant variability among the twenty accessions of moringa for all the characters under study. The highest GCV was recorded for pod weight, number of leaves per rachis, yield per plant. Heritability estimates were high for pod weight, yield per plant, number of pods per plant, length of pod, and number of leaves per rachis. The expected genetic advance as a percentage of mean was high for pod weight, number of leaves per rachis, yield per plant, length of pod, number of seed per pod. Yield per plant had significant and positive association with stem girth fruit setting percentage length of pod, pod girth, pod weight and number of pods per plant indicating their usefulness in selection for yield. Non-significant and negative association with plant height, leaf length, number of leaves per rachis, length of leaf rachis, number of flower per inflorescence and number of seeds per pod. Among the twelve characters studied, six characters, viz., plant height, leaf length, number of leaves per rachis, number of flowers per inflorescence, pod weight and number of seeds per pod showed positive direct effect on yield per plant. Negative direct effect on yield per plant was recorded by stem girth, length of leaf rachis, fruit setting percentage, length of pod, pod girth and number of pods per plant. (Karunakar *et al.*, 2018).

A strong positive and highly significant phenotypic correlation with collar diameter, bark content, fresh shoot weight, dry shoot weight, fresh root weight, dry root weight and total biomass of seedling were exhibited by progenies of thirty candidate plus trees (CPTs) of *T. arjuna* from 30 different provinces of Uttar Pradesh (Kumar *et al.*, 2017).

Dao *et al.*, (2017) studied an analysis of variance, a principal component analysis on seed trait variables (length, thickness and weight) and germination rates at 5 and 12 days after seed sowing of *Moringa oleifera* Lam. The results showed significant variations. After five days the Sahelian provenance (Ouahigouya) recorded the greatest and fastest germination rate of 63 per cent and they recorded variability for germination and seed traits among West African provenances (Burkina Faso).

The accessions of *Moringa oleifera* Lam. showed marked differences in floral, fruit pod and seed characters analysed. The first five principal component axes explained 61.40 per cent of the total variation with PC1 (23.92 %) and PC2 (14.19 %) contributing 38.11 per cent of the total variation. The CA showed that the degree of intraspecific similarity was high (66.82 %) based on Euclidean similarity index. Nevertheless, four clusters were formed indicating intraspecific phenotypic dissimilarity among the 40 accessions especially with the separation of accessions that were collected from similar environments. The phenotypic variations could be explored for utilization, conservation and for future genetic improvement by selection of accessions with promising agronomic characters (Popoola *et al.*, 2016).

PCA carried out in 30 *Ulmus villosa* progenies, proved useful in extracting the most important factors. Three principal components contributed 84.62 per cent of total variance. First principal component contributed 40.97 per cent of total variation whereas the second component accounted for 24.93 per cent variation and the third explained 18.72 per cent of variation. In the first PCA high loadings for traits such as shoot fresh weight (0.853), shoot dry weight (0.843), collar diameter (0.764) and plant height (0.736) represented those which are important for selection and further improvement of the species. The study revealed that maximum weightage should be given to fresh shoot weight due to its maximum variable loading for the initial selection of progenies for the biomass improvement of the species (Thakur and Thakur, 2016).

All the pod and seed traits showed high heritability and progeny growth traits of *Pongamia pinnata* had moderate heritability. The 100-pod weight exhibited highest heritability (more than 98.4 %) followed by 100-seed weight (96.9 %). The 100-pod weight and 100-seed weight expressed high heritability (98.4 %, 96.9 %), accompanied with high genetic advance (46.0 %, 34.9 %). Volume index expressed moderate heritability (47.4 %), accompanied with high genetic advance (48.4 %). This substantial variation can be utilized for genetic resource conservation and further genetic tree improvement programmers of the species (Gawali *et al.*, 2015).

In *Moringa*, the GCV was greater than PCV for all the characters but the differences were low. High GCV and heritability estimates coupled with greater genetic advance was recorded for pod weight and indicated that these characters had additive gene effects and therefore, they are more reliable for effective selection (Tak and Maurya, 2015).

Numerical analysis of the qualitative and quantitative seed and leaf characters of *Moringa oleifera* Lam. grouped the accessions into five clusters. Principal Component Analysis was carried out to augment the cluster analysis which showed large morphological diversity existing in accessions of *Moringa oleifera* Lam. Hence, infraspecific classification is hereby proposed. These analysis particularly traits related to leaf and fruits yield can also be utilised for crop improvement programme (Zhigila *et al.*, 2015).

High heritability and genetic advance for 100-pod weight, 100-seed weight, and seed germination was observed in *Pongamia pinnata*. Positive significant genotypic correlation was recorded between seed thickness and oil content (0.211). Germination percentage showed significant positive correlation with seed weight and oil content. Path analysis revealed that seed thickness contributed directly to oil content (0.272). Based on D² analysis, pongamia accessions were grouped into seven clusters. Maximum diverse Clusters I and VII were identified based on inter-cluster distance (40.19). Thus on the basis of the present findings, it might be suggested that accessions from these clusters could be most suitably used for hybridization and in situ germplasm conservation (Pavithra *et al.*, 2013).

Genetic variability in thirty four genotypes of *Moringa oleifera* Lam. for eight different parameters revealed phenotypic coefficient of variation (PCV) was higher than genotypic coefficient of variation (GCV) for all the eight characters. A positive correlation of length of fruit with fruit girth, fruit weight, number of seeds/fruit and number of pods/tree was found. (Selvakumari, 2013).

Pod length, number of seeds per pod, 100-seed weight and percent germination after harvesting, appearance (straight or curled), colour of fresh pod (green or pale green), colour of dried pod (golden brown or brownish), colour of stem bark (grey or whitish), colour of flowers (white or creamy, with or without purple patches) and branching habit (horizontal branching or vertical branching) divided *Moringa oleifera* Lam. assessions into two distinct clusters (Amoatey *et al.*, 2012).

Kumar (2012) selected seventeen seed sources of *Pongamia pinnata* from natural populations on the basis of morphometric characters to identify suitable seed sources for incorporation in mass afforestation programs. The significant genetic variability and association was observed among seed sources for pod, seed and seedling traits. Path analysis revealed that number of branches; seed thickness, internodal distance and pod breath had the

highest direct effect on 100-seed weight and can be collectively used as selection criteria for improvement of this tree species.

Based on the relative magnitude of D^2 values, twenty superior trees progenies of *Albizia lebbeck* were grouped into six clusters on the basis of morphological and biomass traits. Cluster II exhibited the highest number of six superior tree progenies. Cluster II exhibited maximum intra cluster distance (6.60) indicating that progenies in this cluster were more diverse than the other clusters. The maximum inter cluster D^2 value was obtained between cluster II and V (61.35). The clustering pattern in this study revealed that superior tree progenies from different geographic region were grouped together in a cluster and vice-versa suggesting that geographical diversity did not necessarily represent the genetic diversity. The contribution of different traits towards diversity revealed that seedling height contributed the maximum towards divergence. Phenotypic coefficient of variation (PCV) was higher than the corresponding genotypic coefficient of variation (GCV) for all the morphological and biomass traits which revealed that the traits were sensitive to environmental fluctuations (Wani, 2012).

The phenotypic and genotypic coefficients of variations of *Pongamia pinnata* were also close to each other for all traits, but 100-pod weight and 100-seed weight exhibited higher phenotypic coefficients of variation and genotypic coefficients of variation than the other traits. Estimates of broad sense heritability ranged from 0.82 (for seed length) to 0.98 (for 100-pod weight), genetic advance as percent of the mean ranged between 12.30 per cent and 46.04 per cent with seed length giving the lowest value and 100-pod weight giving the highest value. Germination capacity exhibited positive significant correlation with pod width, 100-pod weight and seed width at both genotypic and phenotypic level. However, pod length, pod thickness and 100-seed weight expressed positive significant correlation only at genotypic level (Divakara *et al.*, 2010).

Heritability and genetic advance were also found moderate to high for 100 seed weight, oil and protein contents, collar diameter, shoot length and root length of *Pongamia pinnata*, representing the effectiveness of these characters for selection and for developing productive trees (Hooda *et al.*, 2009).

Significant correlation was also observed in *Jatropha curcas* among seed weight, germination indices, plant height and collar diameter. The coefficients of variation,

heritability and genetic advance were found comparatively higher for germination than growth traits (Dhillon *et al.*, 2008).

In general, phenotypic coefficient of variation was higher than genotypic coefficient of variation indicating the predominant role of environment. High heritability (broad sense) and genetic gain observed for pod–seed ratio (99.00 %, 87.78 %), 100-seed weight (100.00 %, 66.99 %) and 100-pod weight (98.00 %, 57.38 %), respectively indicate additive gene action. Seed weight and pod weight showed positive and significant correlation with oil content. CPTs 18, 20, 33, 13 and 29 of *Pongamia pinnata* were found to be the best on the basis of oil content and pod–seed characters (Kaushik *et al.*, 2007).

In *Cordia africana*, heritability of 71-98 per cent for seed morphometric traits, 80 per cent for germination capacity, 42 per cent for germination energy, 57-58 per cent for seedling height and 3-13 per cent for root collar diameter was observed. Seed weight showed a significant positive correlation with altitude and negative correlation with mean annual temperature of seed origin. Germination energy was significantly correlated with longitude and mean annual rainfall. Seedling parameters and geo-climatic variables of seed origin were fairly correlated. A significant intercharacter correlation was found between seed length and seed weight, between root collar diameter at the age of 4 months and seed length and weight, as well as between seedling height after 4 and 8 months of growth (Loha *et al.*, 2006).

2.5 Genotyping/Assessment of genetic diversity

By using a modified biotin-streptavidin capturing method, a total of 20 polymorphic microsatellite markers were developed from *Moringa oleifera* Lam. Twenty-four domesticated individuals, with germplasms of India and Myanmar, were used to screen polymorphism of these 20 microsatellite markers. The number of alleles per locus ranged from two to six. The expected and observed heterozygosity varied from 0.3608 to 0.7606 and from 0.0000 to 0.8750, respectively (Wu *et al.*, 2010).

Ravi *et al.*, (2022) used 27 RAPD markers to resolve diversity among 23 superior *Moringa* genotypes mostly collected from Karnataka, Tamil Nadu, and Kerala, states of India. A high level of genetic diversity was observed within the genotypes. Markers came out to be highly polymorphic. Among the 519 bands obtained, 471 (89.61 %) were polymorphic. The discriminatory power of these markers was determined using various parameters like; polymorphism information content (PIC), marker index (MI), and resolving power (Rp). Primer RAPD-3 showed the highest PIC value (0.35), MI (10.28), and Rp (21.65). The

dendrogram generated with UPGMA cluster analysis grouped genotypes into two main clusters with various degrees of sub clustering within the cluster.

Genetic diversity of 30 genotypes from 10 populations of *Moringa* plants originating from several islands in Indonesia was evaluated using 10 SRAP molecular markers and morphological characters. 70 polymorphic bands from 86 total bands (81.40 %) were obtained and Polymorphic Information Content (PIC) values were 0.36 on average. The UPGMA analysis divided 10 accessions into 2 main groups which Java accession was grouped alone and separated from other accessions. The principle component analysis based on morphological characters divided them into 4 groups with Java accession consistently separated from others. Significant intra and inter population variation was observed among population of molecular and morphological analysis. Accession of Java was the most distinct Indonesian accession suggesting the narrow distribution of this accession than others. Leaflets number, tuberous root number and colour were among the most variables that influenced the distinction of *Moringa* accession from Indonesia (Ridwan *et al.*, 2021).

Mature and fresh leaf samples of 57 accessions were collected from 57 different geographical locations within India, including the states of Rajasthan, Haryana, Uttarakhand, Madhya Pradesh, Uttar Pradesh and Goa. Genomic DNA polymorphism and variation in biologically active components of *Moringa oleifera* Lam. were investigated by two different techniques: RAPD-PCR and HPLC analysis. Among 20 RAPD primers, 13 were selected to generate polymorphic amplicons producing an average of 5028 bands, of which 83.7 per cent were found to be polymorphic among 57 accessions of *Moringa oleifera* Lam. and one outgroup (ACB 58) from Banasthali region, India. In total, 57 accessions were clustered into five major groups within the dendrogram (Panwar and Mathur, 2020).

Ravi *et al.*, (2020) concluded that variety of *Moringa oleifera* Lam. was previously accessed using AFLP, RAPD, ISSR, and SSR markers. Using CytP450-based markers, the researchers evaluated 23 accessions grown in various agro-climatic zones of south India. The study showed a high degree of genetic diversity among the accessions, resulting in the identification of genetically distinct germplasm that can be used in crop breeding programs.

Moringa species significantly differed in their nutritional content. *Moringa peregrina* had more chlorophyll content, however *Moringa oleifera* Lam. showed higher protein, Fe, and Zn contents. Significant differences were also found among the genotypes of each species. Chlorophyll, protein, Fe, and Zn were the most pertinent indicators of diversity

between the two species and among the genotypes of each species. Cluster analysis based on nutritional characterisation showed two main clusters for the two species. It also separated three *Moringa oleifera* Lam. genotypes and two *Moringa peregrina* genotypes in independent groups for their variability in nutritional properties. All *Moringa oleifera* Lam. genotypes amplified the nine studied SSR primers except one which amplified 8/9 primers. However, the amplified primers in *Moringa peregrina* genotypes varied from zero to six out of nine per genotype. Cluster analysis based on molecular markers gave the same classification obtained from nutritional characterisation which proves a close relationship between genetic diversity and nutritional prosperities. (Hassanein and Al-Soqeer 2018).

Thirty one accessions of *Moringa oleifera* Lam. from Nigeria were tested for the magnitude of genetic diversity which resulted with a range 0.12 to 0.78 with an average of 0.52, PIC content of the SSR primers ranged from 0.11 to 0.75 with an average of 0.48. The cluster analysis was able to group the thirty one accessions into two main clusters with four sub clusters which exhibited a moderate level of association between genetic divergence and geographical origin of accessions (Amao *et al.*, 2017).

Jacob *et al.*, (2017) examined 70 accessions of *Moringa oleifera* Lam. from six populations from different eco-geographical locations within Nigeria. A total number of 74 alleles with a range of 4 to 15 were detected among the accessions. On the average, 7.4 alleles per locus were amplified in each accession. Allele frequency varied from 0.214 to 0.671 with a mean of 0.477; gene diversity from 0.487 to 0.885 with a mean of 0.669 while the average PIC value was 0.633. Similarity coefficients (SC) of CA ranged from 0.53 to 1.00 and delineated the 70 accessions into seven groups. All accessions were distinguishable from each other at SC of 1.00 except accessions with code (soN066 and taN085) and (anN045 and anN047). The genetic relationships highlighted are significant for conservation, cultivation and genetic improvement of *Moringa oleifera* Lam. in view of the species socio-economic relevance to the people of Nigeria and Africa.

Similarly, a total of 97 accessions of *Moringa oleifera* Lam. collected from different districts of Tamil Nadu, Andhra Pradesh and Odisha were genotyped using 20 simple sequence repeat (SSR) markers to assess the genetic diversity and population structure of *Moringa oleifera* Lam. A total of 140 alleles were detected with the polymorphic information content value of 0.6832 and gene diversity of 0.7292. Population structure analysis through a model-based approach divided the accessions into two subgroups. Molecular variance analysis using principal coordinate analysis (PCoA) summarized a 18.32 per cent variation.

High diversity in the analysed genotypes was found which could be utilized for further exploration based on their nutritional content and for conservation of nutritionally superior germplasm (Rajalakshmi *et al.*, 2017).

Ganesan *et al.*, (2014) reported that analysis of morphological (qualitative and quantitative) and SSR markers indicated that Indian germplasm of *Moringa oleifera* Lam. has a lot of diversity. Based on SSR data, no clear geographical isolation was found in the samples, and genotypes from different populations were placed together in clusters. The study from northern India revealed a particular grouping pattern based on qualitative data, but quantitative and SSR data do not confirm this conclusion. The population structure-based grouping showed that genotypes from north India were only distributed in two of the five clusters produced.

Popoola *et al.*, (2014) evaluated the intra-specific genetic variabilities available among accessions of *Moringa oleifera* Lam. collected from the six eco-geographical areas of Nigeria using 12 fruit morphometric characters and five arbitrary RAPD markers. The results revealed high genetic variability among the accessions. A total number of 224 bands were obtained with 77.86 per cent polymorphism. Cluster analysis of pod and seed characters revealed three distinct groups while dendrogram based on the RAPD data clustered the accessions into four distinct groups with one splinter subgroup. Some accessions exhibited good agronomic features such as long pods, high number of seeds per pod and high seed set percentage. Such accessions could serve as parent plants for breeding for genetic improvement, utilization and conservation.

Genetic diversity assessment of twenty *Moringa oleifera* Lam. using RAPD markers revealed the presence of high genetic divergence with value of 1.80 and 0.13 for Malaysian population and 0.30 and 0.19 for the international population, respectively. Mean of Nei's gene diversity index for the two populations was estimated to be 0.20. In addition, a dendrogram constructed, using UPGMA cluster analysis based on Nei's genetic distance, grouped genotypes into five distinct clusters (Rufai *et al.*, 2013).

Three DNA marker techniques, i.e., RAPD, ISSR and cytochrome P450 gene-based markers were used for the detection of genetic variability in eight Indian cultivars of *Moringa oleifera* Lam. collected from various states of India. A total of 17 RAPD, 6 ISSR and 7 pairs of cytochrome P450-based markers generated 48.68 per cent, 48.57 per cent and 40.00 per cent polymorphisms, respectively. Based on the three types of marker data, the eight cultivars

of *Moringa oleifera* Lam. were grouped into four sub-clusters in a dendrogram, but without any distinct geographical pattern. High bootstrap values (94.4 and 82.3) were obtained at major nodes of the dendrogram using the winboot software. The dendrogram and PCA plots generated from the binary data matrices of the three marker systems were found highly concordant to each other. (Saini *et al.*, 2013).

The grouping of genotypes of *Moringa oleifera* Lam. based on cluster analysis was not similar between morphological data and marker data. The genotypes, viz., MO 1, MO 3 and MO 4 and MO 10, MO 12, MO 20, MO 21 and MO 22 were grouped under I and II cluster respectively in both morphological and SSR analysis. However, some discrepancies between the two dendrograms can be found. In morphological analysis, MO 15 and MO 34 formed a separate cluster were grouped in cluster IV, while in the SSR analysis the genotypes MO 15 and MO 34 grouped under different sub clusters (Selvakumari, 2013).

Shahzad *et al.*, (2013) investigated the genetic diversity and population structure of world-wide collections of 161 accessions of *Moringa oleifera* Lam. collected from Asia, Africa, North and South America, and the Caribbean using 19 SSR markers along with a partial sequence of the chloroplast gene *atpB*. On average, 8.3 alleles/per SSR were amplified in each accession. A total number of 158 alleles were detected in 131 accessions collected from the wild in Pakistan and from 30 accessions obtained from ECHO (Florida). Observed heterozygosity varied from 0.16 to 0.86, with an average of 0.58, while the average PIC value was 0.59. Partial sequencing of chloroplast genes of 43 of 161 plants generated mixed patterns. These findings have demonstrated that there is a large genetic diversity present in wild collections of *Moringa oleifera* Lam. collected in Pakistan; whereas low genetic diversity is detected in cultivated accessions obtained from ECHO.

The genetic diversity of sixteen accessions of *Moringa oleifera* Lam. were evaluated using RAPD markers. The Shannon index and the index of genetic diversity (H) were 0.33 and 0.22, respectively. The accessions MO1 and MO12 were most similar (0.27), and MO13 and MO16 are the most divergent (0.69). By UPGMA and PCoA groupings, assessments MO1, MO2, MO12 and MO13 were identified as most genetically isolated assessments (Da Silva *et al.*, 2012).

Chapter-3

MATERIALS AND METHODS

The present investigation entitled “Genetic diversity in wild populations of *Moringa oleifera* Lam. (Drumstick tree) in north-west India” was carried out to study the magnitude of morphological, nutritional and genetic variation among different genotypes of *Moringa oleifera* Lam. in the Department of Tree Improvement and Genetic Resources, Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Solan (HP) during 2020-2023. The experimental details relating to experimental material, site and methodology adopted for study are described under the following headings:

3.1 EXPERIMENTAL MATERIAL

3.2 EXPERIMENTAL SITE

3.3 EXPERIMENTAL PROCEDURES

3.4 STATISTICAL ANALYSIS

3.1 EXPERIMENTAL MATERIAL

A pilot survey of population of *Moringa oleifera* Lam. was undertaken from November 2020 to February 2021 in the sub-Himalayan regions of Jammu, Himachal Pradesh, Punjab, Haryana and Uttarakhand to identify the sites where this species occurs in the wild stand. The sampling procedure included delineation of the whole area, under the species into a number of sites depending upon the altitudes, aspect and variation in morphological characters. In this way, seven provenances were selected (Figure 1.) in five states of North-West India. Natural occurring trees of *Moringa oleifera* Lam. were selected on the basis of field observation and interaction with local people on the basis of following characters, viz., tree height (9-12 m), tree diameter at breast height (25-35 cm) and crown spread (50-100 m²). These trees were marked within each site for further selection within as per the methods adopted by Savnin (1967), Pozdnjakov (1969) and Dumitriu-Tataranu (1970).

The seed sources selected for study were confined to Samba (Jammu), Dunera (Punjab), Siswa Dam (Punjab), Lamlehri (Himachal Pradesh), Tirla (Himachal Pradesh), Morni Hills (Haryana) and Vikasnagar (Uttarakhand). The latitude, longitude and altitude

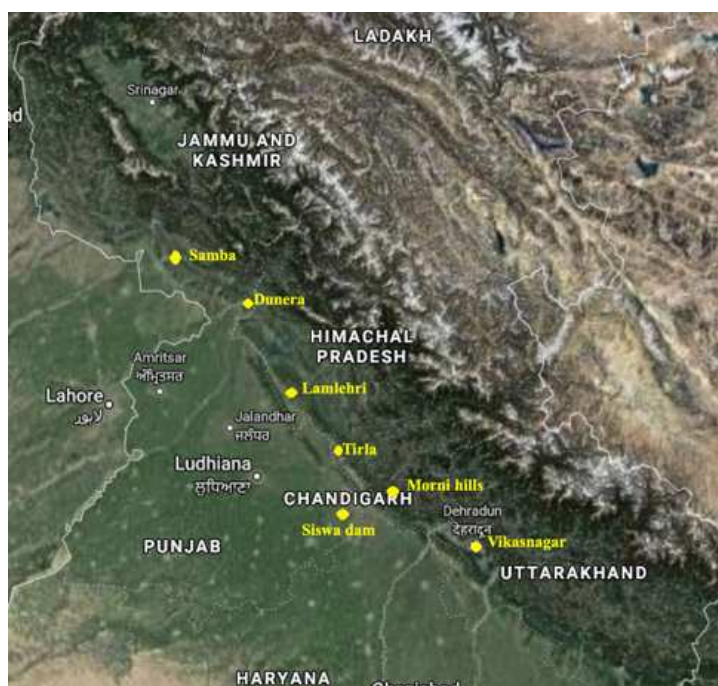
from mean sea level is provided in Table 1. Ten trees per seed source were selected following the selection criteria mentioned above. From these ten trees, five trees having maximum total leaf biomass (kg) were selected per seed source for further studies.

These seven seed sources fall under sub-tropical climate with May-June being the hottest and December-January as the coldest months, experiencing maximum rainfall during July-August months.

Table 1: Selected seed sources of *Moringa oleifera* Lam.

S. No.	Location	District and State/UT	Latitude (° N)	Longitude (° E)	Altitude (above msl)
1	Samba	Samba, Jammu and Kashmir	32.5603	75.1193	423
2	Dunera	Pathankot, Punjab	32.3593	75.7629	463
3	Vikasnagar	Dehradun, Uttarakhand	30.3463	77.9148	549
4	Lamlehri	Una, Himachal Pradesh	31.5232	76.3232	526
5	Tirla	Solan, Himachal Pradesh	30.9239	76.9219	403
6	Siswa Dam	Sahibzada Ajit Singh Nagar, Punjab	30.8671	76.7487	351
7	Morni Hills	Panchkula, Haryana	30.6556	77.0831	516

Figure: 1. Map showing the selected seed sources of *Moringa oleifera* Lam.



3.2 EXPERIMENTAL SITE

This experiment was laid out at Regional Horticulture Research and Training Station, Jachh, Kangra under Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan (HP) 173 230. The station is located at 31.37 N latitude, 77.31 E longitude and 428 m above mean sea level. The experimental location accomplished mean annual rainfall of 1500 mm and mean annual temperature ranged from 29.3 °C in summer and 13.6 °C in winter. Five best trees per location out of ten mother trees on the basis of total leaf biomass (kg) were selected for pod collection in the month of May, 2021. Twenty plants per replication were grown for each mother tree in polybags of size 5x7 inches. Three replication for each mother tree were taken. Randomised Block design was deployed for conducting this experiment in June, 2021.

3.3 EXPERIMENTAL PROCEDURES

3.3.1 Mother tree (genotype) qualitative and quantitative characters

3.3.1.1 Qualitative characters

- (a) **Tree growth habit:** Tree growth habit was observed to be drooping, upright or spreading.
- (b) **Bark colour:** Bark colour was observed to be light grey, grey or dark grey.
- (c) **Anthocyanin coloration of axis and branches of petiole:** The presence or absence of purple colouration of axis and branches of petiole was observed.
- (d) **Leaf stalk surface:** Leaf stalk surface was observed to be either glabrous or hairy.
- (e) **Leaf colour:** Leaf was observed to be green or dark green in colour.
- (f) **Flower bud colour:** Colour of flower bud was observed to be white, pink or dark pink.
- (g) **Seed colour:** The colour of the seed was observed to be either white or cream.
- (h) **Seed wing form:** Wings of the seeds were observed to be either prominent or less prominent.

3.3.1.2 Quantitative characters

- (a) **Tree height (m):** The height of standing mother trees was recorded with the help of Abney's level.
- (b) **Diameter at breast height (DBH) (cm):** The DBH of the individual trees was measured with the help of vernier calliper. All the criteria governing the diameter measurement were followed as describes by Chaturvedi and Khanna (1994).

(c) Crown spread (m²): The points connecting widest and narrowest crown widths were averaged to find average crown spread of the tree.

(d) Total fresh leaf biomass (kg): Total 100 fresh leaves from the mother trees were collected and weighed immediately to minimize the weight loss. Average number of leaves from number of branches was counted and then approximate value of total number of leaves was calculated, which then was multiplied by the weight of leaf in order to get the leaf biomass.

3.3.2 Pod, seed and germination parameters

(a) Pod length (cm): Length of ten pods per mother tree was measured using measuring scale.

(b) Pod breadth (cm): Breadth of above mentioned ten pods per mother tree was measured using measuring scale.

(c) Seeds per pod: Number of seeds in above mentioned pods were counted and averaged to get seeds per pod.

(d) 100 seed weight with wings (g): Weight of random 100 seeds extracted from above mentioned pods was measured on electronic top balance.

(e) 100 seed weight without wings (g): The wings of the above mentioned 100 seeds were removed by a pair of scissors and weighed on electronic top balance.

(f) 100 seed kernel weight (g): The kernels were removed from the above mentioned 100 seeds and weighed on electronic top balance.

(g) Seed length (cm): Randomly 10 seeds from above mentioned 100 seeds were chosen, their seed length was measured using vernier calliper.

(h) Seed breadth (cm): Seed breadth of the above mentioned seeds was measured using vernier calliper.

(i) Seed kernel length (cm): Seed kernel length of the above mentioned seeds was measured using vernier calliper.

(j) Seed kernel breadth (cm): Seed kernel breadth of the above mentioned seeds was measured using vernier calliper.

(k) Germination percentage (%): The number of seeds germinated to the total number of seeds sown was counted.

(l) Survival percentage (%): Number of plants survived after one month of germination was recorded.

(m) Germination index: It was calculated by the following formula: Germination index (GI) = $\sum(G/T)$ where G is the percent of seed germinated per day and T is the germination period.

(n) Germination value: It was calculated by the following formula: Germination value (GV) = Mean daily germination (MDG) x Peak value at mean daily germination (PV).

3.3.3 Growth studies:

3.3.3.1 Morphological growth: The data on morphological growth for below mentioned characters was measured at an interval of 2 months.

(a) Seedling height (cm): The height of the plant was recorded from the collar level to the apex of the leading shoot by using measuring scale.

(b) Collar diameter (mm): Collar diameter of the plant in the nursery was measured above the ground level at collar region with the help of digital calliper.

(c) Internodal length (cm): The internodal length was measured as distance between two nodes, at mid of the plant crown.

(d) Petiole length (cm): The petiole length of 3rd or 4th compound leaf from the bottom end was measured with the help of measuring scale.

(e) Leaf area (cm²): The full grown 3rd or 4th compound leaf from the bottom end was collected from the seedling and leaf area was measured with the help of leaf area meter.

(f) Number of branches: The average number of branches per plant was counted.

(g) Number of leaves/plant: The average number of leaves per plant was counted.

(h) Leaf angle (°): Leaf angle was measured by simple protector from the middle 3rd or 4th compound leaf from the bottom end of the plant and then taking aggregate of them.

3.3.3.2 Biomass studies: The shoot and root portion of the randomly selected five plants of each genotype was destructively sampled and below mentioned characters were measured after one year nursery growth:

(a) Fresh shoot weight (g): The shoot fresh weight for each plant was recorded by using electronic top pan balance.

(b) Fresh root weight (g): The root fresh weight was recorded using electronic top pan balance as that of shoot fresh weight.

(c) Dry shoot weight (g): The fresh shoots after weighing was packed in paper bags and dried at 80 °C in an oven till constant weight and was recorded using electronic top pan balance.

(d) Dry root weight (g): The root dry weight was recorded similar to that of dry shoot weight.

(e) Fresh Root Shoot Ratio: This was calculated by dividing fresh root weight with fresh shoot weight.

(f) Dry Root Shoot Ratio: This was calculated by dividing dry root weight with dry shoot weight.

3.3.4 ASSESSMENT OF NUTRITIONAL VALUES

3.3.4.1 Proximate Principles: Composite samples of five leaves from three branches over different directions of each mother tree and their respective leaf samples of corresponding nursery seedlings during were collected in July, 2022. Here samples collected from each direction were regarded as a replication. These samples were washed with water and treated with 0.1N HCl followed by distilled washing again with distilled water. These samples were oven-dried at 80 °C until they attained constant weight and all moisture was evaporated. The dried samples were grinded and stored with proper labels and analysis for following nutritional parameters was carried out.

(a) Leaf Dry Matter Content (%): The difference in leaf weight due to loss of moisture content before and after drying was denoted as leaf dry matter content.

Dry matter content (%) = (weight of dried leaf samples/weight of fresh leaf samples) x 100

(b) Crude Protein (%): Crude protein was estimated by Microkjeldhal method (Sankaram, 1966). For crude protein estimation, nitrogen content of leaves was estimated and multiplied by standard factor of 6.25.

(c) Ether Extract (%): Two grams of dries leaf samples were extracted with petroleum ether (B.P. 80 °C) in a soxhlet apparatus. Solvent were allowed to

evaporate to constant dryness and the weight of the abstract were expressed as percentage of the weight of the sample taken.

Ether extract (%) = (weight of the fat/original weight of the sample) x 100

(d) Crude Fiber (%): Crude fiber was estimated by acid alkali digestion (ashing) method described by AOAC (1995) and Sankaram (1966). 2 g ether free samples were treated with 200 ml of 1.25 per cent H_2SO_4 for 30 minutes and then filtered through muslin cloth. Samples were added with water to remove acidity and alkali, 200 ml of 1.25 per cent NaOH were added to it and same process was repeated as for acid treatment. The samples were dries overnight and muffled at 500°C for 3-4 hours and weight was recorded. The loss in weight before and after ashing was the weight of crude fiber.

Crude fiber (%) = (weight of crude fiber/weight of sample) X 100

(e) Total Ash (%): Ash content was analysed by taking a known amount of oven dried sample, which were ignited till smoke ended up and then to the muffle furnace at 600°C for 2 hours. The samples were cooled and weight as outlined by AOAC (1995) and Sankaram (1996).

Total ash (%) = (weight of ash/original weight of sample) x 100

(f) Nitrogen Free Extract (%): Nitrogen Free Extract will be determined by subtracting the sum of crude protein, crude fiber, ether extract and ash content from 100 as outlined by AOAC (1995) and Sankaram (1966).

3.3.4.2 Biochemical Estimates:

(a) Amino Acids: Total amino acids were estimated following Lee and Takahashi method (1966). 0.5 M citrate (Na^+) buffer was prepared from analytical- grade citric acid which was free of ammonia or amino acids. pH of the citrate buffer was adjusted to a desired value. A 1 per cent ninhydrin solution was prepared by dissolving 1 g of ninhydrin in 0.5 M citrate buffer solution. 1.0 ml of a reaction mixture which consisted of citrate buffer, ninhydrin, and glycerol was pipetted into a test tube. 1 ml of sample and additional compounds to be tested, if there was any, were introduced into the reaction mixture. The total volume was made up to 2.0 ml with water. After shaking, the test tube was placed in a boiling water bath for a certain time as described in each experiment. After cooling in a tap-water bath at room temperature,

a reading was taken at 570 nm within an hour by colorimeter. The reagent blank and standard amino acid solution were run at the same time to verify and standardize the assay.

3.3.4.3 Mineral Nutrients: Ten mineral nutrients, viz., N, P, K, Ca, Zn, S, Mg, Fe, Cu and Mn were estimated from the dried leaf samples of both mother trees as well as progenies. Nutritive values of the leaves were analysed by oven-drying the leaves at 65 °C until a constant weight was achieved. The leaves were digested with sulphuric acid (98%) at 373 °C in a kjeldahl flask for 1 hr for nitrogen content estimation and with di-acid mixture (conc. HNO₃: HClO₄ = 4:1) for other mineral nutrient content estimation.

5 g of leaf sample was taken in 1500 ml conical flask, 200 ml of di-acid mixture (conc. HNO₃: HClO₄ = 4:1) was added to it and kept for 10-12 hrs for cold digestion. Later, material in the conical flask was heated on the hot plate for two hours till the white fumes stopped and solution became clear. After completion of digestion, it was allowed to cool for some time. Few ml of distilled water was added to the solution, transferred to 1000 ml volumetric flask and the final volume was made to 1000 ml, stored in the plastic bottles for further estimation of mineral nutrients. Brief outline of methods used for the analysis of mineral content is as follows:

(a) Nitrogen (N): Nitrogen was estimated by microkjeldahl method (AOAC, 1995). 1 g of digested sample was taken in a clear digestion flask. To this, 25 ml of concentrated sulphuric acid (H₂SO₄) was added followed by 3 g digestion mixture (20 parts K₂SO₄ + 1 part CuSO₄) carefully from the side. The contents were digested on an electrical heater. The flask from the heater was removed intermittently and shaken carefully in a manner to bring down any stray particles of carbon and undigested material. Leaf digestion was continued 2-2.5 hours for until the light blue colour appeared. Flask contents after cooling were transferred into a 100 ml volumetric flask and the volume was made up to the mark with distilled water.

10 ml of digested material (aliquot) was transferred into microkjeldahl assembly. 20 ml of 40 per cent NaOH solution was added to the distillation assembly to make the contents alkaline and stopper was put immediately. 20 ml of N/100 H₂SO₄ was taken in a conical flask, 2-3 drops of methyl red indicator was added to it. This conical flask was set under the condenser and allowed for distillation for 10-15 minutes. The conical flask was removed after washing the tip of the condenser with distilled water. The flask contents were back titrated with alkali (N/100 NaOH) till the end point was achieved (pink to yellow) Saez-Plaza *et al.*,

(2013). The volume of alkali used in the titration was recorded to calculate the volume of standard H_2SO_4 (N/100) used for ammonia absorption and following calculations were done:

$$1 \text{ ml N/100 } \text{H}_2\text{SO}_4 = 0.00014 \text{ g Nitrogen}$$

$$\text{N (\%)} = (\text{V} \times 0.00014 \times \text{D} \times 100) \times \text{W} \times \text{A}$$

Where,

V	=	Volume of N/100 H_2SO_4 taken or volume of N/100 NaOH taken for titration
D	=	Dilution factor (volume made in volumetric flask)
W	=	Weight (g) of powdered sample taken
A	=	Aliquot taken

(b) Phosphorus (P): Phosphorus was estimated by yellow Vanadomolybdate method (Tatina, 2009). For estimation of phosphorus, five ml of leaf digest was taken into 50 ml volumetric flask and added 10 ml vanadomolybdate reagent (dissolved 22.5 g ammonium molybdate in 400 ml distilled water + dissolved 1.25 g ammonium vanadate in 300 ml boiling distilled water + 250 ml conc. HNO_3 and diluted to one litre) made up the volume with distilled water. shook up thoroughly and read the concentration of solution after 30 minutes in colorimeter. Before taking the reading for the concentration of the plant sample, the instrument was calibrated by the known concentration of standard solution (dissolved 0.2195 g KH_2PO_4 in distilled water and diluted to one litre). Percentage phosphorus was calculated by the following formula:

$$\text{P (\%)} = [(\text{C/wt. of sample}) \times (100/\text{Aliquot (ml) taken}) \times \text{Volume of digest (ml)}/1000]$$

(c) Potassium (K): Five gram of digested plant sample was measured into a wide silica basin and ashed at 45 °C for 11-12 h. The ash was taken up in 10 ml of 6 N hydrochloric acid and evaporated to dryness. To the residue was added 10 ml of 2 N hydrochloric acid, the basin was covered with a watch-glass and digested on the steam bath for 30 min. After the watchglass had rinsed the content of the basin, it was filtered through a Schleicher and Schull filter paper No. 589 into a 250 ml volumetric flask. From this solution, 50 ml was taken for the direct determination of potassium content. Standard solution for K was prepared by dissolving 1.9069 g KCl in distilled water and volume made upto one litre. The solution was further diluted to 100 ppm, 50 ppm, 10 ppm and 5 ppm concentrations, respectively. Thereafter, full scale of flame photometer was set with the solution of highest concentration (i.e., 100 ppm) using filter for potassium. Readings of other standard solution were taken and

standard curve was plotted (Harve, 1961). The readings of digested samples (aliquot) were taken and concentration of potassium was calculated by the following formula:

$$K (\%) = C (\text{ppm}) \times (\text{Vol. of digest/Wt. of sample}) \times (100/1000000)$$

(d) Calcium (Ca): Five grammes of dried plant sample were weighed into a wide silica basin and ashed at 45 °C for 11-12 h. The ash was taken up in 10 ml of 6 N hydrochloric acid and evaporated to dryness. To the residue was added 10 ml of 2 N hydrochloric acid, the basin was covered with a watch-glass and digested on the steam bath for 30 min. After the watchglass had rinsed the content of the basin, it was filtered through a Schleicher and Schull filter paper No. 589 into a 250 ml volumetric flask. The solution was heated to about 80 °C and a few drops of methyl red were added followed by 15 ml of saturated ammonium oxalate solution. Ammonia (15%) was added until the colour changes, and the precipitate was set aside overnight. This solution was filtered through a Schleicher and Schull filter paper No. 589 without washing, the filter was perforated by a glass rod, and the precipitate was washed down into the precipitating beaker. The filter paper was rinsed off with 15 ml of 3 N hydrochloric acid and finally with water. After dissolution of the precipitate, the solution was transferred to a 250 ml volumetric flask and then was determined under the flame photometer. 1000 ppm standard solution was made by dissolving 2.5 g CaCO_3 in one litre distilled water and further diluted to 20, 15, 10, 5 and 2 ppm concentration. The instrument was calibrated by these standard solutions of different concentrations (Harve, 1961). The readings (concentration) of sample solutions were taken from the instrument and per cent calcium was calculated by the following formula:

$$\text{Ca} (\%) = (\text{concentration} \times \text{dilution} \times 100)/1000000$$

(e) Zinc (Zn): In three tubes, 0.5 g of digested leaf sample was measured and 5 ml of the extraction solution: Nitric acid (HNO_3 ; 65 %), sulphuric acid (H_2SO_4 ; 96 %) and perchloric acid (HClO_4 70 %) were added in each tube. A Blanc solution was prepared with 5 ml of the extraction solution. The samples were let to rest during for 2 hrs. After this the samples were heated with temperatures varying between 75-240 °C. The mixture thus got after heating was cooled to the ambient temperature for 24 h and then was diluted to 2/3 of tubes, agitated, cooled again and completed to 75 ml with the distilled water. After agitation and decanting, a quantity of the solution was used to analyse Zn concentration in atomic absorption spectrophotometer at 248.3 nm (Antunes *et al.*, 2017).

(f) Sulphur (S): A 0.24-g/L iodine solution containing 0.2 per cent (v/v) phosphate acid was prepared as follows: 0.06 g of iodine was dissolved in a mixture of water and methanol (50/20, v/v) under ultrasonication and then 0.5 ml of phosphate acid was added. Finally, the mixture was diluted to 250 ml with water. Before use, the mixture was filtered with filter paper. 5 g powdered digested leaf sample was transferred into a 100 ml beaker, then 50 ml of 20 mg/l oxalic acid was added. The mixture was placed into ultrasonic bath for 30 min and then centrifuged for 20 min at 10,000 rpm. The supernatant was filtered through C18 cleanup column and a 0.45 mm syringe filter and injected to ion chromatography system. Eluent was a mixture of 4.5 mmol/l sodium carbonate and 0.8 mmol/l sodium bicarbonate with a flow-rate of 1.0 ml/min. After separation, sulphur-containing species were introduced into a mixing tee, meanwhile derivative solution was also pumped into the mixing tee at a rate of 0.70 mL/min, then the mixture passed through a reaction coil (375 ml), in which sulphite, thiosulfate, thiocyanate, and formaldehyde sulfoxylate reacted with iodine to produce iodic. Iodic would react with the residual iodine to produce triiodide (Chen *et al.*, 2009).

(g) Magnesium (Mg): Magnesium was estimated by atomic absorption spectrophotometer (Dawson and Heaton, 1961). The procedure was same as for calcium, except that the standard solution was made by dissolving 1 g magnesium metal in one litre distilled water and further diluted to make 2, 1.5, 0.5 and 0.25 ppm concentration along with the wavelength of atomic absorption spectrophotometer set at 285.21 nm.

(h) Iron (Fe): The procedure for Fe estimation is same as of Zn estimation. The only difference is the wavelength of atomic absorption spectrophotometer which is 219.9 nm instead of 248.3 nm (Antunes *et al.*, 2017).

(i) Copper (Cu): Two grammes of copper sulphate pentahydrate was weighed and dissolved by adding to it about 100 ml of distilled water in a 250 ml beaker. The final volume was made upto 500 mL in a volumetric flask and mixed well. Different sub-samples of 20, 10, 5, 2.5, 1.25 ppm were prepared directly from the stock standard solution. Two grammes of digested leaf sample were taken and 4 ml concentrated nitric acid was added to it. It was covered with a watch glass with glass hooks and heated in the fumehood until the sample dissolved. When cooled, about 4 ml of 50 per cent sulfuric acid was added to the beaker. It was heated until dense cloudy fumes of sulphur trioxide evolved. The solution was continuously stirred with a stirring rod to avoid bumping. The solution was cooled and was diluted to about 50 mL with hot distilled water. The solution was filtered through a fine filter paper. Each solution was diluted by a dilution factor of 10. The atomic absorption

spectroscopic measurements on the diluted standards and the two diluted unknowns were carried out at wavelength of 324.8 nm and standard curves were drawn for the estimation of Cu in the sample (Antunes *et al.*, 2017).

(i) Manganese (Mn): The procedure for Fe estimation is same as of Zn estimation. The only difference is the wavelength of atomic absorption spectrophotometer which is 219.9 nm instead of 248.3 nm (Stupar and Dolinsek, 1996).

3.3.5 Assessment of the genetic diversity using SSR markers: For molecular level evaluation of genetic diversity in *Moringa oleifera* Lam. mother trees (genotypes), single sequence repeats (SSR) markers (APPENDIX III) were used.

3.3.5.1 Collection of leaf material

Fresh, newly developed leaves were separately excised from different mother trees. Before plucking, the leaves were wiped off the soil with tissue paper and then wrapped in aluminium foil and brought to the laboratory in ice-box and stored in deep freezer at -80 °C till further use.

3.3.5.2 DNA extraction

Two grams of fresh leaf sample were cut into small leaf bits which were transferred into mortar for maceration using 1.5 ml of CTAB buffer. This was then transferred to the Eppendorf tube of 2.0 ml volume and allowed for incubation for one hour at 65 °C in hot water bath and allowed for cooling for 5-10 minutes. Equal volume of Chloroform: Isoamyl alcohol mixture (24:1 V/V) was added and mixed by gentle inversion. It was centrifuged at 12,000 rpm for 20 minutes at room temperature. The clear aqueous phase was transferred to new sterile Eppendorf tube. Equal volume of ice cold isopropanol was added and mixed gently by inversion. It was then kept in the freezer until DNA was precipitated out (optimal at 4 °C for overnight). It was again centrifuged at 12,000 rpm for 20 minutes at room temperature. Only the pellet was taken and the solution was discarded. The pellet was washed with 70 per cent ethanol (200 µl) and was spin at 10,000 rpm for two minutes. The alcohol was discarded carefully and pellet was kept for air drying. 50 µl of TE buffer was added for dissolving and preserving the pellets (Doyle & Doyle, 1990). Preparation of reagents required for DNA extraction is given in APPENDIX IV.

3.3.5.3 Quantitative and Qualitative Assessment of DNA

a. Agarose gel electrophoresis

The isolated genomic DNA was electrophoresed on 0.8 per cent agarose gel (stained with 0.5 µg/ml of ethidium bromide) at 80V –100 V and 70 mA for two hours in 1X TAE buffer and then observed on UV-transilluminator. Quality of DNA samples was judged on the basis of whether sample DNA formed a single high molecular weight band or smear.

b. DNA quantification

The quantity of DNA was assessed by UV/VIS spectrophotometer (Perkin Elmer, Hyderabad, India). Concentration of DNA was quantified using following formula:

$$\text{DNA } (\mu\text{g/ml}) = \frac{\text{OD (260)} \times \text{dilution factor} \times 50}{1000}$$

The ratio of absorbance at 260 nm and at 280 nm was measured to check the contamination of protein.

Purity check of DNA on the basis of $A_{260}:A_{280}$ ratio

Sr. No.	$A_{260}:A_{280}$ ratio (Absorbance ratio)	Indication
1.	Above 1.8	Protein contamination
2.	1.4 to 1.8	Good quality DNA
3.	Below 1.4	RNA contamination

3.3.5.4 DNA amplification

Tests were performed for standardizing polymerase chain reaction amplification conditions mainly the annealing temperature. PCR amplification conducted at different annealing temperatures (APPENDIX-III) using standard concentrations of various components of reaction mixture.

PCR Programme for DNA amplification

S. No.	Reactions		Temperature and time specifications	Number of cycles
1.	Initial denaturation		94 ⁰ C for 3 mins	1
2.	A	Denaturation	92 ⁰ C for 1 min	36
	B	Annealing of primer	55-61 ⁰ C for 1 min	
	C	Primer amplification	72 ⁰ C for 1 min	
3.	Final amplification		72 ⁰ C for 10 mins	1
Holding			4 ⁰ C till electrophoresis	

The amplification reactions were performed in a total volume of 25 µl containing:

Stock	Aliquot
Sterile water	17.5 µl
10X buffer	2.5 µl
MgCl ₂	1.0 µl
2.5 mM dNTPs	0.5 µl
20 mM primer (F)	0.5 µl
20 mM primer (R)	0.5 µl
(1U/µl) Taq DNA polymerase	0.5 µl
DNA	2.0 µl
Total	25.0 µl

3.3.5.5 Gel electrophoresis

PCR amplified products were subjected to gel electrophoresis in ethidium bromide stained 2 per cent agarose gel in 1X TAE at 90 V for 2-3 hours using gel electrophoresis unit. Allele sizes of PCR products were determined by referring to the 50 bp ladder. The materials required and protocol for agarose gel electrophoresis is given in Annexure V.

3.3.5.6 Molecular data analysis

(a) Viewing of amplified DNA: After the run was over, the gel was viewed under the UV light using Gel Documentation system and was photographed.

(b) Scoring: Scoring of SSR marker was carried out by considering only the clear and unambiguous bands. Markers were scored for the presence and absence of the corresponding band among the different genotypes. The scores ‘1’ and ‘0’ were given for the presence and absence of bands, respectively.

(c) Cluster analysis: The data obtained by scoring the SSR profiles of different primers were subject to cluster analysis and dendrogram was constructed by Unweighted Pair-Group Method using Arithmetic averages (UPGMA). Data analysis was done using NTSYS-PC software version 2.02i (Rohlf, 2005).

3.4 STATISTICAL ANALYSIS

3.4.1 Analysis of variance (ANOVA)

Statistical analysis is an important tool for data evaluation and interpretation of the results. The data on pod, seed, germination parameters, morphological characters and nutritional variation was analyzed using RBD (Randomized Block Design). The

statistical analysis for each parameter was carried out on mean values (Panse and Sukhatme, 1989).

ANOVA table for Randomized Block Design (RBD)

Source of Variation	Degree of freedom	Mean Sum of Square	Variance Ratio
Replication (r)	r-1	MSR	MSR/MSE
Seed Source (s)	s-1	$M_t = MSS$	MSS/MSE
Error	$(r-1)(s-1)$	$M_e = MSE$	

where,

r = number of replications

s = number of genotypes or progenies

MSR = Mean Sum of Square due to replication
MSS = Mean Sum of Square due to seed source
MSE = Mean Sum of Square due to error

The critical difference (CD) was calculated as: $CD = SEd \times t_{0.05}$

where,

SEd = and $t_{0.05}$ is the t-table value at error degree of freedom at 5% level of significance.

The calculated 'F' values were compared with tabulated 'F' value for test of significance. Standard Error (SE), Critical Differences (CD) and Coefficient of Variation (CV) were calculated to find out the superiority of one genotype over the others with the help of following formulae:

$$SE(m) \pm = \sqrt{\frac{Me}{r}}$$

$$SE(d) \pm = \sqrt{\frac{2Me}{r}}$$

$$CD_{(0.05)} = SE(d) \times \text{table value of } t_{(0.05)} \text{ at error degree of freedom.}$$

where,

$SE(m) \pm$ = Standard error of mean

$SE(d) \pm$ = Standard error of difference

$CD_{(0.05)}$ = Critical difference at 5% level of significance

All the characters, which showed significant differences, were further subjected to analysis for the following parameters:

3.4.2 Analysis of covariance

Source of variation	df	Sum of squares and sum of products			Adjusted sum of squares	df	Mean sum of squares	F _{cal}
		YY	XX	XY				
Replication	r-1	RYY	RXX	RXY				
Treatment	t-1	T _{YY}	T _{XX}	T _{XY}	T _{YY'}	t-1	T _{adj} = (T' _{YY})/(t-1)	(T _{adj})/SE _{adj}
Error	(r-1)(t-1)	E _{YY}	E _{XX}	E _{XY}	S _E	(r-1)(t-1) - 1 = Ne	SE _{adj} = S _e /n _e	
Total	rt-1	S _{YY}	S _{XX}	S _{XY}				
Treatment + error	r(t-1)	A _{YY}	A _{XX}	A _{XY}	S _{T+E}			

Where,

r = number of replications

t = number of treatments

X = first variable

Y = second variable

SE = standard error of mean

adj = adjusted values

3.4.3 Estimation of coefficient of variance

$$\text{Cov}(X, Y) = \frac{\sum (X_i - \bar{X})(Y_i - \bar{Y})}{n}$$

Where,

X_i is the value of X variable

Y_i is the value of Y variable

X bar is the mean of X variable

Y bar is the mean of Y variable

n is the number of data points

3.4.4 Estimation of coefficient of variability

Coefficient of variability was estimated as per formula given by Burton and Devane (1953).

a. **Phenotypic coefficient of variation (PCV)**

$$\text{PCV (\%)} = \frac{\sqrt{\text{Phenotypic variance (Vp)}}}{\text{General mean of population (GM)}} \times 100$$

b. **Genotypic coefficient of variation (GCV)**

$$\text{GCV (\%)} = \frac{\sqrt{\text{Genotypic variance (Vg)}}}{\text{General mean of population (GM)}} \times 100$$

3.4.5 Estimation of heritability

Heritability in broad sense was calculated as per formula given by Burton and Devane (1953) and Allard (1960)

$$\text{Heritability (h}^2 \text{ \%)} = \frac{\text{Genotypic variance (Vg)}}{\text{Phenotypic variance (Vp)}} \times 100$$

3.4.6 Estimation of genetic advance

The expected genetic advance resulting from selection of five per cent superior individuals were worked out as suggested by Lush (1940) and further used by Burton and Devane (1953) and Johnson *et al.* (1955).

$$\text{Genetic advance} = h^2 \times \sqrt{Vp} \times K$$

Where,

h^2 = Heritability in broad sense

$\sqrt{Vp}$ = Phenotypic variance deviation

K = Selection differential at 5% selection intensity

The value of K = 2.06 (Allard, 1960).

3.4.7 Estimation of genetic gain

Genetic advance expressed as per cent of population mean was suggested by Johnson *et al.* (1955).

$$\text{Genetic gain (\%)} = \frac{\text{Genetic advance}}{\text{General mean of population (}\bar{X}\text{)}} \times 100$$

For categorizing the magnitude of different parameters, the limits used, are given below:

PCV and GCV	> 30 % - High
	15 – 30 % - Moderate
	< 15 % - Low
Heritability (h^2)	> 80 % - High
	50 – 80 % - Moderate
	< 50 % - Low
Genetic gain (GG)	>30 % - High
	15 – 30 % - Moderate
	< 15 % - Low

3.4.8 Correlation coefficient

The correlation between all characters under study, at genotypic, phenotypic and environmental levels was estimated as per the method described by Robinson *et al.* (1951) and Searle (1961).

1. Phenotypic correlation between character X and Y

$$r_{xy(p)} = \frac{Cov_{xy(p)}}{\sqrt{Var_x(p) \times Var_y(p)}}$$

2. Genotypic correlation between character X and Y

$$r_{xy(g)} = \frac{Cov_{xy(g)}}{\sqrt{Var_x(g) \times Var_y(g)}}$$

where,

$Cov_{xy(p)}$ and $Cov_{xy(g)}$ denote phenotypic, genotypic and environmental covariance between characters X and Y, respectively.

$Var_{x(p)}$ and $Var_{y(g)}$ denote phenotypic, genotypic and environmental variances for character X, whereas

$\text{Var}_{y(p)}$ and $\text{Var}_{y(g)}$ denote phenotypic, genotypic and environmental variances for character Y, respectively.

3.4.9 Principal component analysis

A data matrix of various variables of genotype/progenies was prepared and used for analysis. The Eigen values and Eigen vectors were computed from data matrix. Eigen values define the amount of total variation that is displayed on principal components. The Eigen vector (loading) defines the correlation of each variable with the principal components (Pearson, 1901). The biplot of principal component 1 and principal component 2 was prepared using PAST software version 4.0 (Hammer *et al.*, 2001).

3.4.10 Path coefficient analysis

The path coefficient analysis of component traits with total fresh biomass was done by following Dewey and Lu (1959). The direct and indirect effects were calculated by solving the following set of simultaneous equations.

$$\begin{aligned} P_{y_1} + P_{y_2} r_{12} + P_{y_3} r_{13} + \dots + P_{y_n} r_{1n} &= r_{y_1} \\ P_{y_1} r_{12} + P_{y_2} + P_{y_3} r_{23} + \dots + P_{y_n} r_{2n} &= r_{y_2} \\ P_{y_1} r_{1n} + P_{y_2} r_{2n} + P_{y_3} r_{3n} + \dots + P_{y_n} &= r_{y_n} \end{aligned}$$

Where

$P_{y_1}, P_{y_2}, P_{y_3}, \dots, P_{y_n}$ are the direct path effects of 1,2,3, ...n variables on dependent variable 'y'.

$r_{12}, r_{13}, \dots, r_{1n}$ are the genotypic coefficients of correlation between various independent variables.

$r_{y_1}, r_{y_2}, \dots, r_{y_n}$ are the genotypic correlation coefficients of independent variables with dependent variable 'y'.

$P_{y_1} r_{12}, P_{y_3} r_{23}, \dots, P_{y_n} r_{2n}$ are the indirect effects.

The variation in the independent variable, which remained undetermined by including all variables, was assumed to be due to variable (s) not included in the present investigation. The degree of determination (p^2_{yx}) of such variable (s) on dependent variable was calculated as follows:

$$\begin{aligned}
P^2_{yx} = 1 - & (P^2_{y_1} + 2P_{y_1} P_{y_2} r_{12} + 2P_{y_1} P_{y_3} r_{13} + \dots \\
& + P^2_{y_2} + 2P_{y_2} P_{y_3} r_{23} + \dots \\
& + P^2_{y_3} + 2P_{y_3} P_{y_4} r_{34} + \dots + P^2_{y_n})
\end{aligned}$$

and Residual effect (R^2) = $\sqrt{P^2_{yx}}$

3.4.11 Cluster analysis

The data on the characters computed and agglomerative hierarchical clustering was done using PAST software version 4.0 (Hammer *et al.*, 2001) employing Ward's method. The clustering was based on the squared Euclidean distances and the average linkage between groups was taken as the average of the distance between all pairs of cases with one member of each group (Ward, 1963).

Chapter-4

RESULTS AND DISCUSSION

The present research work was carried out in the Department of Tree Improvement and Genetic Resources of Dr. Yashwant Singh Parmar University of Horticulture and Forestry Nauni, Solan (H.P.) by selecting the trees of *Moringa oleifera* Lam. from sub-tropical to mid hills ranges of North-west India. Observations on morphological, biochemical and molecular levels were recorded. The experimental data recorded were subjected to statistical analysis. The results are presented in this chapter under the following headings:

- 4.1 Qualitative and quantitative variation in mother trees**
- 4.2 Pod, seed and germination parameters**
- 4.3 Nursery stage growth performance of progenies**
- 4.4 Variation in biomass characters**
- 4.5 Variation in proximate principles, biochemical estimates and mineral contents of progenies**
- 4.6 Genetic estimates**
- 4.7 Correlation studies**
- 4.8 Principal component analysis**
- 4.9 Path coefficient analysis**
- 4.10 Cluster analysis**
- 4.11 Diversity analysis using SSR markers**
- 4.1 Qualitative and quantitative variation in mother trees**

Credentials in Table 2. illustrated noteworthy variation among mother trees for qualitative characters. The visual examination of these mother trees under study revealed that anthocyanin coloration of axis and branches of petiole was present in M2, M7, M8, M17, M33 and M35 mother trees, whereas it was absent in other mother trees. Hairy leaf stalk was found in M1, M3, M23, M24, M26, M28, M32, M34 and M35 mother trees while leaf stalk was glabrous in remaining mother trees. Colour of flower bud was observed dark pink in M11 and M30 mother trees; pink in M12, M13, M14, M29, M31, M33, M34 and M35 mother trees while other mother trees had white floral bud. Cream coloured seeds were

Table: 2. Qualitative characters of *Moringa oleifera* Lam. mother trees under study sites.

Seed sources (District, State/UT)	Mother tree	Anthocyanin coloration of axis and branches of petiole	Leaf stalk surface	Leaf colour	Flower bud colour	Seed colour	Wing form	Bark colour	Tree growth habit
Samba (Samba, Jammu and Kashmir)	M1	Absent	Glabrous	Green	White	White	Prominent	Grey	Spreading
	M2	Present	Glabrous	Dark green	White	White	Less prominent	Light grey	Upright
	M3	Absent	Glabrous	Green	White	White	Prominent	Light grey	Spreading
	M4	Absent	Glabrous	Green	White	White	Prominent	Light grey	Upright
	M5	Absent	Glabrous	Dark green	White	White	Prominent	Light grey	Spreading
Dunera (Pathankot, Punjab)	M6	Absent	Glabrous	Green	White	White	Prominent	Grey	Drooping
	M7	Present	Glabrous	Green	White	White	Prominent	Dark grey	Drooping
	M8	Present	Glabrous	Green	White	White	Prominent	Dark grey	Upright
	M9	Absent	Glabrous	Green	White	White	Prominent	Dark grey	Drooping
	M10	Absent	Glabrous	Green	White	White	Less prominent	Dark grey	Upright
Vikasnagar (Dehradun, Uttarakhand)	M11	Absent	Glabrous	Dark green	Dark pink	White	Prominent	Dark grey	Upright
	M12	Absent	Glabrous	Dark green	Pink	White	Prominent	Light grey	Upright
	M13	Absent	Hairy	Green	Pink	White	Prominent	Dark grey	Spreading
	M14	Absent	Glabrous	Dark green	Pink	White	Prominent	Light grey	Upright
	M15	Absent	Glabrous	Green	Pink	White	Prominent	Grey	Upright
Lamlehri (Una, Himachal Pradesh)	M16	Absent	Glabrous	Green	White	Cream	Less prominent	Dark grey	Spreading
	M17	Present	Glabrous	Green	White	Cream	Less prominent	Dark grey	Upright
	M18	Absent	Glabrous	Green	White	White	Prominent	Dark grey	Spreading
	M19	Absent	Glabrous	Green	White	Cream	Less prominent	Light grey	Upright
	M20	Absent	Glabrous	Green	White	Cream	Less prominent	Light grey	Spreading
Tirla (Solan, Himachal Pradesh)	M21	Absent	Glabrous	Dark green	White	White	Prominent	Dark grey	Spreading
	M22	Absent	Glabrous	Green	White	White	Less prominent	Light grey	Spreading
	M23	Absent	Hairy	Green	White	Cream	Less prominent	Dark grey	Upright
	M24	Absent	Hairy	Dark green	White	Cream	Less prominent	Dark grey	Spreading
	M25	Absent	Glabrous	Green	White	White	Prominent	Dark grey	Upright
Siswa Dam (Sahibzada Ajit Singh Nagar, Punjab)	M26	Absent	Hairy	Green	White	White	Prominent	Light grey	Drooping
	M27	Absent	Glabrous	Green	White	White	Prominent	Grey	Spreading
	M28	Absent	Hairy	Green	White	White	Prominent	Light grey	Drooping
	M29	Absent	Glabrous	Dark green	Pink	White	Prominent	Dark grey	Spreading
	M30	Absent	Glabrous	Dark green	Dark pink	White	Prominent	Light grey	Spreading
Morni Hills (Panchkula, Haryana)	M31	Present	Glabrous	Green	Pink	White	Prominent	Light grey	Spreading
	M32	Absent	Hairy	Green	White	White	Prominent	Light grey	Drooping
	M33	Present	Glabrous	Dark green	Pink	White	Prominent	Light grey	Spreading
	M34	Absent	Hairy	Green	Pink	White	Prominent	Light grey	Spreading
	M35	Present	Hairy	Dark green	Pink	Cream	Prominent	Light grey	Drooping



Plate: 1. Seed size and colour variations in *Moringa oleifera* Lam.



2a



2b



2c

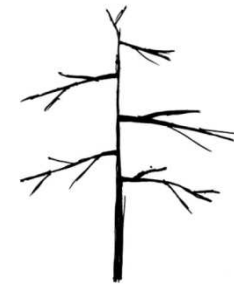
Plate: 2. Colour of the bark of *Moringa oleifera* Lam. mother trees: 2a. Light grey 2b. Grey, 2c. Dark grey



Upright



Spreading



Drooping

Plate: 3. Tree growth habit of *Moringa oleifera* Lam. mother trees

observed in M16, M17, M19, M20, M23, M24 and M35 whereas rest of the mother trees had white coloured seeds (Plate 1.) Bark colour was observed light grey (Plate 2a.) in M2, M3, M4, M5, M2, M14, M19, M20, M22, M26, M28, M30, M31, M32, M33, M34 and M35, grey (Plate 2b.) in M1, M6, M15 and M27 while dark grey bark (Plate 2c.) was observed in M7, M8, M9, M10, M11, M13, M16, M17, M18, M21, M23, M24, M25 and M29 mother trees. In case of tree growing habit (Plate 3.); M6, M7, M9, M26, M28 and M32 mother trees were observed having drooping growth habit where M2, M4, M8, M10, M11, M12, M14, M15, M17, M19, M23 and M25 mother trees were observed to have upright growing habit. The rest of the mother trees were following spreading growth habit.

The variability persisting in the qualitative characters among mother trees may be due to the existence of species differences and the prevalence of different agro-climatic conditions. The variation could also be due to differences in age and environmental effects of the trees. The results are in accordance with the findings of Kurian *et al.*, (2020), Selvakumari (2013) and Amoatey *et al.*, (2012) for *Moringa oleifera* Lam.; Singh *et al.*, (2021) and Deepanjli (2018) for *Toona ciliata* M. Roem.

It is apparent from the Table 3. that average height of mother trees was 10.49 m with an range of 9.01 m and 13.41 m for M13 and M15, respectively. Diameter at breast height ranged from 25.19 cm for M11 to 33.97 cm for M28 with an average DBH of 29.19 cm. Crown spread was maximum observed for M15 (76.80 m²) whereas minimum crow spread of 56.00 m² was observed in M3. The average crown spread and total leaf biomass of the selected 35 mother trees were 67.51 m² and 50.96 kg, respectively. For total leaf biomass, a range 35.21 kg (M18) to 61.14 kg (M20) was observed.

Morphological characterization provides information to aid the identification of genotypes and to recognize important traits related to the tree. The variability noted in the present study may be due to environment factors, as trees tend to grow tall and have great leaf biomass where resources are abundant, stresses are minor and competition for light is less. Vast variability for tree height and diameter was reported by Kumar *et al.*, (2005) and Sidhu (1997) in *D. sissoo* and Dhillon (2001) in *Azadirachta indica*. The variation for crown spread and total leaf biomass in the present study may be due to environmental factors. Similar finding about the variability in respect of tree dimensions including crown area was also reported by Deepanjli (2018) who also found a wide range of variability in the different morphological characters like DBH and total tree height of selected CPTs of *Toona ciliata* M. Roem. Similar findings were observed by Singh *et al.*, (2021) in *Toona ciliata* M. Roem.

Paray *et al.*, (2017) selected hundred CPTs of *Salix alba* based on Height, DBH, bole height and crown diameter. Selvakumari (2013) selected 34 plus trees of *Moringa oleifera* Lam. on the basis of plant height, trunk girth and leaf area. The results are in accordance with the findings of Redhu (2021) for tree height, DBH and crown spread in *Moringa oleifera* Lam. and Kurian *et al.*, (2020) for tree height and DBH. Similar results were observed by Thangjam *et al.*, (2020) in *Parkia timoriana*.

Table: 3. Growth characters of *Moringa oleifera* Lam. mother trees

Seed sources (District, State/UT)	Mother tree	Tree height (m)	Diameter at breast height (cm)	Crown spread (m ²)	Total leaf biomass (kg)
Samba (Samba, Jammu and Kashmir)	M1	10.48	27.91	65.53	48.53
	M2	10.33	31.64	59.62	44.26
	M3	11.76	26.94	56.00	47.67
	M4	9.50	25.66	66.37	51.74
	M5	9.24	30.77	62.47	43.24
Dunera (Pathankot, Punjab)	M6	9.39	33.35	67.92	48.77
	M7	9.25	26.19	76.70	46.14
	M8	9.34	29.68	63.84	54.13
	M9	10.33	25.84	76.64	57.08
	M10	11.65	29.78	64.57	58.22
Vikasnagar (Dehradun, Uttarakhand)	M11	9.25	25.19	68.50	52.94
	M12	10.65	26.56	72.90	53.04
	M13	9.01	26.15	62.93	48.61
	M14	12.62	31.09	67.30	54.67
	M15	13.41	25.21	76.80	60.41
Lamlehri (Una, Himachal Pradesh)	M16	10.74	29.11	66.75	48.62
	M17	9.84	27.26	66.53	50.56
	M18	11.60	31.82	57.85	35.21
	M19	11.82	28.59	68.51	55.27
	M20	10.16	33.08	68.03	61.14
Tirla (Solan, Himachal Pradesh)	M21	10.72	32.92	65.58	52.45
	M22	9.36	29.34	63.60	52.94
	M23	10.26	27.69	57.16	46.87
	M24	9.30	32.47	63.05	54.92
	M25	11.14	27.11	65.94	57.52
Siswa Dam (Sahibzada Ajit Singh Nagar, Punjab)	M26	9.79	33.37	72.87	46.88
	M27	11.28	33.07	74.49	48.47
	M28	9.52	33.97	69.26	50.66
	M29	11.34	31.12	73.02	45.17
	M30	9.74	25.70	66.69	53.66
Morni Hills (Panchkula, Haryana)	M31	11.45	28.34	72.60	44.91
	M32	9.80	27.83	69.90	46.51
	M33	10.44	32.43	76.31	43.45
	M34	11.58	25.98	68.94	58.38
	M35	10.93	28.36	67.83	60.66
	Mean	10.49	29.19	67.51	50.96

Analysis of variance revealed significant variation for all the proximate principals, viz., crude protein (%), ether extract (%), crude fiber (%), ash content (%) and nitrogen free extract (%) along with biochemical estimates, viz., total amino acids among mother trees except leaf dry matter content. (Table 4).

It is evident from the Table 4. that maximum crude protein content (%) was observed in M2 (24.94) which was statistically at par with M8 (24.63), M6 (24.38), M20 (24.21), M24 (24.19), M9 (24.17), M4 (24.15) and M15 (23.76). However, minimum crude protein content (%) was observed in M14 (17.65 %).

Maximum ether extract (%) was observed in M19 (6.29) which was statistically at par with M19 (6.29), M24 (6.12), M18 (6.11), M16 (5.84), M15 (5.82) and M6 (5.76). Minimum ether extract (%) was observed in M3 (2.73).

Maximum crude fiber content (%) was observed in M28 (15.32) which was statistically at par with M14 (14.72), M13 (14.30) and M4 (10.58). Minimum crude fiber content (%) was observed in M1 (3.43).

Data pertaining to Table 4. illustrated that maximum total ash content (%) was observed in M7 (11.86) which was statistically at par with M34 (11.48), M3 (11.36), M20 (11.34) and M12 (11.17). Minimum total ash content (%) was observed of M24 (7.08).

Maximum nitrogen free extract (%) was observed in M11 (65.63) followed by in M1 (64.18) whereas, minimum nitrogen free extract (%) was observed in M31 (45.88).

The results are in accordance with the findings of Tak (2012) and Moyo (2011) in *Moringa oleifera* Lam. In additions, Osman and Abohassan (2012) gave similar results for crude protein, crude fiber, ether extract and ash content in *Moringa peregrina*.

It is apparent from Table 4. that maximum total amino acid content (%) was observed in M2 (16.30) which was statistically at par with M9 (14.98), M31 (14.95), M24 (14.93), M8 (14.86), M20 (14.80), M4 (14.74), M6 (14.72), M27 (14.63), M15 (14.42), M16 (14.41), M19 (14.36), M34 (14.33) and M35 (14.22). Minimum total amino acid content (%) was observed in M5 (11.49).

The results of total amino acids content are in line with the findings of Moyo (2011) in *Moringa oleifera* Lam.

Table: 4. Variation in leaf dry matter content, proximate principles and biochemical estimates of leaf samples of *Moringa oleifera* Lam. mother trees.

Seed sources (District, State/UT)	Mother tree	Leaf dry matter content (%)	Crude protein (%)	Ether extract (%)	Crude fiber (%)	Ash content (%)	Nitrogen free extract (%)	Total amino acids (%)
Samba (Samba, Jammu and Kashmir)	M1	19.30 (4.51)	20.11 (4.59)	4.35 (2.31)	3.43 (2.10)	7.93 (2.99)	64.18	12.12 (3.62)
	M2	18.99 (4.47)	24.94 (5.09)	3.41 (2.10)	7.93 (2.98)	10.58 (3.40)	53.14	15.19 (4.02)
	M3	19.13 (4.49)	18.86 (4.46)	2.73 (1.93)	12.84 (3.72)	11.36 (3.51)	54.21	11.47 (3.53)
	M4	19.12 (4.49)	24.15 (5.01)	3.26 (2.06)	14.80 (3.98)	10.99 (3.46)	46.80	14.74 (3.97)
	M5	18.99 (4.47)	17.64 (4.32)	5.13 (2.47)	7.23 (2.86)	9.12 (3.18)	60.87	10.96 (3.46)
Dunera (Pathankot, Punjab)	M6	18.60 (4.43)	24.38 (5.04)	5.76 (2.60)	10.65 (3.41)	9.82 (3.29)	49.40	14.72 (3.96)
	M7	19.13 (4.49)	21.36 (4.73)	2.85 (1.96)	11.17 (3.49)	11.86 (3.58)	52.76	13.26 (3.78)
	M8	18.67 (4.44)	24.63 (5.06)	3.22 (2.05)	9.18 (3.18)	10.46 (3.38)	52.50	14.86 (3.98)
	M9	18.99 (4.47)	24.17 (5.02)	3.77 (2.18)	8.19 (3.02)	9.34 (3.22)	54.52	14.98 (4.00)
	M10	18.75 (4.44)	21.35 (4.73)	3.40 (2.10)	5.26 (2.50)	10.94 (3.46)	59.05	13.36 (3.79)
Vikasnagar (Dehradun, Uttarakhand)	M11	19.13 (4.49)	18.57 (4.42)	3.68 (2.16)	4.61 (2.37)	7.51 (2.92)	65.63	11.44 (3.53)
	M12	19.18 (4.49)	22.50 (4.85)	3.51 (2.12)	11.13 (3.48)	11.17 (3.49)	51.70	14.05 (3.88)
	M13	18.66 (4.43)	17.61 (4.31)	5.32 (2.51)	14.30 (3.91)	7.75 (2.96)	55.02	11.01 (3.46)
	M14	18.88 (4.46)	17.65 (4.31)	2.96 (1.99)	14.72 (3.97)	10.00 (3.32)	54.65	10.75 (3.42)
	M15	19.06 (4.48)	23.76 (4.98)	5.82 (2.61)	4.13 (2.26)	8.20 (3.03)	58.09	14.42 (3.93)
Lamlehri (Una, Himachal Pradesh)	M16	19.17 (4.49)	23.12 (4.91)	5.84 (2.61)	4.35 (2.31)	7.27 (2.88)	59.42	14.41 (3.93)
	M17	19.27 (4.50)	22.32 (4.83)	2.86 (1.97)	8.47 (3.07)	9.87 (3.30)	56.47	13.77 (3.84)
	M18	19.33 (4.51)	21.95 (4.79)	6.11 (2.67)	10.29 (3.36)	9.98 (3.31)	51.67	13.20 (3.77)
	M19	19.13 (4.49)	22.96 (4.89)	6.29 (2.70)	4.89 (2.42)	9.77 (3.28)	56.09	14.36 (3.92)
	M20	19.19 (4.49)	24.21 (5.02)	3.24 (2.06)	11.78 (3.57)	11.34 (3.51)	49.43	14.80 (3.97)

Tirla (Solan, Himachal Pradesh)	M21	18.88 (4.46)	19.50 (4.53)	6.07 (2.66)	11.34 (3.51)	8.72 (3.12)	54.37	12.14 (3.62)
	M22	18.93 (4.46)	18.48 (4.41)	5.55 (2.56)	5.78 (2.60)	8.13 (3.02)	62.06	11.29 (3.51)
	M23	19.01 (4.47)	18.74 (4.44)	3.47 (2.11)	12.21 (3.63)	10.37 (3.37)	55.21	11.65 (3.56)
	M24	18.56 (4.42)	24.19 (5.02)	6.12 (2.67)	7.71 (2.94)	7.08 (2.84)	54.90	14.93 (3.99)
	M25	18.74 (4.44)	19.77 (4.56)	4.42 (2.33)	8.73 (3.11)	9.40 (3.22)	57.68	12.35 (3.65)
Siswa Dam (Sahibzada Ajit Singh Nagar, Punjab)	M26	19.17 (4.49)	17.64 (4.32)	4.86 (2.42)	5.02 (2.45)	8.92 (3.15)	63.56	11.04 (3.47)
	M27	19.09 (4.48)	22.89 (4.89)	4.09 (2.26)	6.65 (2.76)	8.48 (3.08)	57.89	14.63 (3.95)
	M28	18.83 (4.45)	21.11 (4.70)	4.30 (2.30)	15.32 (4.04)	8.04 (3.00)	51.24	12.65 (3.69)
	M29	19.41 (4.52)	19.87 (4.57)	4.63 (2.37)	13.31 (3.78)	8.58 (3.10)	53.62	12.07 (3.61)
	M30	19.20 (4.49)	19.63 (4.54)	5.17 (2.48)	9.69 (3.27)	8.30 (3.05)	57.22	11.64 (3.56)
Morni Hills (Panchkula, Haryana)	M31	18.76 (4.45)	23.68 (4.97)	5.08 (2.46)	15.08 (4.01)	10.28 (3.36)	45.88	14.95 (3.99)
	M32	19.03 (4.53)	18.64 (4.43)	3.60 (2.14)	12.93 (3.73)	8.78 (3.13)	56.05	11.01 (3.47)
	M33	18.96 (4.47)	18.78 (4.45)	4.55 (2.35)	11.62 (3.55)	8.78 (3.13)	56.27	11.84 (3.58)
	M34	19.36 (4.51)	23.21 (4.92)	4.67 (2.38)	3.60 (2.14)	11.48 (3.53)	57.04	14.33 (3.91)
	M35	19.52 (4.48)	23.27 (4.92)	4.21 (2.28)	9.30 (3.20)	9.26 (3.20)	53.95	14.22 (3.90)
	Mean	19.03 (4.48)	21.30 (4.71)	4.41 (2.31)	9.36 (3.16)	9.42 (3.22)	55.50	13.10 (3.75)
	CD_{0.05}	NS	0.11	0.10	0.22	0.10	1.90	0.12
	CV		1.36	2.61	4.19	0.95	2.10	1.88

(The values in parenthesis are square root transformed values)

The perusal data in Table 5. in significant results for nitrogen, phosphorus, potassium, calcium, magnesium, sulphur, zinc, iron, copper and manganese contents in leaf samples of mother trees. Maximum nitrogen content (mg/100g) was observed in M2 (3990.42) which was statistically at par with M8 (3941.12), M6 (3901.00), M9 (3867.72), M20 (3873.43), M24 (3869.84) and M4 (3863.66). Minimum nitrogen content (mg/100g) of (2817.70) was observed in M13.

Maximum phosphorus content (mg/100g) was observed in M19 (81.85) which was statistically in accordance with M35 (80.81) whereas, minimum phosphorus content (mg/100g) was observed in M2 (27.00).

Maximum potassium content (mg/100g) was observed in M7 (1861.33) which was statistically at par with M14 (1826.33), M3 (1810.33), M8 (1801.00), M17 (1777.00), M4 (1773.67), M10 (1766.67), M2 (1756.33), M20 (1751.67), M23 (1736.00) and M32 (1732.00). Minimum potassium content (mg/100g) was observed in M24 (1356.33).

Maximum calcium content (mg/100g) was observed in M18 (2.10) which was statistically at par with M22 (2.08), M24 (2.08), M16 (2.07), M26 (2.07), M19 (2.05) and M25 (2.05). Minimum calcium content (mg/100g) was observed in M7 (1.74) (Table 5).

Data obtained from Table 5. revealed that maximum magnesium content (mg/100g) was observed in M20 (772.00) which was statistically at par with M14 (766.33), M15 (762.33), M12 (749.33), M18 (747.67), M11 (734.33), M17 (734.00) and M21 (727.67). Minimum magnesium content (mg/100g) was observed in M29 (356.67).

Maximum sulphur content (mg/100g) was observed in M7 (1421.67) which was statistically followed by M14 (1387.33) whereas minimum sulphur content (mg/100g) was observed in M24 (641.33).

Maximum zinc content (mg/100g) was observed in M7 (3.25) which was statistically at par with M14 (3.22), M3 (3.19) and M8 (3.16). Minimum zinc content (mg/100g) was observed in M24 (2.21).

Maximum iron content (mg/100g) was observed in M27 (43.67) which was statistically at par with M18 (43.49) and M33 (41.64). Minimum iron content (mg/100g) was observed in M13 (21.50).

Maximum copper content (mg/100g) was observed in M6 (0.87) which was statistically at par with M10 (0.85), M19 (0.85), M18 (0.84), M21 (0.83) and M13 (0.82). Minimum copper content (mg/100g) was observed in M7 (0.38).

Maximum manganese content (mg/100g) was observed in M18 (4.62) which was statistically at par with M8 (4.59), M24 (4.53), M16 (4.49), M21 (4.47), M19 (4.45), M25 (4.40) and M15 (4.34). The variation in the nutritional values differ for a wide range of reasons, such as cultivated regions, growing conditions, nature of soil, seasonal changes, genetically different cultivars, storage conditions or due to the period of analysis. Minimum

manganese content (mg/100g) was observed in M7 (3.00). The results are in line with the findings of Tak (2012) and Moyo (2011) in *Moringa oleifera* Lam.

Table: 5. Variation in mineral nutrient contents of leaf samples of *Moringa oleifera* Lam. mother trees.

Seed sources (District, State/UT)	Mother tree	Mineral nutrient content (mg/100 g)									
		N	P	K	Ca	Mg	S	Zn	Fe	Cu	Mn
Samba (Samba, Jammu and Kashmir)	M1	3217.72	67.17	1631.67	1.91	551.00	1043.67	2.78	25.29	0.61	3.78
	M2	3990.42	27.00	1756.33	1.86	673.00	1250.67	3.07	24.10	0.55	3.60
	M3	3017.43	39.21	1810.33	1.76	660.67	1350.67	3.19	23.53	0.42	3.11
	M4	3863.66	43.43	1773.67	1.81	512.00	1316.00	3.11	38.09	0.43	3.06
	M5	2822.98	75.34	1570.33	1.95	639.67	963.00	2.71	24.86	0.45	4.06
Dunera (Pathankot, Punjab)	M6	3901.00	36.35	1678.00	1.91	628.67	1103.33	2.89	24.80	0.87	3.69
	M7	3417.21	34.04	1861.33	1.74	667.33	1421.67	3.25	29.62	0.38	3.00
	M8	3941.12	53.35	1801.00	1.97	709.33	842.67	3.17	31.85	0.60	4.59
	M9	3867.72	42.37	1602.33	1.94	555.00	801.67	2.78	31.51	0.64	3.42
	M10	3416.42	65.12	1766.67	1.80	645.67	1291.67	2.46	31.38	0.85	3.33
Vikasnagar (Dehradun, Uttarakhand)	M11	2970.53	49.04	1583.33	1.92	734.33	973.00	2.52	38.69	0.52	3.84
	M12	3599.57	70.69	1670.00	1.83	749.33	1069.67	2.83	33.25	0.50	3.51
	M13	2817.70	55.71	1456.33	2.03	391.67	786.67	2.44	21.50	0.82	4.18
	M14	2824.77	45.01	1826.33	1.78	766.33	1387.33	3.22	28.56	0.43	3.22
	M15	3801.56	58.84	1473.67	2.04	762.33	1007.33	2.50	27.76	0.39	4.34
Lamlehri (Una, Himachal Pradesh)	M16	3699.04	74.53	1386.00	2.08	473.00	707.33	2.34	22.61	0.69	4.49
	M17	3571.01	31.82	1777.00	1.82	734.00	1321.67	3.14	34.77	0.48	3.89
	M18	3511.61	60.00	1364.00	2.10	747.67	661.67	2.28	43.49	0.84	4.62
	M19	3673.93	81.85	1488.67	2.05	438.00	865.33	2.91	37.28	0.85	4.45
	M20	3873.43	45.94	1751.67	1.82	772.00	1224.33	3.05	32.40	0.50	3.46
Tirla (Solan, Himachal Pradesh)	M21	3120.58	69.07	1500.67	1.84	727.67	882.00	2.58	22.23	0.83	4.47
	M22	2956.82	54.88	1377.00	2.08	385.00	680.67	2.30	36.28	0.47	3.28
	M23	2998.21	56.78	1736.00	1.79	652.33	1201.67	3.03	28.12	0.72	4.25
	M24	3869.84	72.18	1356.33	2.08	510.67	641.33	2.21	28.93	0.63	4.53
	M25	3162.67	40.60	1401.00	2.05	590.67	736.33	2.36	39.04	0.81	4.40
Siswa Dam (Sahibzada Ajit Singh Nagar, Punjab)	M26	2822.71	28.95	1545.33	2.07	407.00	947.00	2.67	35.74	0.73	4.31
	M27	3662.19	54.07	1711.33	1.89	614.67	1125.33	2.41	43.67	0.56	3.67
	M28	3377.06	68.61	1409.33	2.03	353.67	749.00	2.39	40.12	0.76	3.65
	M29	3178.85	77.08	1534.67	2.02	356.67	1335.33	2.65	38.42	0.67	4.27
	M30	3140.10	47.24	1642.00	1.95	577.33	1055.00	2.80	27.29	0.73	3.98
Morni Hills (Panchkula, Haryana)	M31	3788.13	63.30	1463.00	1.96	630.00	927.33	2.76	37.43	0.78	4.09
	M32	2982.48	57.92	1732.00	1.98	414.67	1163.00	2.98	26.11	0.68	3.55
	M33	3005.35	51.96	1513.00	1.75	378.67	908.00	2.63	41.64	0.71	4.23
	M34	3713.99	50.28	1433.00	1.89	534.00	774.33	3.08	30.70	0.74	3.38
	M35	3723.45	80.81	1716.00	1.99	568.67	1155.33	2.95	33.21	0.56	4.13
	Mean	3408.61	55.16	1602.84	1.93	586.08	1019.17	2.76	31.84	0.63	3.88
	CD _{0.05}	154.38	4.11	131.99	0.05	46.21	52.92	0.09	3.26	0.05	0.28
	CV	2.78	4.57	5.05	1.48	4.84	3.19	2.09	6.28	4.37	4.38

4.2 Pod, seed and germination parameters

Analysis of variance revealed significant variation for pod and seed characters among different progenies. It is evident from the Table 6. that maximum pod length was observed in P32 (50.60 cm) which was statistically at par with P35 (49.40 cm). The minimum pod length was observed in P15 (33.80 cm). Maximum pod breadth was observed in P11 (2.40 cm) whereas, minimum pod breadth of 1.20 cm was observed for P7, P14, P30 and P33. Maximum seeds per pod was observed in P35 (29) whereas minimum seeds per pod was observed in P1 (16).

Appraisal of the data presented in Table 6. elucidated that maximum 100 seed weight with wings was observed in P1 (16.13 g) whereas minimum 100 seed weight with wings was observed in P23 (9.11 g). Maximum 100 seed weight without wings was observed in P1 (15.34 g) whereas minimum 100 seed weight with wings was observed in P23 (8.22 g). Maximum 100 seed kernel weight was observed in P11 (13.41 g) which was statistically at par with P5 (13.29 g), P2 (13.24 g), P15 (13.21 g). Minimum 100 seed kernel weight was observed in P17 (6.83 g).

Maximum seed length was observed in P11 (1.28 cm) which was statistically at par with P30 (1.27 cm), P16 (1.26 cm), P1 (1.25 cm), P18 (1.25 cm), P19 (1.25 cm), P34 (1.25 cm), P4 (1.24 cm), P15 (1.24 cm), P23 (1.23 cm) and P26 (1.23 cm). Minimum seed length was observed for P9 (0.76 cm). Maximum seed breadth was observed in P6 (0.99 cm) whereas minimum seed breadth was observed in P7 (0.53 cm). Maximum seed kernel length was observed in P30 (0.83 cm) whereas minimum seed kernel length was observed in P9 (0.49 cm). Maximum seed kernel breadth was observed in P30 (0.58 cm) whereas minimum seed kernel breadth of 0.34 cm was observed in both P15 and P19.

It is clear from the results that the nature and extent of observed variations for traits under consideration was large. The variations observed, might have occurred due to climatic and microclimatic conditions as this species grows over the wide range of climatic conditions. Seed weight depends on the reserve food material (endosperm) formed as a result of double fertilization which is controlled by the maternal traits, and is also affected by the nutrient availability during seed setting and other geoclimatic conditions. Harper *et al.*, (1970) reported that seed size may vary due to external (environmental) and internal conditions (maternal and hereditary) performing at the time of seed development. Moreover, the seeds collected from the different locations, from trees of approximately same age, the

Table: 6. Variation in pod and seed morphometric characters of *Moringa oleifera* Lam.

Seed sources (District, State/UT)	Progeny	PL	PB	S/P	SWW	SW	SK	SL	SB	SKL	SKB
Samba (Samba, Jammu and Kashmir)	P1	41.20	2.30	16.00	16.13	15.34	12.63	1.35	0.99	0.74	0.52
	P2	39.80	2.20	18.00	14.47	13.92	13.24	0.89	0.53	0.65	0.46
	P3	41.30	1.70	19.00	15.34	14.79	12.57	1.02	0.66	0.75	0.52
	P4	40.90	1.70	21.00	13.62	12.42	9.66	0.76	0.55	0.49	0.34
	P5	41.50	1.30	19.00	14.27	13.81	13.29	1.12	0.63	0.75	0.53
Dunera (Pathankot, Punjab)	P6	41.40	1.70	21.00	9.49	9.17	8.21	1.25	0.78	0.67	0.46
	P7	40.50	1.20	17.00	11.61	11.37	10.92	1.20	0.66	0.67	0.44
	P8	47.80	1.60	22.00	13.13	12.41	10.22	1.10	0.85	0.68	0.48
	P9	37.30	1.80	19.00	12.63	12.13	10.72	1.24	0.77	0.67	0.47
	P10	42.60	2.20	24.00	12.42	12.19	10.46	1.04	0.74	0.66	0.46
Vikasnagar (Dehradun, Uttarakhand)	P11	35.50	2.40	23.00	14.26	13.89	13.41	1.28	0.76	0.75	0.53
	P12	37.20	2.30	21.00	12.11	11.61	11.18	1.13	0.68	0.71	0.49
	P13	41.60	2.10	17.00	11.45	10.82	10.45	1.15	0.71	0.75	0.52
	P14	38.60	1.20	19.00	12.77	12.32	11.64	1.18	0.78	0.75	0.53
	P15	33.80	1.80	22.00	14.05	13.76	13.21	1.24	0.79	0.76	0.34
Lamlehri (Una, Himachal Pradesh)	P16	36.40	1.80	19.00	10.17	9.67	9.19	1.26	0.81	0.73	0.51
	P17	37.80	1.30	17.00	9.14	8.22	6.83	1.05	0.63	0.64	0.46
	P18	35.20	1.80	19.00	12.63	12.30	11.80	1.25	0.84	0.72	0.50
	P19	37.60	1.70	17.00	12.19	11.72	11.30	1.25	0.72	0.71	0.50
	P20	38.70	1.60	23.00	8.26	8.05	7.73	1.05	0.61	0.63	0.45
Tirla (Solan, Himachal Pradesh)	P21	40.40	2.00	19.00	10.80	10.32	9.88	1.19	0.66	0.73	0.51
	P22	43.10	1.80	24.00	9.11	8.73	7.28	1.08	0.62	0.68	0.48
	P23	39.40	1.90	19.00	11.60	11.28	10.27	1.23	0.73	0.74	0.52
	P24	41.50	1.90	16.00	10.80	10.46	10.03	1.06	0.69	0.72	0.51
	P25	39.50	2.10	18.00	12.20	11.83	10.61	1.15	0.75	0.75	0.54
Siswa Dam (Sahibzada Ajit Singh Nagar, Punjab)	P26	46.30	1.30	21.00	11.37	10.58	9.25	1.23	0.92	0.79	0.55
	P27	47.40	1.70	19.00	10.66	10.37	8.97	1.13	0.78	0.73	0.51
	P28	45.70	1.30	19.00	11.63	11.31	10.21	1.12	0.82	0.72	0.51
	P29	44.20	1.30	20.00	10.69	10.38	9.49	1.07	0.72	0.68	0.48
	P30	43.30	1.20	19.00	10.57	10.33	8.26	1.27	0.78	0.83	0.58
Morni Hills (Panchkula, Haryana)	P31	48.30	1.40	22.00	10.92	10.47	9.64	1.17	0.79	0.63	0.45
	P32	50.60	1.40	23.00	10.41	10.13	8.59	1.08	0.61	0.68	0.47
	P33	45.70	1.20	24.00	10.65	10.22	7.83	1.13	0.74	0.73	0.52
	P34	46.80	1.30	28.00	10.34	9.74	8.23	1.25	0.87	0.77	0.54
	P35	49.40	1.50	29.00	10.83	10.58	10.05	1.17	0.73	0.70	0.49
	Mean	41.67	1.69	20.37	11.79	11.33	10.21	1.15	0.74	0.71	0.49
	CD _{0.05}	1.71	0.07	0.78	0.51	0.46	0.40	0.05	0.03	0.03	0.02
	CV	2.52	2.55	2.35	2.68	2.48	2.40	2.51	2.40	2.69	2.42

Where: PL is pod length (cm), PB is pod breadth (cm), S/P is seeds per pod, SWW is 100 seed weight with wings (g), SW is 100 seed weight without wings (g), SK is 100 seed kernel weight (g), SL is seed length (cm), SB is seed breadth (cm), SKL is seed kernel length (cm) and SKB is seed kernel breadth (cm).

variations recorded in seed traits may be associated to different genetic architecture advanced as a consequences of adaptation to varied environmental conditions prevalent throughout their distribution range (Salazar and Quesda, 1987). Similar results have been reported for pod length, pod girth, number of seeds and 100 seed weight (Tak, 2012) and Selvakumari (2013) for *Moringa oleifera* Lam. Results also agree with the findings in *Pongamia pinnata* (Divakara *et al.*, 2010) which also exhibited significant differences for seed length, and 100 seed weight. Kaushik *et al.*, (2007) reported significant differences for pod traits (length, breadth, thickness and 100 pod weight) and seed traits (length, breadth and 100 seed weight) among the 40 CPTs of *Pongamia pinnata*. Dao *et al.*, (2017) reported significant seed trait variations (length, thickness and weight) among West African provenances of *Moringa oleifera* Lam. Shaltout *et al.*, (2017) reposted similar findings in *Moringa oleifera* Lam. for pod length, pod breadth, seeds per pod and 100 seed weight. Osman and Abohassan (2012) reported significant results in pod length, seeds per pod and 100 seed weight for *Moringa peregrina*. Findings of Deepanjli (2018) showed significant seed length, seed breadth and 100 seed weight for *Toona ciliata* M. Roem.

Data pertaining to Table 7. indicated that maximum germination percentage of 93.33 was observed in P10 which was found to be statistically at par with P27 (91.67), P35 (91.67), P28 (90.00), P34 (86.67), P26 (85.00), P7 (83.33), P13 (83.33), P29 (88.33), P30 (88.33), P31 (88.33), P8 (81.67), P12 (81.67), P16 (81.67), P17 (80.00) and P24 (80.00). Minimum germination percentage was observed in P23 (25.00).

Non-significant differences were observed for survival percentage among the nursery genotypes Table 7.

Maximum germination index was observed in P27 (148.67) which was statistically at par with P29 (146.00), P30 (145.00), P28 (144.00), P26 (143.33), P35 (143.33), P34 (140.67), P31 (139.33), P16 (135.33), P10 (135.00), P8 (132.00), P24 (129.33) and P17 (129.00). Minimum germination index was observed in P23 (33.67).

Table 7. revealed that maximum germination value was observed in P27 (75.00) which was statistically at par with P26 (73.38), P29 (72.62), P34 (71.09), P30 (70.15), P35 (69.10), P28 (67.90), P31 (66.22), P10 (63.16), P24 (58.67), P17 (58.38), P8 (56.25) and P16 (54.30). Minimum germination value was observed in P23 (4.24).

Whatsoever variations observed might have been occurred either due to the genetic structure of plant or impact of parent plant environment (Bewely and Black (1994) and

Table: 7. Variation in germination parameters of *Moringa oleifera* Lam.

Seed sources (District, State/UT)	Progeny	Germination per cent (%)	Survival per cent (%)	Germination index	Germination value
Samba (Samba, Jammu and Kashmir)	P1	55.00	91.16	87.00	23.78
	P2	33.33	85.00	49.33	8.43
	P3	43.33	93.33	69.33	15.80
	P4	45.00	95.83	64.00	14.88
	P5	41.67	90.00	57.00	11.78
Dunera (Pathankot, Punjab)	P6	35.00	66.67	52.67	9.20
	P7	83.33	98.15	126.67	52.37
	P8	81.67	97.62	132.00	56.25
	P9	71.67	96.08	105.33	38.26
	P10	93.33	98.04	135.00	63.16
Vikasnagar (Dehradun, Uttarakhand)	P11	76.67	100.00	114.00	49.05
	P12	81.67	96.67	118.33	50.45
	P13	83.33	100.00	120.00	51.47
	P14	46.67	96.67	65.00	15.60
	P15	66.67	100.00	105.33	36.17
Lamlehri (Una, Himachal Pradesh)	P16	81.67	95.24	130.33	54.30
	P17	80.00	100.00	129.00	58.38
	P18	75.00	100.00	123.33	51.19
	P19	68.33	100.00	105.33	36.53
	P20	68.33	97.62	113.33	44.72
Tirla (Solan, Himachal Pradesh)	P21	41.67	92.59	64.67	15.22
	P22	61.67	97.22	96.67	33.70
	P23	25.00	83.33	33.67	4.24
	P24	80.00	94.41	129.33	58.67
	P25	73.33	100.00	116.33	49.28
Siswa Dam (Sahibzada Ajit Singh Nagar, Punjab)	P26	85.00	100.00	143.33	73.38
	P27	91.67	100.00	148.67	75.00
	P28	90.00	98.25	144.00	67.90
	P29	88.33	100.00	146.00	72.62
	P30	88.33	100.00	145.00	70.15
Morni Hills (Panchkula, Haryana)	P31	88.33	98.25	139.33	66.22
	P32	78.33	100.00	119.00	48.85
	P33	66.67	90.48	108.67	42.18
	P34	86.67	100.00	140.67	71.09
	P35	91.67	98.25	143.33	69.10
	Mean	69.95	95.74	109.17	44.55
	CD _{0.05}	14.81	NS	23.54	20.72
	CV	12.97		13.23	28.54

Fenner (1991). Isik (1986) stated that germination characters are assumed to be inherited traits affected by age, growth, macro and micro habitat of the mother tree. According to Guttermann (2000), seed germination can be greatly affected by maternal factors (position of seed in fruit, age of mother plant during seed maturation) as well as environmental factors (day length, light quality, moisture availability and altitude).

The results are in accordance with the findings of Deepanjli (2018) in *Toona ciliata* M. Roem and Fredrick *et al.*, (2015) in *Faidherbia albida*.

4.3 Nursery stage growth performance of progenies

a. Plant height (cm)

Examination of Table 8. illustrated that significant variation for plant height (cm) was observed at every 2 month intervals (Plate 4.).

After 2 months

Maximum plant height was observed in P34 (37.91 cm) which was statistically at par with P35 (37.12 cm), P28 (36.94 cm) and P26 (36.88 cm). Minimum plant height was observed in P5 (28.08 cm).

After 4 months

Maximum plant height was observed in P28 (78.03 cm) which was statistically at par with P35 (77.70 cm), P34 (76.41 cm), P29 (76.13 cm), P32 (75.63 cm), P33 (74.22 cm) and P27 (74.21 cm). Minimum plant height was observed in P5 (54.20 cm).

After 6 months

Maximum plant height was observed in P34 (115.82 cm) which was statistically at par with P35 (112.95 cm), P26 (111.91 cm), P29 (109.83 cm), P33 (108.82 cm), P28 (108.22) and P32 (105.93 cm). Minimum plant height was observed in P5 (67.08 cm).

After 8 months

Maximum plant height was observed in P35 (145.55 cm) which was statistically at par with P34 (143.92 cm), P28 (141.76 cm), P33 (136.77 cm), P29 (136.55 cm) and P26 (135.99 cm). Minimum plant height was observed in P1 (81.33 cm).

After 10 months

Maximum plant height was observed in P35 (180.33 cm) which was statistically at par with P34 (143.92 cm), P29 (136.35 cm), P28 (171.05 cm) and P33 (171.00 cm). Minimum plant height was observed in P1 (107.20 cm).



Plate: 4. *Moringa oleifera* Lam. progeny trial established at RHR&TS, Jachh

After 12 months

Maximum plant height was observed in P35 (234.44 cm) which was statistically at par with P34 (226.96 cm), P28 (219.42 cm), P29 (218.20 cm) and P26 (216.79 cm). Minimum plant height was observed in P1 (124.39 cm).

Table: 8. Nursery growth performance of *Moringa oleifera* Lam. progenies for plant height (cm)

Seed sources (District, State/UT)	Progeny	After 2 months	After 4 months	After 6 months	After 8 months	After 10 months	After 12 months
Samba (Samba, Jammu and Kashmir)	P1	30.33	56.63	67.78	81.33	107.20	124.39
	P2	32.52	62.20	69.94	97.29	114.33	135.89
	P3	31.28	57.98	76.21	99.45	113.91	148.36
	P4	32.31	62.13	77.86	95.04	121.98	150.84
	P5	28.08	54.20	67.08	92.20	110.38	133.29
Dunera (Pathankot, Punjab)	P6	30.01	57.63	76.51	92.96	112.89	140.88
	P7	30.47	57.76	76.57	93.50	119.08	142.77
	P8	31.36	60.87	81.03	96.92	123.27	150.03
	P9	29.68	56.92	72.31	97.13	113.70	142.22
	P10	28.84	56.93	71.53	89.60	111.58	132.07
Vikasnagar (Dehradun, Uttarakhand)	P11	34.52	69.42	89.27	115.81	134.49	173.03
	P12	35.55	73.87	93.94	116.33	146.47	190.53
	P13	35.51	70.97	91.67	120.73	138.67	178.13
	P14	33.45	69.11	85.92	103.45	138.54	169.79
	P15	34.65	69.98	91.06	100.69	134.45	167.58
Lamlehri (Una, Himachal Pradesh)	P16	31.78	62.73	81.98	101.09	116.00	153.96
	P17	31.70	62.70	74.78	98.27	122.52	142.98
	P18	31.69	62.00	79.73	94.13	122.21	150.30
	P19	30.54	57.90	79.58	98.33	120.39	148.42
	P20	31.76	60.67	74.49	99.06	117.41	155.58
Tirla (Solan, Himachal Pradesh)	P21	33.96	69.72	88.03	115.46	139.01	165.66
	P22	32.54	65.57	84.36	102.98	128.24	158.25
	P23	32.57	67.43	88.63	107.99	128.96	159.05
	P24	34.93	70.29	95.86	115.69	143.99	177.85
	P25	32.73	68.48	84.31	110.76	130.33	159.81
Siswa Dam (Sahibzada Ajit Singh Nagar, Punjab)	P26	36.88	73.20	111.91	135.99	164.69	216.79
	P27	35.89	74.21	100.28	131.88	160.13	211.14
	P28	36.94	78.03	108.22	141.76	171.05	219.42
	P29	36.61	76.13	109.83	136.35	172.29	218.20
	P30	36.00	73.70	105.71	128.45	165.92	208.90
Morni Hills (Panchkula, Haryana)	P31	32.68	66.18	88.27	109.60	131.16	161.72
	P32	36.28	75.63	105.93	132.42	166.88	205.72
	P33	36.37	74.22	108.82	136.77	171.00	213.50
	P34	37.91	76.41	115.82	143.92	175.55	226.96
	P35	37.12	77.70	112.95	145.55	180.33	234.44
	Mean	33.30	66.56	88.23	110.82	136.26	170.53
	CD _{0.05}	1.26	4.00	9.93	12.00	13.33	18.96
	CV	2.33	3.692	6.91	6.65	6.00	6.83

b. Collar diameter (mm): Data pertaining to collar diameter (mm) showed significant variation at every month interval (Table 9).

After 2 months

Maximum collar diameter was observed in P34 (4.37 mm) which was statistically at par with P35 (4.29 mm), P33 (4.16 mm), P32 (4.15 mm) and P30 (4.09 mm). Minimum collar diameter was observed in P3 (2.40 mm).

After 4 months

Maximum collar diameter was observed in P34 (10.99 mm) which was statistically at par with P35 (10.34 mm) and P33 (10.41 mm). Minimum collar diameter was observed in P3 (5.57 mm).

After 6 months

Maximum collar diameter was observed in P34 (17.12 mm) whereas minimum collar diameter was observed in P3 (8.85 mm).

After 8 months

Maximum collar diameter was observed in P34 (22.42 mm) which was statistically at par with P35 (20.82 mm). Minimum collar diameter was observed in P3 (11.54 mm).

After 10 months

Maximum collar diameter was observed in P34 (27.56 mm) which was statistically at par with P35 (25.20 mm) and P33 (25.19 mm). Minimum collar diameter was observed in P3 (13.49 mm).

After 12 months

Maximum collar diameter was observed in P34 (34.85 mm) whereas minimum collar diameter was observed in P1 (16.75 mm).

c. Internodal length (mm): Data presented in Table 10. showed significant variation in internodal length (cm) for every 2 month interval.

After 2 months

Maximum internodal length was observed in P34 (3.31 cm) which was statistically at par with P35 (3.27 cm), P32 (3.26 cm), P30 (3.17 cm), P29 (3.15 cm), P28 (3.13 cm), P27 (3.12 cm), P33 (3.10 cm), P31 (3.09 cm), P9 (3.07 cm), P10 (3.04 cm), P7 (3.03 cm), P16 (3.01 cm) and P15 (2.99 cm). Minimum internodal length was observed in P18 (2.22 cm).

Table: 9. Nursery growth performance of *Moringa oleifera* Lam. progenies for collar diameter (mm)

Seed sources (District, State/UT)	Progeny	After 2 months	After 4 months	After 6 months	After 8 months	After 10 months	After 12 months
Samba (Samba, Jammu and Kashmir)	P1	2.88	6.76	10.21	13.24	15.73	16.75
	P2	3.47	7.61	12.60	16.05	19.95	21.48
	P3	2.40	5.57	8.85	11.54	13.49	16.92
	P4	2.87	6.57	10.17	13.33	16.38	18.68
	P5	3.42	7.75	11.70	16.76	20.41	22.00
Dunera (Pathankot, Punjab)	P6	3.27	8.35	12.22	16.57	20.29	22.69
	P7	2.90	7.00	10.65	12.95	16.61	20.90
	P8	3.21	7.73	11.93	15.49	19.34	22.35
	P9	2.62	6.03	9.37	11.95	14.57	17.76
	P10	2.99	7.66	11.67	15.01	18.39	22.34
Vikasnagar (Dehradun, Uttarakhand)	P11	2.54	5.84	9.89	11.29	14.36	17.53
	P12	3.24	7.92	12.08	16.02	20.04	24.55
	P13	3.28	8.38	11.90	16.57	20.30	23.54
	P14	3.48	8.45	12.93	17.09	20.77	24.42
	P15	3.53	8.54	13.13	17.25	21.10	25.57
Lamlehri (Una, Himachal Pradesh)	P16	2.63	6.33	9.62	12.32	15.00	17.65
	P17	3.55	7.95	12.54	16.92	20.69	22.52
	P18	3.03	7.44	11.26	14.93	18.78	22.01
	P19	2.89	6.85	10.55	13.92	17.01	20.38
	P20	3.18	7.62	12.21	15.32	19.15	22.11
Tirla (Solan, Himachal Pradesh)	P21	3.02	7.99	11.79	14.48	18.55	22.72
	P22	3.57	8.35	12.96	17.15	21.37	24.78
	P23	3.16	8.13	11.80	15.08	18.92	22.46
	P24	2.94	6.44	10.27	12.61	15.35	18.14
	P25	3.52	8.67	13.17	17.77	21.75	26.51
Siswa Dam (Sahibzada Ajit Singh Nagar, Punjab)	P26	3.97	9.48	14.59	18.58	23.53	27.57
	P27	3.71	9.35	14.06	18.46	22.60	24.72
	P28	3.80	9.07	13.46	18.42	22.67	27.43
	P29	3.51	9.08	13.59	17.83	21.81	25.85
	P30	4.09	9.73	15.35	19.69	24.05	27.90
Morni Hills (Panchkula, Haryana)	P31	3.65	9.17	13.71	17.98	21.99	26.89
	P32	4.15	9.84	14.52	19.84	24.91	30.28
	P33	4.16	10.41	15.28	19.94	25.19	31.26
	P34	4.37	10.99	17.12	22.42	27.56	34.85
	P35	4.29	10.34	15.45	20.82	25.20	31.11
	Mean	3.35	8.10	12.36	16.16	19.94	23.56
	CD_{0.05}	0.33	0.89	1.37	2.02	2.73	3.49
	CV	6.10	6.756	6.79	7.67	8.40	9.08

After 4 months

Maximum internodal length was observed in P35 (4.49 cm) which was statistically at par with P34 (4.43 cm), P28 (4.31 cm), P3 (4.23 cm), P29 (4.07 cm), P33 (4.07 cm), P32 (4.06 cm), P5 (4.04 cm), P27 (4.04 cm), P30 (4.04 cm), P26 (4.03 cm), P24 (40.1 cm), P2 (3.98 cm), P6 (3.98 cm), P1(3.96 cm), P7 (3.95 cm), P14 (3.95 cm), P19 (3.93 cm), P31 (3.91 cm), P4 (3.88 cm), P8 (3.88 cm), P10 (3.87 cm), P13 (3.85 cm) and P23 (3.84 cm). Minimum internodal length was observed in P20 (3.37 cm).

After 6 months

Maximum internodal length was observed in P35 (4.77 cm) which was statistically at par with P28 (4.75 cm), P3 (4.74 cm), P29 (4.73 cm), P32 (4.69 cm), P30 (4.68 cm), P27 (4.66 cm), P5 (4.64 cm), P26 (4.59 cm), P24 (4.57 cm), P6 (4.49 cm), P34 (4.43 cm), P2 (4.41 cm) and P1 (4.28 cm). Minimum internodal length was observed in (3.62 cm).

After 8 months

Maximum internodal length was observed in P35 (5.12 cm) which was statistically at par with P34 (5.09 cm), P28 (5.05 cm), P3 (5.02 cm), P29 (5.01 cm), P33 (4.89 cm), P32 (4.85 cm) and P30 (4.76 cm). Minimum internodal length was observed in (3.89 cm).

After 10 months

Maximum internodal length was observed in P35 (5.50 cm) which was statistically at par with P28 (5.48 cm), P3 (5.42 cm), P29 (5.42 cm), P33 (5.21 cm) and P34 (5.19 cm), P30 (5.17 cm), P32 (5.17 cm) and P1 (5.15 cm). Minimum internodal length was observed in (4.23 cm).

After 12 months

Maximum internodal length was observed in P35 (5.54 cm) which was statistically at par with P34 (5.53 cm), P28 (5.48 cm), P3 (5.44 cm), P29 (5.42 cm), P33 (5.40 cm), P32 (5.37 cm), P30 (5.36 cm), P27 (5.30 cm) and P5 (5.29 cm). Minimum internodal length was observed in P20 (4.82 cm).

Table: 10. Nursery growth performance of *Moringa oleifera* Lam. progenies for internodal length (cm)

Seed sources (District, State/UT)	Progeny	After 2 months	After 4 months	After 6 months	After 8 months	After 10 months	After 12 months
Samba (Samba, Jammu and Kashmir)	P1	2.69	3.96	4.38	4.70	5.15	5.15
	P2	2.77	3.98	4.41	4.58	4.93	5.17
	P3	3.02	4.23	4.74	5.02	5.42	5.44
	P4	2.69	3.88	4.11	4.43	4.99	5.05
	P5	2.73	4.04	4.64	4.70	4.96	5.29
Dunera (Pathankot, Punjab)	P6	2.65	3.98	4.49	4.68	5.00	5.17
	P7	3.03	3.95	4.29	4.54	5.03	5.15
	P8	2.92	3.88	4.12	4.43	4.81	5.07
	P9	3.07	3.78	4.02	4.24	4.54	4.98
	P10	3.04	3.87	4.06	4.37	4.87	5.03
Vikasnagar (Dehradun, Uttarakhand)	P11	2.46	3.52	3.68	3.96	4.32	4.87
	P12	2.38	3.82	4.03	4.47	4.99	4.99
	P13	2.89	3.85	4.06	4.36	4.79	5.03
	P14	2.88	3.95	4.25	4.49	4.94	5.10
	P15	2.99	3.77	3.98	4.23	4.59	4.97
Lamlehri (Una, Himachal Pradesh)	P16	3.01	3.58	3.89	4.02	4.39	4.94
	P17	2.25	3.59	3.91	4.13	4.48	4.95
	P18	2.22	3.54	3.84	3.99	4.35	4.93
	P19	2.23	3.93	4.15	4.45	4.96	5.08
	P20	2.28	3.37	3.62	3.89	4.31	4.82
Tirla (Solan, Himachal Pradesh)	P21	2.44	3.61	3.92	4.19	4.65	4.95
	P22	2.34	3.53	3.69	3.95	4.43	4.91
	P23	2.26	3.84	4.04	4.35	4.82	5.02
	P24	3.09	4.01	4.57	4.64	4.94	5.21
	P25	2.39	3.44	3.67	3.91	4.23	4.84
Siswa Dam (Sahibzada Ajit Singh Nagar, Punjab)	P26	2.73	4.03	4.59	4.70	4.94	5.22
	P27	3.12	4.04	4.66	4.73	4.98	5.30
	P28	3.13	4.31	4.75	5.05	5.48	5.48
	P29	3.15	4.07	4.73	5.01	5.42	5.42
	P30	3.17	4.05	4.68	4.76	5.17	5.36
Morni Hills (Panchkula, Haryana)	P31	3.09	3.93	4.22	4.60	5.09	5.09
	P32	3.26	4.06	4.69	4.85	5.17	5.37
	P33	3.10	4.07	4.71	4.89	5.21	5.40
	P34	3.31	4.43	4.76	5.09	5.49	5.53
	P35	3.27	4.49	4.77	5.12	5.50	5.54
	Mean	2.80	3.90	4.26	4.50	4.90	5.14
	CD_{0.05}	0.37	0.65	0.39	0.38	0.38	0.31
	CV	8.06	10.20	5.54	5.17	4.79	3.66

d. Petiole length (cm): It is evident from the Table 11. that significant variation was observed in petiole length (cm) for every 2 month interval.

After 2 months

Maximum petiole length was observed in P34 (3.16 cm) whereas minimum petiole length of 2.14 cm was observed for both P14 and P19.

After 4 months

Maximum petiole length was observed in P33 (5.55 cm) which was statistically at par with P30 (5.46 cm), P31 (5.34 cm), P29 (5.24 cm) and P35 (5.24 cm). Minimum petiole length was observed in P8 (3.34 cm).

After 6 months

Maximum petiole length was observed in P33 (7.28 cm) which was statistically at par with P34 (7.13 cm), P30 (7.07 cm), P31 (7.06 cm), P29 (7.03 cm) and P35 (6.96 cm). Minimum petiole length was observed in P8 (4.73 cm).

After 8 months

Maximum petiole length was observed in P33 (9.26 cm) which was statistically at par with P30 (9.00 cm), P31 (8.87 cm) and P35 (8.77 cm). Minimum petiole length was observed in P8 (5.93 cm).

After 10 months

Maximum petiole length was observed in P3 (10.49 cm) which was statistically at par with P28 (10.41 cm), P31 (10.37 cm), P35 (10.34 cm), P30 (10.27 cm), P29 (10.13 cm), P34 (10.05 cm) and P32 (10.00 cm). Minimum petiole length was observed in P8 (7.89 cm).

After 12 months

Maximum petiole length was observed in P33 (11.32 cm) which was statistically at par with P30 (11.29 cm), P31 (11.13 cm), P35 (11.08 cm), P29 (11.06 cm), P34 (11.05 cm), P32 (10.93 cm), P28 (10.86 cm) and P26 (10.79 cm). Minimum petiole length was observed in P8 (8.41 cm).

Table: 11. Nursery growth performance of *Moringa oleifera* Lam. progenies for petiole length (cm)

Seed sources (District, State/UT)	Progeny	After 2 months	After 4 months	After 6 months	After 8 months	After 10 months	After 12 months
Samba (Samba, Jammu and Kashmir)	P1	2.21	3.88	4.94	6.12	8.09	8.63
	P2	2.55	3.65	5.19	6.35	8.23	9.11
	P3	2.15	3.46	4.87	6.21	8.04	8.57
	P4	2.68	3.75	5.24	6.39	8.46	9.22
	P5	2.37	3.94	5.44	6.64	8.53	9.75
Dunera (Pathankot, Punjab)	P6	3.50	4.15	5.82	6.93	8.73	10.03
	P7	2.28	3.54	4.83	6.34	7.94	8.51
	P8	2.20	3.34	4.73	5.93	7.89	8.41
	P9	2.51	3.83	5.32	6.40	8.34	9.33
	P10	2.47	4.06	5.64	6.81	8.72	9.83
Vikasnagar (Dehradun, Uttarakhand)	P11	2.55	3.61	5.01	6.37	8.24	8.76
	P12	2.23	3.99	5.38	6.46	8.46	9.56
	P13	2.59	3.52	4.97	6.35	8.12	8.68
	P14	2.14	4.01	5.59	6.74	8.65	9.48
	P15	2.75	4.26	5.90	7.05	8.86	10.06
Lamlehri (Una, Himachal Pradesh)	P16	2.91	4.21	5.86	6.99	8.81	10.03
	P17	2.67	4.46	5.91	7.08	9.28	10.00
	P18	2.56	4.27	5.96	7.06	9.15	10.07
	P19	2.14	4.54	6.13	7.16	9.46	10.23
	P20	2.76	4.51	6.01	7.20	9.39	10.16
Tirla (Soln, Himachal Pradesh)	P21	2.66	4.62	6.07	7.47	9.50	10.34
	P22	2.51	4.78	6.68	8.10	9.74	10.74
	P23	2.73	4.67	6.25	7.69	9.45	10.46
	P24	2.85	4.65	6.24	7.78	9.33	10.33
	P25	2.85	4.70	6.65	7.90	9.66	10.71
Siswa Dam (Sahibzada Ajit Singh Nagar, Punjab)	P26	2.58	4.99	6.58	8.18	9.81	10.79
	P27	2.33	4.69	6.10	7.54	9.25	10.41
	P28	2.63	5.07	6.76	8.26	10.41	10.86
	P29	2.44	5.24	7.03	8.63	10.13	11.06
	P30	2.50	5.46	7.07	9.00	10.27	11.29
Morni Hills (Panchkula, Haryana)	P31	2.62	5.34	7.06	8.87	10.37	11.13
	P32	2.49	5.13	6.71	8.45	10.00	10.93
	P33	2.60	5.55	7.28	9.26	10.49	11.32
	P34	3.16	5.11	7.13	8.63	10.05	11.05
	P35	2.34	5.24	6.96	8.77	10.34	11.08
	Mean	2.56	4.41	5.98	7.35	9.15	10.03
	CD _{0.05}	0.16	0.38	0.45	0.57	0.52	0.57
	CV	3.83	5.23	4.64	4.75	3.50	3.48

e. Leaf angle ($^{\circ}$): Data related to leaf angle ($^{\circ}$) revealed significant variation at every 2 month interval (Table 12).

After 2 months

Maximum leaf angle was observed in P26 (76.73°) which was statistically at par with P27 (76.70°), P35 (74.37°) and P2 (74.17°). Minimum leaf angle was observed in P10 (56.03°).

After 4 months

Maximum leaf angle was observed in P26 (71.60°) which was statistically at par with P14 (71.30°), P22 (70.07°), P35 (69.97°), P27 (69.53°) and P30 (68.27°). Minimum leaf angle was observed in P9 (56.33°).

After 6 months

Maximum leaf angle was observed in P26 (71.07°) which was statistically at par with P25 (70.83°), P18 (70.77°), P14 (70.43°), P17 (70.40°), P22 (70.10°) and P11 (69.77°). Minimum leaf angle was observed in P1 (56.73°).

After 8 months

Maximum leaf angle was observed in P26 (72.23°) which was statistically at par with P35 (71.63°), P22 (70.20°) and P27 (68.73°). Minimum leaf angle was observed in P4 (56.27°).

After 10 months

Maximum leaf angle was observed in P22 (74.00°) which was statistically at par with P26 (73.80°), P27 (73.77°), P35 (72.27°), P14 (71.37°), P25 (71.10°) and P17 (70.30°). Minimum leaf angle was observed in P7 (55.57°).

After 12 months

Maximum leaf angle was observed in P27 (75.17°) which was statistically at par with P14 (74.97°), P35 (74.40°), P24 (73.80°), P26 (73.00°) and P22 (72.10°). Minimum leaf angle was observed in P23 (56.07°).

Table: 12. Nursery growth performance of *Moringa oleifera* Lam. progenies for leaf angle ($^{\circ}$)

Seed sources (District, State/UT)	Progeny	After 2 months	After 4 months	After 6 months	After 8 months	After 10 months	After 12 months
Samba (Samba, Jammu and Kashmir)	P1	59.43	63.80	56.73	59.87	57.83	56.10
	P2	65.60	56.73	61.60	59.20	67.20	62.73
	P3	65.53	67.33	64.40	56.67	67.20	66.20
	P4	57.93	58.27	60.27	56.27	59.20	58.53
	P5	58.13	58.40	60.07	57.13	58.60	59.07
Dunera (Pathankot, Punjab)	P6	66.67	57.80	62.43	60.37	63.97	60.37
	P7	57.97	61.20	58.00	59.87	55.57	59.60
	P8	58.93	59.27	58.67	57.13	58.73	58.20
	P9	56.67	56.33	60.20	60.53	60.23	55.23
	P10	56.03	60.13	59.53	58.13	61.40	61.30
Vikasnagar (Dehradun, Uttarakhand)	P11	70.13	66.53	69.77	65.50	68.43	68.93
	P12	66.67	57.57	58.97	59.67	65.10	57.80
	P13	58.17	61.27	59.00	58.87	59.90	63.43
	P14	72.37	71.30	70.43	72.03	71.37	74.97
	P15	66.23	64.97	65.53	57.80	65.20	65.27
Lamlehri (Una, Himachal Pradesh)	P16	59.13	58.60	57.53	56.87	58.67	58.87
	P17	70.80	67.13	70.40	66.23	70.30	70.13
	P18	70.17	64.07	70.77	66.23	68.53	69.07
	P19	59.10	60.73	59.07	59.93	61.77	56.13
	P20	65.57	59.03	61.70	59.17	65.97	59.93
Tirla (Solan, Himachal Pradesh)	P21	58.93	59.80	59.53	57.47	58.53	59.87
	P22	74.17	70.07	70.10	70.20	74.00	72.10
	P23	65.00	61.97	59.83	60.93	66.90	56.07
	P24	57.53	61.23	61.63	61.47	55.07	73.80
	P25	69.13	65.53	70.83	66.03	71.10	69.30
Siswa Dam (Sahibzada Ajit Singh Nagar, Punjab)	P26	76.73	71.60	71.07	72.23	73.80	73.00
	P27	76.70	69.53	69.17	68.73	73.77	75.17
	P28	67.60	67.80	66.40	61.43	66.53	64.57
	P29	67.53	66.13	66.93	62.43	66.53	65.77
	P30	66.80	68.27	65.43	61.20	65.13	66.30
Morni Hills (Panchkula, Haryana)	P31	59.23	58.33	59.87	59.27	62.63	60.50
	P32	59.60	60.00	62.23	60.67	59.00	61.33
	P33	59.57	58.57	56.67	60.33	55.70	57.23
	P34	70.47	66.90	68.53	64.73	69.73	68.87
	P35	74.37	69.97	69.40	71.63	72.27	74.40
	Mean	64.70	63.03	63.51	61.89	64.45	64.00
	CD_{0.05}	4.18	3.60	4.13	4.12	4.47	4.76
	CV	3.97	3.50	3.99	4.09	4.26	4.56

f. Number of leaves: It is apparent from the Table 13. that significant variation was obtained number of leaves for every 2 month interval.

After 2 months

Maximum number of leaves was observed in P28 (7.33) which was statistically at par with P26 (7.27), P32 (7.27), P14 (7.20), P27 (7.20), P29 (7.20), P30 (7.20), P31 (7.20), P13 (7.07), P33 (7.07), P34 (7.07), P35 (7.07), P11 (6.93), P15 (6.93), P23 (6.93), P3 (6.87), P8 (6.87), P10 (6.87), P12 (6.87), P18 (6.87), P22 (6.87) and P25 (6.87). Minimum number of leaves (6.27) was observed both P1 and P4.

After 4 months

Maximum number of leaves was observed in P35 (14.74) which was statistically at par with P31 (13.95) and P27 (13.92). Minimum number of leaves was observed in P1 (9.57).

After 6 months

Maximum number of leaves was P35 (23.41) which was statistically at par with P33 (22.59), P31 (22.37) and P34 (21.91). Minimum number of leaves was observed in P1 (14.09).

After 8 months

Maximum number of leaves was observed in P33 (27.75) which was statistically at par with P34 (27.67), P31 (27.46), P32 (27.33), P30 (26.96), and P35 (26.94), P29 (26.92), P26 (26.48), P27 (26.35) and P28 (26.33). Minimum number of leaves was observed in P3 (16.56).

After 10 months

Maximum number of leaves was observed in P33 (34.22) which was statistically at par with P35 (33.83), P31 (33.42), P34 (32.83), P29 (32.47), P30 (32.46), P32 (32.18), P28 (31.73), and P27 (31.55). Minimum number of leaves was observed in P2 (21.18).

After 12 months

Maximum number of leaves was observed in P35 (43.20) which was statistically at par with P34 (42.73), P33 (41.87), P32 (41.60), P31 (40.67), P30 (40.47), P29 (40.07), P28 (39.80), P27 (39.78). Minimum number of leaves was observed in P2 (26.13).

Table: 13. Nursery growth performance of *Moringa oleifera* Lam. progenies for number of leaves

Seed sources (District, State/UT)	Progeny	After 2 months	After 4 months	After 6 months	After 8 months	After 10 months	After 12 months
Samba (Samba, Jammu and Kashmir)	P1	6.27	9.57	14.09	16.80	22.12	27.28
	P2	6.60	9.85	14.43	17.68	21.18	26.33
	P3	6.87	9.99	14.77	16.56	21.43	27.06
	P4	6.27	9.85	14.83	17.11	22.15	26.39
	P5	6.60	10.23	15.45	18.88	23.36	27.56
Dunera (Pathankot, Punjab)	P6	6.53	10.16	15.11	17.73	22.72	28.06
	P7	6.80	10.91	15.25	17.81	22.77	28.50
	P8	6.87	10.79	15.38	18.64	22.36	29.44
	P9	6.80	11.43	15.38	18.68	24.00	29.56
	P10	6.87	10.69	15.19	17.68	23.54	30.06
Vikasnagar (Dehradun, Uttarakhand)	P11	6.93	11.00	15.73	18.47	22.96	30.48
	P12	6.87	12.60	18.25	23.75	27.85	33.80
	P13	7.07	12.01	18.28	22.23	27.65	34.20
	P14	7.20	12.76	19.66	23.23	28.53	34.33
	P15	6.93	12.12	18.63	23.48	28.72	34.93
Lamlehri (Una, Himachal Pradesh)	P16	6.67	11.61	17.23	22.51	28.50	35.07
	P17	6.80	12.24	18.36	23.13	29.21	35.33
	P18	6.87	12.53	18.85	23.85	28.86	35.53
	P19	6.67	12.29	18.58	23.20	28.82	35.87
	P20	6.73	12.21	18.36	23.17	29.40	35.87
Tirla (Solun, Himachal Pradesh)	P21	6.80	12.21	18.63	23.04	29.01	36.00
	P22	6.87	12.39	18.57	23.43	28.67	36.33
	P23	6.93	12.81	18.69	23.58	29.06	36.40
	P24	6.80	12.26	17.72	23.18	29.54	36.80
	P25	6.87	12.39	17.85	22.44	28.63	36.80
Siswa Dam (Sahibzada Ajit Singh Nagar, Punjab)	P26	7.27	13.67	20.41	26.48	31.21	38.53
	P27	7.20	13.92	20.64	26.35	31.55	39.78
	P28	7.33	13.66	20.30	26.33	31.73	39.80
	P29	7.20	13.77	20.71	26.92	32.47	40.07
	P30	7.20	13.79	21.71	26.96	32.46	40.47
Morni Hills (Panchkula, Haryana)	P31	7.20	13.95	22.37	27.46	33.42	40.67
	P32	7.27	13.73	21.53	27.33	32.18	41.60
	P33	7.07	13.83	22.59	27.75	34.22	41.87
	P34	7.07	13.49	21.91	27.67	32.83	42.73
	P35	7.07	14.74	23.41	26.94	33.83	43.20
	Mean	6.90	12.16	18.25	22.58	27.91	34.72
	CD_{0.05}	0.46	0.87	1.51	2.14	2.99	4.27
	CV	4.06	4.41	5.08	5.81	6.56	7.53

g. Leaf area (cm²): Table 14. revealed significant variation in leaf area (cm²) after every 2 month interval (Plate 5.).

After 2 months

Maximum leaf area was observed in P35 (106.70 cm²) which was statistically at par with P30 (105.35 cm²), P32 (102.86 cm²), P33 (102.30 cm²) and P34 (102.04 cm²). Minimum leaf area was observed in P3 (74.72 cm²).

After 4 months

Maximum leaf area was observed in P30 (198.03 cm²) which was statistically at par with P35 (192.30 cm²), P33 (190.06 cm²) and P32 (189.14 cm²). Minimum leaf area was observed in P3 (131.79 cm²).

After 6 months

Maximum leaf area was observed in P30 (356.52 cm²) which was statistically at par with P35 (356.26 cm²), P32 (349.54 cm²) and P33 (349.35 cm²). Minimum leaf area was observed in P3 (260.89 cm²).

After 8 months

Maximum leaf area was observed in P30 (647.47 cm²) which was statistically at par with P33 (641.56 cm²), P32 (638.55 cm²), P35 (637.71 cm²) and P22 (627.48 cm²). Minimum leaf area was observed in P3 (422.45 cm²).

After 10 months

Maximum leaf area was observed in P30 (841.63 cm²) which was statistically at par with P33 (837.94 cm²), P32 (834.68 cm²), P35 (830.92 cm²) and P34 (813.78 cm²). Minimum leaf area was observed in P3 (558.20 cm²).

After 12 months

Maximum leaf area was observed in P30 (1014.09 cm²) which was statistically followed by P35 (998.48 cm²) whereas minimum leaf area was observed in P3 (654.04 cm²).

Plants require certain amount of time to exhibit consistent performance. Morphological traits, viz., seedling height, number of leaves per plant and root length varied significantly among seed sources. In overall performance, the progenies selected from Siswa Dam and Morni hills exhibited outstanding growth in terms of morphological characters as well as biomass characters. Juvenile growth traits are controlled by genetic structure, environment and the seed quality or seed characters (Dunlap and Barnett 1983, Pathak *et al.*,



Plate: 5. Measuring leaf area using leaf area meter



6a



6b

Plate: 6. Proximate principles analysis of dried leaves
6a. ether extract using soxhlet apparatus
6b. ash content using muffle furnace

Table: 14. Nursery growth performance of *Moringa oleifera* Lam. progenies for leaf area (cm²)

Seed sources (District, State/UT)	Progeny	After 2 months	After 4 months	After 6 months	After 8 months	After 10 months	After 12 months
Samba (Samba, Jammu and Kashmir)	P1	88.87	166.04	281.62	540.24	698.47	848.27
	P2	80.06	141.35	263.38	463.73	604.51	697.49
	P3	74.72	131.79	260.89	422.45	558.20	654.04
	P4	88.35	160.58	276.69	549.36	716.12	851.01
	P5	83.87	152.16	273.45	474.55	615.08	736.51
Dunera (Pathankot, Punjab)	P6	82.62	146.80	274.93	456.35	592.31	703.08
	P7	78.69	141.12	266.31	454.55	595.71	695.42
	P8	87.76	160.59	288.49	523.35	679.21	816.10
	P9	88.20	169.58	294.67	537.18	698.28	849.07
	P10	76.62	134.61	279.46	435.75	574.59	663.66
Vikasnagar (Dehradun, Uttarakhand)	P11	86.81	162.59	289.87	518.99	671.46	837.03
	P12	78.12	139.01	279.53	445.16	585.53	678.66
	P13	92.26	169.46	299.48	576.38	747.75	879.57
	P14	87.03	160.02	291.94	523.36	678.21	825.45
	P15	78.63	141.16	285.64	449.32	590.66	685.64
Lamlehri (Una, Himachal Pradesh)	P16	94.07	178.27	322.35	579.19	746.67	910.28
	P17	85.97	156.92	297.01	501.81	652.57	771.85
	P18	87.41	157.72	303.46	517.99	675.07	795.31
	P19	81.10	140.42	301.56	458.06	603.31	686.83
	P20	83.51	149.14	298.79	488.79	638.55	745.28
Tirla (Solán, Himachal Pradesh)	P21	86.39	157.46	306.85	500.01	647.07	783.90
	P22	98.62	185.21	332.04	627.48	820.26	951.08
	P23	98.20	182.69	333.83	615.05	801.26	929.41
	P24	92.85	177.30	318.61	587.44	759.61	898.94
	P25	98.43	183.72	332.05	600.86	781.07	937.73
Siswa Dam (Sahibzada Ajit Singh Nagar, Punjab)	P26	91.23	171.42	302.20	555.28	715.07	856.01
	P27	83.14	145.67	293.72	464.30	603.80	718.63
	P28	91.80	172.89	301.93	576.95	747.35	883.51
	P29	95.15	182.12	342.40	612.32	794.46	923.45
	P30	105.35	198.03	356.52	647.47	841.63	1014.09
Morni Hills (Panchkula, Haryana)	P31	94.12	181.02	336.52	596.73	773.68	917.56
	P32	102.86	189.14	349.54	638.55	834.68	980.36
	P33	102.30	190.06	349.35	641.56	837.94	975.91
	P34	102.04	184.33	342.84	623.36	813.78	963.87
	P35	106.70	192.30	356.26	637.71	830.92	988.48
	Mean	89.54	164.36	305.27	538.33	700.71	830.10
	CD_{0.05}	4.51	9.07	13.07	22.90	28.74	33.68
	CV	3.09	3.39	2.63	2.61	2.52	2.49

1984). Since the seedlings from the seeds of plus trees of all 35 mother trees were raised under the similar nursery conditions, so variation observed among seed sources as well as among the genotypes might be attributed to genetic potential of individual progenies and the variations noticed in seed traits. Similarly, significant variations among progenies for growth traits were reported in *Acacia nilotica* (Gupta *et al.*, 2020), *Cedrus deodara* (Banyal and Sharma 2015), *Acacia nilotica* (Arya and Toky 2006), *Celtis australis* (Singh *et al.*, 2006) and *Magnolia officinalis* (Shu *et al.*, 2012). Gupta *et al.*, (2020) found significant results for seedling height, collar diameter, internodal length, fresh shoot weight, fresh root weight, dry shoot weight, dry root weight, root: shoot ratio and seedling biomass for *Acacia nilotica*.

4.4 Variation in biomass characters

Variations between the progenies of naturally and widely distributed species growing under the different geographic conditions are expected to be occurred partly due to genotypic and environmental differences. Analysis of variance revealed the significant differences among the progenies for biomass characters and their ratios thus predicting the occurrence of considerable variability (Table 15.).

a. Fresh shoot weight (g)

Maximum fresh shoot weight (g) was observed in P30 (1509.01 g) which was statistically at par with P29 (1458.92 g), P32 (1433.41 g), P33 (1403.03 g), P28 (1386.28 g), P34 (1376.79 g) and P31 (1362.39 g). Minimum fresh shoot weight was observed in P7 (792.16 g).

b. Fresh root weight (g)

Maximum fresh root weight (g) was observed in P35 (634.41 g) which was statistically at par with P27 (630.09 g), P33 (624.50 g), P22 (590.93 g), P32 (586.39 g), P29 (570.50 g), P28 (558.84 g) and P31 (552.04 g). Minimum fresh root biomass was observed in P3 (332.77 g).

c. Total fresh weight (g)

Maximum total fresh weight (g) was observed in P29 (2029.43 g) which was statistically at par with P33 (2027.53 g), P32 (2019.80 g) and P30 (2007.87 g). Minimum total fresh weight was observed in P3 (1175.66 g).

d. Dry shoot weight (g)

Maximum dry shoot weight (g) was observed in P35 (412.44 g) which was statistically at par with P29 (375.51 g), P23 (373.70 g), P22 (371.48 g), P18 (361.45 g) and P33 (358.28 g). Minimum dry shoot weight was observed in P2 (193.23 g).

Table: 15. Variation in shoot and root biomass and their ratios of *Moringa oleifera* Lam. progenies

Seed sources (District, State/UT)	Progeny	Fresh shoot weight (g)	Fresh root weight (g)	Total fresh weight (g)	Dry shoot weight (g)	Dry root weight (g)	Fresh root shoot ratio	Dry root shoot ratio
Samba (Samba, Jammu and Kashmir)	P1	948.56	369.45	1318.01	246.79	85.98	0.39	0.35
	P2	897.03	407.13	1304.15	193.23	90.74	0.46	0.47
	P3	842.89	332.77	1175.66	244.27	102.75	0.40	0.42
	P4	961.43	454.21	1415.64	276.00	91.80	0.47	0.33
	P5	877.38	371.06	1248.45	246.15	89.23	0.43	0.38
Dunera (Pathankot, Punjab)	P6	845.68	510.28	1355.96	270.39	114.02	0.60	0.44
	P7	792.16	396.41	1188.57	229.19	91.41	0.50	0.40
	P8	896.45	386.08	1282.53	314.52	127.54	0.43	0.41
	P9	871.75	446.37	1318.11	215.51	99.39	0.53	0.46
	P10	931.70	351.56	1283.26	229.67	116.44	0.38	0.53
Vikasnagar (Dehradun, Uttarakhand)	P11	907.09	357.08	1264.17	247.96	91.83	0.39	0.37
	P12	1092.79	508.35	1601.14	225.80	93.37	0.47	0.42
	P13	957.96	394.52	1352.48	207.29	109.42	0.41	0.53
	P14	1111.63	364.77	1476.40	311.13	124.76	0.33	0.40
	P15	1051.66	414.30	1465.96	281.48	108.62	0.40	0.39
Lamlehri (Una, Himachal Pradesh)	P16	976.71	443.53	1420.24	262.13	104.16	0.45	0.40
	P17	1233.52	421.97	1655.48	281.05	105.45	0.35	0.38
	P18	874.95	414.36	1289.31	361.45	109.16	0.48	0.31
	P19	1067.57	429.55	1497.11	257.34	100.88	0.41	0.40
	P20	1191.52	448.18	1639.69	238.16	93.34	0.38	0.40
Tirla (Solan, Himachal Pradesh)	P21	975.83	405.84	1381.66	294.29	103.40	0.42	0.35
	P22	1066.03	590.93	1656.96	371.98	107.48	0.57	0.29
	P23	1020.31	415.34	1435.64	373.70	163.47	0.41	0.44
	P24	1195.29	408.29	1603.59	232.50	101.64	0.35	0.48
	P25	980.24	402.51	1382.75	283.69	135.74	0.41	0.48
Siswa Dam (Sahibzada Ajit Singh Nagar, Punjab)	P26	1081.29	484.67	1565.96	251.45	102.53	0.45	0.41
	P27	1280.70	630.09	1910.79	236.50	153.61	0.50	0.65
	P28	1386.28	558.84	1945.13	252.54	122.06	0.40	0.49
	P29	1458.92	570.50	2029.43	357.51	160.35	0.40	0.45
	P30	1509.01	498.86	2007.87	338.58	104.62	0.34	0.31
Morni Hills (Panchkula, Haryana)	P31	1362.39	552.04	1914.43	283.48	144.57	0.41	0.52
	P32	1433.41	586.39	2019.80	322.10	156.92	0.41	0.49
	P33	1403.03	624.50	2027.53	358.28	154.07	0.44	0.43
	P34	1376.79	416.07	1792.86	354.95	156.34	0.31	0.46
	P35	1220.38	634.41	1854.80	412.44	118.76	0.52	0.30
	Mean	1088.01	457.18	1545.19	281.81	115.31	0.42	0.41
	CD _{0.05}	179.77	109.29	55.09	55.09	32.14	0.12	0.16
	CV	10.14	14.67	7.79	12.00	17.10	17.57	23.30

e. Dry root weight (g)

Maximum dry root weight (g) was observed in P29 (160.35) which was statistically at par with P32 (156.92), P34 (156.34), P33 (154.07), P27 (153.61), P31 (144.57) and P25 (135.74). Minimum dry root weight was observed in P1 (85.98).

f. Fresh root: shoot ratio

Maximum fresh root: shoot ratio was observed in P6 (0.60) which was statistically at par with P22 (0.57), P9 (0.53), P35 (0.52), P7 (0.50) and P18 (0.48). Minimum fresh root: shoot ratio was observed in P34 (0.34).

g. Dry root: shoot ratio

Maximum dry root: shoot ratio was observed in P27 (0.65) which was statistically at par with P13 (0.53), P31 (0.52), P28 (0.49) and P32 (0.49). Minimum dry root: shoot ratio was observed in P22 (0.29).

It is apparent from the results that the progenies from Siswa dam and Morni hills performed better indicating their genetic superiority. Since seeds were collected from different seed sources and seedlings were raised from the seeds in the same experimental field under the similar nursery conditions the variation in biomass characters may be attributed to the genotypic differences amongst the different progenies. Similar findings were observed by Dao and Kabore (2015) and Shreehari *et al.*, (2022) in *Moringa oleifera* Lam. whereas Deepanjli (2018) found similar results for *Toona ciliata* M. Roem.

4.5 Variation in proximate principles, biochemical estimates and mineral contents of progenies

Data pertaining to Table 16. revealed non-significant differences for leaf dry matter content (%) among the nursery genotypes.

Maximum crude protein content (%) was observed in P7 (27.49) which was statistically at par with P18 (27.11), P16 (26.73), P9 (26.98) and P10 (26.67). Minimum crude protein content (%) was observed in P34 (18.93).

Maximum ether extract content (%) was observed in P19 (7.07) which was statistically at par with in P21 (6.89), P24 (6.85), P18 (6.78) and P16 (6.60). Minimum ether extract content (%) was observed in P7 (3.20) (Plate 6a).

Maximum crude fiber content (%) was observed in P3 (13.60) which was statistically at par with P7 (13.51), P14 (13.41), P8 (13.39) and P17 (12.95). Minimum crude fiber content (%) was observed in P24 (3.08).

Maximum total ash content (%) was observed in P22 (10.39) which was statistically at par with P33 (10.20), P11 (10.19), P35 (10.01), P13 (9.73), P15 (9.95) and P16 (9.55). Minimum total ash content (%) was observed in P14 (6.35) (Plate 6b).

Table: 16. Variation in leaf dry matter content, proximate principles and biochemical estimates of leaf samples of *Moringa oleifera* Lam. progenies

Seed sources (District, State/UT)	Progeny	Leaf dry matter content (%)	Crude protein (%)	Ether extract (%)	Crude fiber (%)	Total ash content (%)	Nitrogen free extract (%)	Total amino acids (%)
Samba (Samba, Jammu and Kashmir)	P1	19.06 (4.48)	22.52 (4.85)	4.88 (2.42)	8.40 (3.06)	7.81 (2.97)	56.38	15.51 (4.06)
	P2	19.22 (4.50)	19.11 (4.48)	3.79 (2.19)	10.91 (3.45)	7.09 (2.84)	59.10	13.16 (3.76)
	P3	18.89 (4.46)	19.61 (4.54)	3.02 (1.00)	13.60 (3.82)	8.05 (3.01)	55.71	13.55 (3.81)
	P4	19.08 (4.48)	24.07 (5.01)	3.63 (2.15)	11.85 (3.58)	8.67 (3.11)	51.79	16.67 (4.20)
	P5	19.01 (4.47)	20.44 (4.63)	5.55 (2.56)	8.00 (2.99)	6.73 (2.78)	59.28	14.13 (3.89)
Dunera (Pathankot, Punjab)	P6	19.58 (4.54)	26.26 (5.22)	6.21 (2.68)	10.09 (3.33)	7.73 (2.95)	49.72	18.14 (4.37)
	P7	18.72 (4.44)	27.49 (5.34)	3.20 (2.05)	13.51 (3.81)	9.23 (3.20)	46.56	19.12 (4.49)
	P8	18.83 (4.45)	20.70 (4.66)	3.47 (2.11)	13.39 (3.79)	9.29 (3.21)	53.15	14.22 (3.90)
	P9	19.25 (4.50)	26.98 (5.29)	4.32 (2.31)	8.61 (3.10)	6.65 (2.77)	53.44	18.63 (4.43)
	P10	19.02 (4.47)	26.67 (5.26)	3.74 (2.18)	11.92 (3.59)	7.65 (2.94)	50.02	18.42 (4.41)
Vikasnagar (Dehradun, Uttarakhand)	P11	19.05 (4.48)	23.08 (4.91)	4.09 (2.25)	8.09 (3.01)	10.19 (3.34)	54.56	15.84 (4.10)
	P12	19.25 (4.50)	21.28 (4.72)	3.99 (2.23)	9.60 (3.26)	7.34 (2.89)	57.79	14.78 (3.97)
	P13	19.16 (4.49)	19.51 (4.53)	5.78 (2.60)	4.59 (2.36)	9.73 (3.27)	60.38	13.46 (3.80)
	P14	19.03 (4.48)	20.25 (4.61)	3.43 (2.10)	13.41 (3.80)	6.35 (2.71)	56.56	14.02 (3.88)
	P15	18.98 (4.47)	24.50 (5.05)	6.36 (2.71)	4.54 (2.35)	9.95 (3.31)	54.64	16.91 (4.23)
Lamlehri (Una, Himachal Pradesh)	P16	19.02 (4.47)	26.73 (5.27)	6.60 (2.75)	3.95 (2.22)	9.55 (3.25)	53.17	18.37 (4.40)
	P17	19.05 (4.48)	20.28 (4.61)	3.25 (2.06)	12.95 (3.74)	6.53 (2.74)	56.98	13.87 (3.86)

	P18	18.70 (4.44)	27.11 (5.30)	6.76 (2.78)	3.31 (2.08)	9.31 (3.21)	53.52	18.80 (4.45)
	P19	18.70 (4.44)	26.48 (5.24)	7.07 (2.84)	6.00 (2.65)	9.03 (3.16)	51.42	18.26 (4.39)
	P20	19.10 (4.48)	20.56 (4.64)	3.56 (2.13)	11.65 (3.56)	9.37 (3.22)	54.86	14.08 (3.88)
Tirla (Solani, Himachal Pradesh)	P21	19.15 (4.49)	25.23 (5.12)	6.89 (2.81)	6.77 (2.78)	8.68 (3.11)	52.42	17.47 (4.30)
	P22	19.05 (4.48)	21.54 (4.75)	6.11 (2.67)	3.71 (2.17)	10.39 (3.37)	58.24	14.97 (4.00)
	P23	19.36 (4.51)	24.84 (5.08)	3.88 (2.21)	10.59 (3.40)	8.10 (3.01)	52.58	17.08 (4.25)
	P24	19.10 (4.48)	25.43 (5.14)	6.85 (2.80)	3.08 (2.02)	8.27 (3.04)	56.38	17.51 (4.30)
	P25	19.41 (4.52)	21.92 (4.79)	4.99 (2.45)	4.17 (2.27)	7.65 (2.94)	61.26	15.19 (4.02)
Siswa Dam (Sahibzada Ajit Singh Nagar, Punjab)	P26	19.16 (4.90)	24.71 (5.07)	5.41 (2.53)	7.34 (2.88)	7.59 (2.93)	54.95	16.99 (4.24)
	P27	18.82 (4.45)	22.22 (4.82)	4.52 (2.35)	10.24 (3.35)	9.50 (3.24)	53.51	15.44 (4.05)
	P28	19.30 (4.51)	25.89 (5.18)	4.79 (2.40)	6.58 (2.75)	8.72 (3.12)	54.02	17.68 (4.32)
	P29	19.47 (4.53)	25.10 (5.11)	5.16 (2.48)	7.21 (2.86)	8.22 (3.03)	54.31	17.32 (4.28)
	P30	19.01 (4.47)	24.00 (5.00)	5.94 (2.63)	9.21 (3.20)	8.20 (3.03)	52.65	16.52 (4.19)
Morni Hills (Panchkula, Haryana)	P31	18.97 (4.47)	23.46 (4.95)	5.72 (2.59)	4.44 (2.33)	7.04 (2.83)	59.34	16.17 (4.14)
	P32	19.33 (4.51)	20.75 (4.66)	4.13 (2.26)	10.54 (3.40)	7.40 (2.90)	57.18	14.43 (3.93)
	P33	18.79 (4.45)	25.61 (5.16)	5.12 (2.47)	7.73 (2.95)	10.20 (3.34)	51.35	17.69 (4.32)
	P34	19.29 (4.50)	18.93 (4.46)	5.23 (2.50)	5.15 (2.48)	7.18 (2.86)	63.51	13.07 (3.75)
	P35	18.68 (4.44)	26.13 (5.21)	4.67 (2.38)	10.28 (3.36)	10.01 (3.32)	48.90	17.98 (4.36)
	Mean	19.07 (4.48)	23.41 (4.93)	4.92 (2.42)	8.44 (2.94)	8.38 (3.06)	54.85	16.16 (4.13)
	CD_{0.05}	NS	0.09	0.10	0.21	0.12	1.89	0.14
	CV		1.17	2.54	4.23	2.35	2.12	2.08

(The values in parenthesis are square root transformed values)

Maximum nitrogen free extract (%) was observed in P34 (63.51) whereas minimum nitrogen free extract (%) was observed in P7 (46.56).

Maximum total amino acid content (%) was observed in P7 (19.12) which was statistically at par with in P18 (18.80), P9 (18.63), P10 (18.42), P16 (18.37), P19 (18.26), P6 (18.14) and P35 (17.98). Minimum total amino acid content (%) was observed in P34 (13.07).

The perusal data in Table 17. revealed maximum nitrogen content (mg/100 g) was observed in P7 (4397.87) which was statistically at par with P18 (4337.07), P9 (4317.33), P16 (4277.33), P10 (4267.73), P19 (4236.27), P6 (4201.07), P35 (4181.33) and P28 (4142.93). Minimum nitrogen content (mg/100g) was observed in P34 (3028.80).

Maximum phosphorus content (mg/100 g) was observed in P22 (74.68) which was statistically followed by P18 (74.63) and P24 (70.73) whereas, P7 had the minimum value for phosphorus content (25.09 mg/100 g).

Maximum potassium content (mg/100 g) was observed in P29 (1761.02) which was statistically at par with P28 (1708.04), P33 (1708.84), P19 (1696.56), P22 (1695.45), P26 (1677.17), P32 (1675.46), P4 (1661.11), P13 (1653.30), P16 (1647.14), P34 (1644.08), P1 (1634.98), P9 (1619.67), P30 (1597.68) and P24 (1569.82). Minimum potassium content was observed in P12 (1272.59 mg/100 g).

Maximum calcium content (mg/100 g) was observed in P20 (1.89) which was statistically at par with P17 (1.88), P18 (1.88), P8 (1.87), P14 (1.87), P15 (1.87) P12 (1.85), P2 (1.83), P11 (1.82) and P7 (1.81). Minimum calcium content was observed in P29 (1.58 mg/100 g).

Maximum magnesium content (mg/100 g) was observed in P16 (777.88) which was statistically at par with P21 (775.16), P22 (758.94), P25 (731.82), P19 (727.14), P18 (723.71), P24 (716.57), P15 (673.18), P28 (672.63) and P13 (668.91). Minimum magnesium content was observed in P7 (340.45 mg/100 g).

Maximum sulphur content (mg/100 g) was observed in P7 (1279.19) whereas minimum sulphur content was observed in P24 (568.27 mg/100 g).

Maximum zinc content (mg/100 g) was observed in P7 (3.87) which was statistically at par with P14 (3.84), P3 (3.79) and P8 (3.74) however minimum zinc content was observed in P24 (2.25 mg/100 g).

Table: 17. Variation in mineral nutrient contents of leaf samples of *Moringa oleifera* Lam. progenies

Seed sources (District, State/UT)	Progeny	Mineral nutrient content (mg/100g)									
		N	P	K	Ca	Mg	S	Zn	Fe	Cu	Mn
Samba (Samba, Jammu and Kashmir)	P1	3603.20	49.16	1634.98	1.69	545.02	930.22	3.10	29.96	0.50	3.50
	P2	3057.07	44.33	1356.43	1.83	537.73	1095.17	3.54	26.76	0.65	3.49
	P3	3138.13	28.89	1425.95	1.79	394.92	1212.27	3.79	26.06	0.65	3.07
	P4	3850.67	37.26	1661.11	1.65	398.67	1179.14	3.62	41.87	0.49	3.30
	P5	3270.93	51.78	1362.37	1.78	601.56	839.62	2.95	28.76	0.61	3.94
Dunera (Pathankot, Punjab)	P6	4201.07	48.18	1535.54	1.77	551.97	980.35	3.27	29.08	0.62	3.63
	P7	4397.87	25.09	1471.26	1.81	340.45	1279.19	3.87	33.03	0.67	2.79
	P8	3311.47	26.58	1325.02	1.87	344.98	755.84	3.74	34.47	0.69	2.82
	P9	4317.33	50.89	1619.67	1.72	628.42	731.06	3.04	36.02	0.50	4.22
	P10	4267.73	36.12	1454.82	1.77	384.54	1152.03	3.58	35.08	0.63	3.47
Vikasnagar (Dehradun, Uttarakhand)	P11	3692.80	49.92	1320.82	1.82	594.20	902.36	3.01	43.62	0.74	3.97
	P12	3405.33	41.30	1272.59	1.85	475.49	981.55	3.21	37.74	0.77	3.41
	P13	3122.13	55.21	1653.30	1.65	688.91	711.60	2.61	24.56	0.40	4.36
	P14	3240.53	31.53	1311.17	1.87	371.75	1206.60	3.84	32.41	0.78	3.44
	P15	3920.53	64.01	1417.00	1.87	673.18	944.96	2.68	30.89	0.74	4.27
Lamlehri (Una, Himachal Pradesh)	P16	4277.33	69.42	1647.14	1.64	777.88	623.09	2.41	25.70	0.43	4.39
	P17	3245.33	39.14	1416.99	1.88	446.31	1178.74	3.69	39.44	0.72	3.28
	P18	4337.07	74.63	1306.92	1.88	723.71	583.21	2.34	49.60	0.78	4.57
	P19	4236.27	66.15	1696.56	1.62	727.14	781.21	2.74	42.40	0.45	4.32
	P20	3289.60	40.08	1298.64	1.89	473.53	1062.88	3.50	37.12	0.74	3.15
Tirla (Solan, Himachal Pradesh)	P21	4036.80	69.52	1393.42	1.86	775.16	826.16	2.77	25.13	0.72	4.59
	P22	3446.40	74.68	1695.45	1.62	758.94	617.06	2.39	42.65	0.37	4.82
	P23	3974.93	33.27	1378.15	1.78	377.73	1088.08	3.46	31.32	0.62	3.29
	P24	4068.27	70.73	1569.82	1.64	716.57	568.27	2.25	33.35	0.44	4.36
	P25	3507.73	65.07	1495.77	1.72	731.82	680.51	2.45	46.33	0.58	4.33
Siswa Dam (Sahibzada Ajit Singh Nagar, Punjab)	P26	3954.13	54.48	1677.17	1.64	657.57	855.80	2.92	41.04	0.41	3.97
	P27	3555.73	46.47	1515.11	1.78	519.95	1010.08	3.33	48.80	0.59	3.49
	P28	4142.93	63.26	1708.04	1.59	672.63	652.39	2.53	46.89	0.35	4.27
	P29	4015.47	60.97	1761.02	1.58	649.37	1213.32	2.85	45.87	0.35	4.37
	P30	3839.47	51.68	1597.68	1.72	637.21	959.09	3.16	30.53	0.54	4.06
Morni Hills (Panchkula, Haryana)	P31	3753.07	52.84	1470.96	1.75	596.20	813.91	2.66	42.14	0.61	4.06
	P32	3319.47	42.36	1675.46	1.65	484.26	1075.78	3.40	29.45	0.42	3.58
	P33	4097.60	57.87	1708.84	1.61	638.10	831.81	2.81	47.49	0.38	4.29
	P34	3028.80	45.84	1644.08	1.66	651.12	697.67	2.56	34.22	0.49	3.93
	P35	4181.33	61.06	1549.13	1.70	529.49	1043.56	3.37	38.31	0.55	3.57
	Mean	3745.84	50.85	1515.10	1.74	573.61	916.13	3.07	36.23	0.57	3.84
	CD _{0.05}	261.42	4.22	149.50	0.09	112.01	55.11	0.14	2.87	0.06	0.56
	CV	4.28	5.10	6.06	3.07	11.99	2.69	2.89	4.86	6.36	8.97

Maximum iron content (mg/100 g) was observed in P18 (49.60) statistically followed by P27 (48.80), P33 (47.49) and P28 (46.89). Minimum iron content was observed in P13 (24.56) mg/100 g.

Maximum copper content (mg/100 g) was observed in P14 and P18 (0.78 each) which was statistically at par with P12 (0.77), P11 (0.74), P15 (0.74), P20 (0.74), P17 (0.72) and P21 (0.72). Minimum copper content was observed for both P28 and P29 (0.35 mg/100 g).

Maximum manganese content (mg/100 g) was observed in P22 (4.82) which was statistically at par with P21 (4.59), P18 (4.57), P16 (4.39), P29 (4.37), P13 (4.36), P24 (4.36), P25 (4.33), P19 (4.32), P33 (4.29), P15 (4.27) and P28 (4.27). Minimum manganese content was observed in P7 (2.79 mg/100 g).

Variation in leaf proximate principles, leaf biochemical estimated and leaf mineral contents could be due to genetic makeup of the genotypes, environmental conditions, or their interaction. Since all the genotypes were planted in the nursery, the growing conditions for all the genotypes were same. Hence the genetic makeup of these genotypes under study could have created the variations. Similar results were found by Okiki *et al.*, (2015) for dry matter content, crude protein, ether extract, crude fiber, ash content, nitrogen free extract, zinc, iron, magnesium, calcium, potassium and phosphorus in *Moringa oleifera* Lam. Offor *et al.*, (2014) also found similar results for dry matter content, crude protein, ether extract, crude fiber, ash content, nitrogen free extract, zinc, iron, magnesium, calcium, copper and manganese in *Moringa oleifera* Lam. Balaguru *et al.*, (2020) found similar results for crude fiber, iron and calcium whereas Selvakumari (2013) found similar results for crude fiber, iron and magnesium in *Moringa oleifera* Lam. Singh (2011) reported that all part of the *Moringa* plant have medicinal value and are used as drug for the treatment of various disease and ailments.

4.6 Genetic estimates

Variability was estimated for seed and germination parameters, morphological characters, leaf proximate principles, leaf biochemical estimates and leaf mineral contents in terms of mean, range, genotypic coefficient of variation and phenotypic coefficient of variation. Genetic parameters were worked out in terms of heritability (broad sense), genetic advance and genetic gain as per cent of mean.

All the seed and germination parameters exhibited wide range of values (Table 18.) indicating the extent of variation present in the plants. The mean value for pod length (cm), Pod breadth (cm) and seeds per pod, 100 Seed weight with wings (g), 100 Seed weight without wings (g), 100 Seed kernel weight (g), Seed length (cm), Seed breadth (cm), Seed kernel length (cm), Seed kernel breadth (cm) germination percentage (%), germination index and germination value was observed to be 41.67, 1.69, 20.37, 11.79, 11.33, 10.21, 1.15, 0.74, 0.71, 0.49, 69.95, 109.17 and 44.55, respectively. The values for pod length ranged between 33.80-50.60 cm, 1.20-2.40 cm for pod breadth, 16.00-29.00 for seeds per pod, 8.26-16.13 g for 100 seed weight with wings, 8.05-15.34 g for 100 seed weight without wings, 6.83-13.41 g for 100 seed kernel weight, 0.76-1.35 cm seed length, 0.53-0.99 cm for seed breadth, 0.49-0.83 cm for seed kernel length, 0.34-0.58 cm for seed kernel breadth, 33.33-91.67 % for germination percentage, 33.67-148.67 for germination index and 4.24-75.00 for germination value.

Table 18: Genetic estimates for pod, seed and germination characters of *Moringa oleifera* Lam.

Character	Range	Mean	CV	GCV	PCV	Heritability (broad sense) per cent (%)	Genetic Advance	Genetic advance (per cent) of mean
Pod length (cm)	33.80-50.60	41.67	2.52	10.16	10.47	94.21	8.47	20.32
Pod breadth (cm)	1.20-2.40	1.69	2.55	21.40	21.55	98.61	0.74	43.76
Seeds per pod	16.00-29.00	20.37	2.35	14.96	15.14	97.59	6.20	30.43
100 Seed weight with wings (g)	8.26-16.3	11.79	2.68	15.33	15.56	97.04	3.67	31.11
100 Seed weight without wings (g)	8.05-15.34	11.33	2.48	15.52	15.72	97.51	3.58	31.57
100 Seed kernel weight (g)	6.83-13.41	10.21	2.40	17.41	17.58	98.14	3.63	35.53
Seed length (cm)	0.76-1.35	1.15	2.51	9.95	10.26	94.01	0.23	19.86
Seed breadth (cm)	0.53-0.99	0.74	2.40	13.48	13.69	96.93	0.20	27.34
Seed kernel length (cm)	0.49-0.83	0.71	2.69	8.20	8.63	90.29	0.11	16.06
Seed kernel breadth (cm)	0.34-0.58	0.49	2.42	10.00	10.29	94.45	0.10	20.01
Germination percentage (%)	33.33-91.67	69.95	12.90	26.84	29.80	81.07	34.82	49.77
Germination index	33.67-148.67	109.17	13.23	28.99	31.87	82.76	59.32	54.33
Germination value	4.24-75.00	44.55	28.54	46.03	54.16	72.22	35.90	80.57

Regarding coefficient of variation, it was found maximum in germination value (28.54 %) and minimum was found in seeds per pod (2.35 %). Both genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) were found maximum for germination value (46.03 and 54.16, respectively) and minimum GCV and PCV was found in seed kernel length (8.20 and 8.63, respectively). The phenotypic coefficient of variation

values were found slightly higher for all the parameters than genotypic coefficient of variation (GCV).

Heritability percentage was recorded to be maximum (98.61 %) for pod breadth followed by 100 seed kernel weight (98.14 %), seeds per pod (97.59 %) and 100 seed weight without wings (97.51), whereas germination value recorded the minimum heritability percentage (72.22 %).

Genetic advance was recorded maximum (59.32) for germination index whereas, minimum value (0.10) was recorded for seed kernel breadth.

Genetic advance (per cent) of mean was recorded maximum for germination value (80.57) whereas, minimum value (16.06) was recorded for seed kernel length.

High heritability with high genetic gain was observed for all the characters except seed kernel length which indicated the presence of additive genes in those traits which offers the most suitable condition for selection.

Deepanjli (2018) found similar findings with seed length, seed breadth, 100 seed weight in *Toona ciliata* M. Roem. Selvakumari (2013) found similar results for pod length, pod breadth, seeds per pod and 100 seed weight in *Moringa oleifera* Lam. The results are in accordance with the findings of Redhu (2021) for pod length, pod breadth, seeds/pod and germination percentage in *Moringa oleifera* Lam.

The critical investigation of variability estimates of seed traits namely seed length, seed width and seed weight represented in (Table 19.) revealed that GCV values were quite close to PCV values, and recorded very high heritability which indicates that these traits were least influenced by environment. The high variability and heritability values exhibited the additive type of gene action.

The mean value for plant height (cm), collar diameter (cm), internodal length (cm), petiole length (cm), branch angle ($^{\circ}$), number of branches, leaf area (cm^2), fresh shoot weight (g), fresh root weight (g), total fresh biomass (g), dry shoot weight (g), dry root weight (g), fresh root shoot ratio and dry root shoot ratio was observed to be 170.53, 23.56, 5.14, 10.03, 64.00, 34.76, 830.10, 1088.01, 457.18, 1545.19, 281.81, 115.31, 0.43 and 0.42, respectively.

The values for plant height ranged between 124.39-234.44 cm, 16.75-34.85 mm for collar diameter, 4.82-5.54 cm for internodal length, 8.41-11.32 cm for petiole length, 55.23-

75.17⁰ for branch angle, 26.33-43.20 for number of branches, 654.04-1014.09 cm² for leaf area, 792.16-1509.01 g for fresh shoot weight, 332.77-634.41 g for fresh root weight, 1175.66-2029.43 g for fresh total biomass, 193.23-412.44 g for dry shoot weight, 85.98-163.47 g for dry root weight, 0.31-0.60 for fresh root: shoot ratio and 0.29-0.65 for dry root: shoot ratio.

Coefficient of variation was found maximum in dry root: shoot ratio (23.30) whereas it was found minimum in leaf area (2.49). GCV was found maximum in dry shoot weight (18.43) which was followed by fresh shoot weight (18.05), plant height (17.95) and collar diameter (17.90). Minimum GCV was found in internodal length (3.67). Maximum PCV was estimated for dry root: shoot ratio (26.13) followed by dry root weight (24.68) and fresh root weight (22.48) while minimum PCV was estimated for internodal length (4.59).

Heritability percentage (%) was recorded to be maximum (96.47) for leaf area. It was followed by number of branches (91.64), plant height (87.37), petiole length (85.84) and total fresh biomass (82.65) whereas, dry root: shoot ratio recorded the minimum heritability percentage (20.44).

Genetic advance was recorded to be maximum (352.60) for fresh shoot weight followed by leaf area (218.58) and fresh root weight (121.48) while minimum genetic advance (0.05) was recorded for dry root: shoot ratio.

Table 19: Genetic estimates for morphological characters of *Moringa oleifera* Lam. progenies

Character	Range	Mean	CV	GCV	PCV	Heritability (broad sense) per cent	Genetic Advance	Genetic advance (per cent) of mean
Plant height (cm)	124.39-234.44	170.53	6.83	17.95	19.21	87.37	58.95	34.57
Collar diameter (mm)	16.75-34.85	23.56	9.08	17.90	20.07	79.53	7.75	32.88
Internodal length (cm)	4.82-5.54	5.14	3.66	3.67	4.59	63.81	0.31	6.04
Petiole length (cm)	8.41-11.32	10.03	3.48	8.56	9.24	85.84	1.64	16.34
Leaf angle (°)	55.23-75.17	64.00	4.56	9.32	10.38	80.70	11.04	17.26
Number of leaves	26.33-43.20	34.76	7.53	14.59	15.24	91.64	10.00	28.76
Leaf area (cm ²)	654.04-1014.09	830.10	2.49	13.01	13.25	96.47	218.58	26.33
Fresh shoot weight (g)	792.16-1509.01	1088.01	10.14	18.05	20.70	76.00	352.60	32.41
Fresh root weight (g)	332.77-634.41	457.18	14.67	17.03	22.48	57.39	121.48	26.57
Total fresh biomass (g)	1175.66-2029.43	1545.19	7.79	17.01	18.71	82.65	0.49	31.86
Dry shoot weight (g)	193.23-412.44	281.81	12.00	18.43	21.99	70.23	89.64	31.81
Dry root weight (g)	85.98-163.47	115.31	17.10	17.79	24.68	51.96	30.46	26.41
Fresh root shoot ratio	0.306-0.603	0.428	17.57	11.44	20.97	29.77	0.06	12.86
Dry root shoot ratio	0.289-0.647	0.421	23.30	11.81	26.13	20.44	0.05	11.00

Genetic advance (per cent) of mean was recorded to be maximum (34.57) for plant height which was followed by collar diameter (32.88), fresh shoot weight (32.41), total fresh biomass (31.86) and dry shoot weight (31.81). Internodal length recorded the minimum genetic advance (per cent) of mean value of 6.04.

High heritability with high genetic gain was observed for plant height and total fresh biomass which offers the most suitable condition for selection indicated by the presence of additive genes in those traits.

Based on the higher values of variability and heritability, seedling height, collar diameter and leaf area came out to be amenable for judicious screening/ selection of superior progenies. Similar results have been reported for plant height, collar diameter and petiole length in *Moringa oleifera* Lam. by Nair *et al.*, (2021). Gawali *et al.*, (2015) found similar findings for plant height and collar diameter in *Pongamia pinnata*. Deepanjli (2018) found similar findings with plant height, collar diameter, dry shoot weight and dry root weight in *Toona ciliata* M. Roem. Mandal *et al.*, (2022) found similar results for plant height, collar diameter and internodal length in *Moringa oleifera* Lam. Selvakumari (2013) found similar results for plant height and leaf area in *Moringa oleifera* Lam. The results are in accordance with the findings of Redhu (2021) for shoot length, collar diameter, number of leaves, fresh seedling weight and dry seedling weight in *Moringa oleifera* Lam.

Thus the characters with higher estimates of heritability and variability can be exploited well for further advanced tree improvement programme

It is apparent from the Table 20. that the mean value for crude protein (%), ether extract (%), crude fiber (%), total ash content (%), nitrogen free extract (%), total amino acids (%), nitrogen (mg/100 g), phosphorus (mg/100 g), potassium (mg/100 g), calcium (mg/100 g), magnesium (mg/100 g), zinc (mg/100 g), sulphur (mg/100 g), iron (mg/100 g), copper (mg/100 g) and manganese (mg/100 g) was observed to be 23.41, 4.92, 8.44, 8.38, 54.85, 16.16, 3745.84, 50.85, 1515.10, 1.74, 573.61, 916.13, 3.07, 36.23, 0.57 and 3.84, respectively.

The value for crude protein (%) ranged between 18.93-27.49, 3.02-7.07 for ether extract (%), 3.08-13.60 for crude fiber (%), 6.35-10.39 for total ash content (%), 46.56-63.51 for nitrogen free extract (%), 13.07-19.12 for total amino acids (%), 3028.80-4397.87 for nitrogen (mg/100 g), 25.09-74.68 for phosphorus (mg/100 g), 1272.59-1761.02 for potassium (mg/100 g), 1.58-1.89 for calcium (mg/100 g), 340.45-777.88 for magnesium (mg/100 g),

2.25-3.87 for zinc (mg/100 g), 568.27-1279.19 for sulphur (mg/100 g), 24.56-49.60 for iron (mg/100 g), 0.35-0.78 for copper (mg/100 g) and 2.79-4.82 for manganese (mg/100 g).

Coefficient of variation was found maximum in manganese (8.97) whereas minimum was found in crude protein (1.17). Maximum GCV and PCV were found maximum in crude fiber (38.79 and 39.76, respectively) followed by phosphorus (27.27 and 27.74, respectively) whereas minimum GCV and PCV were found in calcium (5.30 and 6.13, respectively).

Heritability percentage (%) was recorded to be maximum (97.39) for sulphur. It was followed by zinc (96.71), phosphorus (96.62), crude protein (95.94) and crude fiber (95.15) whereas, manganese content recorded the minimum heritability percentage (67.43).

Genetic advance was recorded to be maximum (813.65) for phosphorus while minimum genetic advance (0.27) was recorded for copper content.

Genetic advance (per cent) of mean was recorded to be maximum (77.94) for crude fiber. Calcium content recorded the minimum genetic advance (per cent) of mean value of 9.44.

Table 20: Genetic estimates for leaf proximate principles, leaf biochemical estimates and leaf mineral contents of *Moringa oleifera* Lam. progenies

Character	Range	Mean	CV	GCV	PCV	Heritability (broad sense) per cent	Genetic Advance	Genetic advance (per cent) of mean
Crude protein (%)	18.93-27.49	23.41	1.17	11.47	11.71	95.94	5.42	23.14
Ether extract (%)	3.02-7.07	4.92	2.54	24.51	25.36	93.41	3.00	48.79
Crude fiber (%)	3.08-13.60	8.44	4.23	38.79	39.76	95.15	6.58	77.94
Total ash content (%)	6.35-10.39	8.38	2.35	13.73	14.69	87.45	2.22	26.46
Nitrogen free extract (%)	46.56-63.51	54.85	2.12	6.58	6.91	90.63	7.08	12.91
Total amino acids (%)	13.07-19.12	16.16	2.08	11.28	12.08	87.17	3.50	21.69
Nitrogen (mg/100g)	3028.80-4397.87	3745.84	4.28	11.28	12.07	87.40	813.65	21.72
Phosphorus(mg/100g)	25.09-74.68	50.85	5.10	27.27	27.74	96.62	28.80	55.21
Potassium (mg/100g)	1272.09-1761.02	1515.10	6.06	9.13	10.95	69.43	237.33	15.66
Calcium(mg/100g)	1.58-1.89	1.74	3.07	5.30	6.13	74.81	0.16	9.44
Magnesium(mg/100g)	340.45-777.88	573.61	11.99	22.27	25.29	77.54	231.49	40.39
Zinc(mg/100g)	2.25-3.87	3.07	2.89	15.69	15.95	96.71	0.98	31.78
Sulphur(mg/100g)	568.27-1279.19	916.13	2.69	22.57	22.87	97.39	420.31	45.88
Iron(mg/100g)	24.56-49.60	36.23	4.86	20.42	20.99	94.63	14.82	40.91
Copper (mg/100g)	0.35-0.78	0.57	6.36	23.63	24.47	93.24	0.27	47.00
Manganese (mg/100g)	2.79-4.82	3.84	8.97	12.90	15.71	67.43	0.84	21.82

High heritability with high genetic gain was observed for ether extract, crude fiber, phosphorus, zinc, sulphur, iron and manganese content which offers the most suitable condition for selection indicated by the presence of additive genes in those trait.

The results are in accordance with the findings of Redhu (2021) for dry matter content, crude protein, ether extract, crude fiber and ash content in *Moringa oleifera* Lam. Balaguru *et al.*, (2020) found similar results in *Moringa oleifera* Lam. for crude fiber, iron and calcium contents whereas Tak (2012) found similar findings for crude protein, nitrogen, phosphorus, potassium, calcium, iron and magnesium. Okon *et al.*, (2022) found similar findings for crude protein, crude fiber, ether extract, ash content, nitrogen free extract, calcium, magnesium, potassium, iron and zinc for *Cucurbita pepo*.

4.7 Correlation studies

Data presented in Table 21. revealed that pod length showed significant and positive genotypic as well as phenotypic correlation with seeds per pod (0.446, 0.421), germination percentage (0.351, 0.333), germination index (0.376, 0.355) and germination value (0.437, 0.395) whereas it showed significant and negative genotypic as well as phenotypic correlation with pod breadth (-0.461, -0.444), 100 seed weight with wings (-0.280, -0.276), 100 seed weight without wings (-0.288, -0.272) and 100 seed kernel weight (-0.436, -0.419).

Pod breadth showed significant and positive genotypic as well as phenotypic correlation with 100 seed weight with wings (0.399, 0.387), 100 seed weight without wings (0.403, 0.396) and 100 seed kernel weight (0.450, 0.439). Pod breadth showed significant and negative genotypic correlation with germination index (-0.273), germination value (-0.299) and germination percentage (-0.212) whereas it showed significant and negative phenotypic correlation with germination index (-0.240) and germination value (-0.249).

Seeds per pod showed significant and positive genotypic as well as phenotypic correlation with germination percentage (0.253, 0.223), germination index (0.248, 0.213) and germination value (0.288, 0.228) whereas significant and negative correlation were observed for 100 seed weight with wings (-0.269, -0.257), 100 seed weight without wings (-0.251, -0.250) and 100 seed kernel weight (-0.294, -0.288).

100 seed weight with wings showed significant and positive genotypic as well as phenotypic correlation with 100 seed weight without wings (0.995, 0.975) and 100 seed kernel weight (0.898, 0.878), whereas it showed significant and negative genotypic as well as phenotypic correlation with germination percentage (-0.352, -0.320), germination index (-0.387, -0.360) and germination value (-0.433, -0.376).

100 seed weight without wings showed significant and positive genotypic as well as phenotypic correlation with 100 seed kernel weight (0.925, 0.904) whereas it showed significant and negative genotypic as well as phenotypic correlation with germination percentage (-0.338, -0.303), germination index (-0.378, -0.341) and germination value (-0.424, -0.357).

100 seed kernel weight showed significant and positive genotypic as well as phenotypic correlation with seed kernel length (0.225, 0.203) whereas it showed highly significant and negative genotypic as well as phenotypic correlation with germination percentage (-0.314, -0.272), germination index (-0.366, -0.323) and germination value (-0.409, -0.334).

Seed length showed significant and positive genotypic as well as phenotypic correlation with seed breadth (0.750, 0.712), seed kernel length (0.712, 0.642) and seed kernel breadth (0.484, 0.457).

Seed breadth showed significant and positive genotypic as well as phenotypic correlation with seed kernel length (0.542, 0.505), seed kernel breadth (0.391, 0.381), germination percentage (0.338, 0.305), germination index (0.377, 0.344) and germination value (0.376, 0.331).

Seed kernel length showed significant and positive genotypic as well as phenotypic correlation with seed kernel breadth (0.752, 0.715) whereas seed kernel breadth showed significant and positive genotypic correlation with germination value (0.201).

Germination percentage showed highly significant and positive genotypic as well as phenotypic correlation with germination index (0.991, 0.980) and germination value (0.984, 0.953).

Germination index showed highly significant and positive genotypic as well as phenotypic correlation with germination value (0.995, 0.975).

In the present investigation, mostly the genotypic correlation coefficients were slightly higher than the phenotypic correlation coefficients. A similar trend was reported by Ali *et al.*, (2005); Roy *et al.*, (2006); Golani *et al.*, (2007) and Bendale *et al.*, (2008). This may be due to effect of environment in modifying the total expression of the genotypes, thus altering the phenotypic expression (Nandpuri and Kumar, 1973).

Table: 21. Genotypic and phenotypic correlation coefficients for pod, seed and germination parameters of *Moringa oleifera* Lam. progenies

		PL	PB	SP	SWW	SW	SK	SL	SB	SKL	SKB	GP	GI	GV
PL	G	1.000												
	P	1.000												
PB	G	-0.461**	1.000											
	P	-0.444**	1.000											
SP	G	0.446**	-0.170	1.000										
	P	0.421**	-0.171	1.000										
SWW	G	-0.280**	0.399**	-0.269**	1.000									
	P	-0.276**	0.387**	-0.257**	1.000									
SW	G	-0.288**	0.403**	-0.251**	0.995**	1.000								
	P	-0.272**	0.396**	-0.250*	0.975**	1.000								
SK	G	-0.436**	0.450**	-0.294**	0.898**	0.925**	1.000							
	P	-0.419**	0.439**	-0.288**	0.878**	0.904**	1.000							
SL	G	-0.175	0.018	-0.044	-0.019	0.019	0.111	1.000						
	P	-0.148	0.022	-0.034	-0.019	0.020	0.108	1.000						
SB	G	0.110	-0.027	0.034	0.148	0.152	0.079	0.750**	1.000					
	P	0.106	-0.029	0.036	0.143	0.147	0.079	0.712**	1.000					
SKL	G	-0.035	-0.030	-0.079	0.118	0.159	0.225*	0.712**	0.542**	1.000				
	P	-0.038	-0.023	-0.085	0.110	0.163	0.203*	0.642**	0.505**	1.000				
SKB	G	0.177	-0.055	-0.131	-0.057	-0.037	-0.029	0.484**	0.391**	0.752**	1.000			
	P	0.176	-0.053	-0.125	-0.056	-0.038	-0.031	0.457**	0.381**	0.715**	1.000			
GP	G	0.351**	-0.212*	0.253**	-0.352**	-0.338**	-0.314**	0.177	0.338**	0.126	0.126	1.000		
	P	0.333**	-0.188	0.223*	-0.320**	-0.303**	-0.272**	0.153	0.305**	0.097	0.095	1.000		
GI	G	0.376**	-0.273**	0.248*	-0.387**	-0.378**	-0.366**	0.189	0.377**	0.157	0.147	0.991**	1.000	
	P	0.355**	-0.240*	0.213*	-0.360**	-0.341**	-0.323**	0.164	0.344**	0.122	0.123	0.980**	1.000	
GV	G	0.437**	-0.299**	0.288**	-0.433**	-0.424**	-0.409**	0.171	0.376**	0.180	0.201*	0.984**	0.995**	1.000
	P	0.395**	-0.249*	0.228*	-0.376**	-0.357**	-0.334**	0.137	0.331**	0.132	0.168	0.953**	0.975**	1.000

** Significant at 1% level * Significant at 5% level; G = genotypic correlation, P = phenotypic correlation

Where: PL is pod length, PB is pod breadth, S/P is seeds per pod, SWW is 100 seed weight with wings, SW is 100 seed weight without wings, SK is 100 seed kernel weight, SL is seed length, SB is seed breadth, SKL is seed kernel length, SKB is seed kernel breadth, GP is germination percentage, GI is germination index and GV is germination value.

Thangjam *et al.*, (2020) showed similar findings with pod length, pod breadth, seeds per pod, germination percentage and germination energy in *Parkia timoriana*. Asati *et al.*, (2021) showed similar findings with pod length, pod breadth and seeds per pod; Chan *et al.*, (2018) showed similar results for 100 seed weight with wings, 100 seed weight without wings, seed kernel weight, seed length, seed breadth and seeds per pod in *Moringa oleifera* Lam. Loha *et al.*, (2006) showed similar results for seed length, seed breadth, 100 seed weight and germination energy in *Cordia africana*. Selvakumari (2013) showed similar results for pod length, pod breadth and seeds/pod in *Moringa oleifera* Lam.

It is evident from the Table 22. that plant height showed significant and positive genotypic as well as phenotypic correlation with collar diameter (0.774, 0.734), internodal length (0.643, 0.594), petiole length (0.666, 0.593), leaf angle (0.452, 0.438), number of branches (0.832, 0.783), leaf area (0.558, 0.536), fresh shoot weight (0.787, 0.716), fresh root weight (0.681, 0.608), dry shoot weight (0.441, 0.427) and dry root weight (0.549, 0.492).

Collar diameter also showed significant and positive genotypic as well as phenotypic correlation with internodal length (0.526, 0.476), petiole length (0.765, 0.666), leaf angle (0.307, 0.284), number of branches (0.807, 0.725), leaf area (0.506, 0.473), fresh shoot weight (0.723, 0.659), fresh root weight (0.622, 0.543), dry shoot weight (0.580, 0.545) and dry root weight (0.676, 0.573).

Internodal length showed significant and positive genotypic as well as phenotypic correlation with petiole length (0.356, 0.334), number of branches (0.375, 0.342), leaf area (0.284, 0.266), fresh shoot weight (0.542, 0.477), fresh root weight (0.432, 0.395), dry shoot weight (0.288, 0.256) and dry root weight (0.420, 0.331) whereas it showed significant genotypic correlation with leaf angle (0.198).

Petiole length showed significant and positive genotypic as well as phenotypic correlation with leaf angle (0.293, 0.255), number of branches (0.902, 0.792), leaf area (0.587, 0.531), fresh shoot weight (0.811, 0.707), fresh root weight (0.731, 0.601), dry shoot weight (0.607, 0.524) and dry root weight (0.633, 0.476).

Leaf angle showed significant and positive genotypic as well as phenotypic correlation with number of branches (0.402, 0.382), fresh shoot weight (0.281, 0.240) and dry

shoot weight (0.242, 0.225) whereas it showed significant genotypic correlation with leaf area (0.198).

Number of branches showed significant and positive genotypic as well as phenotypic correlation with leaf area (0.646, 0.612), fresh shoot weight (0.877, 0.782), fresh root weight (0.692, 0.596), dry shoot weight (0.601, 0.532) and dry root weight (0.692, 0.572). On the other hand, it showed significant and negative genotypic correlation as well as phenotypic correlation with fresh root: shoot ratio (-0.268, -0.195).

Leaf area showed highly significant and positive genotypic as well as phenotypic correlation with fresh shoot weight (0.591, 0.545), fresh root weight (0.469, 0.397), dry shoot weight (0.644, 0.592) and dry root weight (0.492, 0.453).

Fresh shoot weight showed highly significant and positive genotypic as well as phenotypic correlation with fresh root weight (0.717, 0.570), dry shoot weight (0.450, 0.396) and dry root weight (0.611, 0.543) whereas it showed highly significant and negative genotypic as well as phenotypic correlation with fresh root: shoot ratio (-0.388, -0.415).

Fresh root weight showed highly significant and positive genotypic as well as phenotypic correlation with dry shoot weight (0.441, 0.399), dry root weight (0.527, 0.392) and fresh root: shoot ratio (0.354, 0.486).

Dry shoot weight showed highly significant and positive genotypic as well as phenotypic correlation with dry root weight (0.597, 0.507) whereas it showed highly significant and negative genotypic as well as phenotypic correlation with dry root: shoot ratio (-0.426, -0.434).

Dry root weight showed highly significant and positive genotypic as well as phenotypic correlation with dry root: shoot ratio (0.453, 0.526).

Similar findings were observed in the study by Kumar *et al.*, (2017) for plant height, collar diameter, internodal length, leaf area, fresh shoot weight, fresh root weight, dry shoot weight, dry root weight, fresh root: shoot ratio and dry root: shoot ratio in *Terminalia arjuna*. Findings of Deepanjli (2018) had similar results for plant height, collar diameter, number of leaves, dry shoot weight and dry shoot weight in *Toona ciliata* M. Roem. Loha *et al.*, (2006) had similar results for plant height and collar diameter in *Cordia africana*.

Table: 22. Genotypic and phenotypic correlation coefficients for morphological characters of *Moringa oleifera* Lam. progenies

		PH	CD	IL	PTL	BA	NL	LA	FSW	FRW	DSW	DRW	FRS	DRS
PH	G	1.000												
	P	1.000												
CD	G	0.774**	1.000											
	P	0.734**	1.000											
IL	G	0.643**	0.526**	1.000										
	P	0.594**	0.476**	1.000										
PTL	G	0.666**	0.765**	0.356**	1.000									
	P	0.593**	0.666**	0.334**	1.000									
BA	G	0.452**	0.307**	0.198*	0.293**	1.000								
	P	0.438**	0.284**	0.188	0.255**	1.000								
NL	G	0.832**	0.807**	0.375**	0.902**	0.402**	1.000							
	P	0.783**	0.725**	0.342**	0.792**	0.382**	1.000							
LA	G	0.558**	0.506**	0.284**	0.587**	0.198*	0.646**	1.000						
	P	0.536**	0.473**	0.266**	0.531**	0.190	0.612**	1.000						
FSW	G	0.787**	0.723**	0.542**	0.811**	0.281**	0.877**	0.591**	1.000					
	P	0.716**	0.659**	0.477**	0.707**	0.240*	0.782**	0.545**	1.000					
FRW	G	0.681**	0.622**	0.432**	0.731**	0.174	0.692**	0.469**	0.717**	1.000				
	P	0.608**	0.543**	0.395**	0.601**	0.156	0.596**	0.397**	0.570**	1.000				
DSW	G	0.441**	0.580**	0.288**	0.607**	0.242*	0.601**	0.644**	0.450**	0.441**	1.000			
	P	0.427**	0.545**	0.256**	0.524**	0.225*	0.532**	0.592**	0.396**	0.399**	1.000			
DRW	G	0.549**	0.676**	0.420**	0.633**	0.136	0.692**	0.492**	0.611**	0.527**	0.597**	1.000		
	P	0.492**	0.573**	0.331**	0.476**	0.131	0.572**	0.453**	0.543**	0.392**	0.507**	1.000		
FRS	G	-0.161	-0.142	-0.128	-0.100	-0.169	-0.268**	-0.152	-0.388**	0.354**	-0.010	-0.143	1.000	
	P	-0.101	-0.077	-0.059	-0.075	-0.110	-0.195*	-0.127	-0.415**	0.486**	0.028	-0.157	1.000	
DRS	G	0.156	0.134	0.158	0.050	-0.006	0.141	-0.160	0.171	0.160	-0.426**	0.453**	-0.067	1.000
	P	0.128	0.092	0.114	-0.001	-0.001	0.102	-0.109	0.167	0.079	-0.434**	0.526**	-0.123	1.000

** Significant at 1% level * Significant at 5% level; G = genotypic correlation, P = phenotypic correlation

Where, PH is plant height, CD is collar diameter, IL is internodal length, PTL is petiole length, BA is leaf angle, NL is number of leaves, LA is leaf area, FSW is fresh shoot weight, FRW is fresh root weight, DSW is dry shoot weight, DRW is dry root weight, FSR fresh root: shoot ratio and DRS is dry root: shoot ratio.

Data present in the Table 23. showed that crude protein showed significant and positive genotypic as well as phenotypic correlation with ether extract (0.398, 0.383), total ash content (0.249, 0.238), total amino acids (0.920, 0.837), nitrogen content (1.000, 1.000), potassium content (0.339, 0.263), magnesium content (0.391, 0.364), phosphorus content (0.501, 0.482), iron content (0.403, 0.375) and manganese content (0.470, 0.367). On the other hand, crude protein showed significant and negative genotypic as well as phenotypic correlation with crude fiber (-0.368, -0.355), nitrogen free extract (-0.693, -0.693), sulphur content (-0.272, -0.269), calcium content (-0.342, -0.280), zinc content (-0.392, -0.371) and copper content (-0.268, -0.243).

Ether extract showed significant and positive genotypic as well as phenotypic correlation with total ash content (0.269, 0.252), total amino acids (0.406, 0.351), nitrogen content (0.398, 0.383), potassium content (0.391, 0.277), magnesium content (0.904, 0.809), phosphorus content (0.866, 0.848) and manganese content (0.913, 0.696). On the other hand, ether extract showed significant and negative genotypic as well as phenotypic correlation with crude fiber (-0.849, -0.828), sulphur content (-0.694, -0.676), calcium content (-0.388, -0.387), zinc content (-0.850, -0.800) and copper content (-0.331, -0.317).

Crude fiber showed significant and positive genotypic as well as phenotypic correlation with calcium content (0.476, 0.387), zinc content (0.997, 0.946) and copper content (0.410, 0.389) whereas it showed significant and negative genotypic as well as phenotypic correlation with nitrogen free extract (-0.300, -0.307), total amino acids (-0.232, -0.205), nitrogen content (-0.368, -0.355), potassium content (-0.394, -0.310), magnesium content (-0.953, -0.843), phosphorus content (-0.887, -0.869), iron content (-0.207, -0.200) and manganese content (-0.969, -0.781).

Total ash content showed highly significant and positive genotypic as well as phenotypic correlation with total amino acids (0.307, 0.258), nitrogen content (0.249, 0.238) and phosphorus content (0.363, 0.345) showed significant and negative genotypic as well as phenotypic correlation with nitrogen free extract (-0.401, -0.405).

Nitrogen free extract showed significant and negative genotypic as well as phenotypic correlation with total amino acids (-0.789, -0.704), nitrogen content (-0.693, -0.693), sulphur content (-0.263, -0.242) and zinc content (-0.286, -0.251). In addition, it showed significant

and positive genotypic correlation with magnesium content (0.206) whereas significant and negative genotypic correlation with iron content (-0.215).

Total amino acids showed significant and positive genotypic as well as phenotypic correlation with nitrogen content (0.920, 0.837), magnesium content (0.270, 0.200), potassium content (0.289, 0.263), phosphorus content (0.388, 0.342) iron content (0.254, 0.250) and manganese content (0.308, 0.290) whereas it showed significant and negative genotypic as well as phenotypic correlation with calcium content (-0.247, -0.237) and zinc content (-0.259, -0.216).

Nitrogen content showed significant and positive genotypic as well as phenotypic correlation with potassium content (0.339, 0.263), magnesium content (0.391, 0.364), phosphorus content (0.501, 0.482), iron content (0.403, 0.375) and manganese content (0.470, 0.367), whereas it showed significant and negative genotypic as well as phenotypic correlation with sulphur content (-0.272, -0.269), calcium content (-0.342, -0.280), zinc content (-0.392, -0.371) and copper content (-0.268, -0.243).

Potassium content showed significant and positive genotypic as well as phenotypic correlation with magnesium content (0.491, 0.328), phosphorus (0.423, 0.330) and manganese content (0.493, 0.348) whereas it showed significant and negative genotypic as well as phenotypic correlation with sulphur content (-0.260, -0.209), calcium content (-0.998, -0.857), zinc content (-0.431, -0.343) and copper content (-0.998, -0.819).

Magnesium content showed significant and positive genotypic as well as phenotypic correlation with phosphorus content (0.983, 0.871) and manganese content (0.865, 0.771) whereas it showed significant and negative genotypic as well as phenotypic correlation with sulphur content (-0.808, -0.705), calcium content (-0.500, -0.365), zinc content (-0.982, -0.841) and copper content (-0.469, -0.381).

Sulphur content showed significant and positive genotypic as well as phenotypic correlation with calcium content (0.355, 0.288), zinc content (0.866, 0.822) and copper content (0.345, 0.329) whereas, it showed significant and negative genotypic as well as phenotypic correlation with phosphorus content (-0.718, -0.697) and manganese content (-0.779, -0.617).

Phosphorus content showed significant and positive genotypic as well as phenotypic correlation with iron content (0.274, 0.266) and manganese content (0.813, 0.788) whereas, it showed significant and negative genotypic as well as phenotypic correlation with calcium content (-0.449, -0.383), zinc content (-0.917, 0.856) and copper content (-0.409, -0.382).

Calcium content showed significant and positive genotypic as well as phenotypic correlation with zinc content (0.515, 0.429) and copper content (0.932, 0.877) whereas, it showed significant and negative genotypic as well as phenotypic correlation with iron content (-0.218, -0.200) and manganese content (-0.516, -0.402).

Zinc content showed significant and positive genotypic as well as phenotypic correlation with copper content (0.458, 0.427) whereas, it showed significant and negative genotypic as well as phenotypic correlation with iron content (-0.256, -0.233) and manganese content (-0.806, -0.765).

Copper content showed significant and positive genotypic as well as phenotypic correlation with iron content (0.243, 0.202) and significant and negative genotypic as well as phenotypic correlation with manganese content (-0.484, -0.389).

The present results are in line with the findings of Selvakumari (2013) in *Moringa oleifera* Lam.

4.8 Principal component analysis

In this PCA, first four principal components had eigen value greater than one and they cumulatively explained 81.23 per cent of the total variation present in the original data set. So, these four principal components were considered important for further explanation (Table 24.)

Data presented in the Table 24. showed that the first principal component contributed 36.12 per cent while, the second, third and fourth principal component exhibited 23.82 per cent, 12.51 per cent and 8.781 per cent variability, respectively with eigen value more than one. First and second principal components (PC1 and PC2) of the 35 progenies of *Moringa oleifera* Lam. generated 59.94 % of total variation.

Table: 23. Genotypic and phenotypic correlation coefficients for leaf proximate principles, leaf biochemical estimates and leaf mineral contents of *Moringa oleifera* Lam. progenies

		CP	EE	CF	AC	NFE	TAA	N	K	Mg	S	P	Ca	Zn	Fe	Cu	Mn
CP	G	1.000															
	P	1.000															
EE	G	0.398**	1.000														
	P	0.383**	1.000														
CF	G	-0.368**	-0.849**	1.000													
	P	-0.355**	-0.828**	1.000													
AC	G	0.249*	0.269**	-0.260**	1.000												
	P	0.238*	0.252**	-0.246*	1.000												
NFE	G	-0.693**	0.063 ^{NS}	-0.300**	-0.401**	1.000											
	P	-0.693**	0.049 ^{NS}	-0.307**	-0.405**	1.000											
TAA	G	0.920**	0.406**	-0.232*	0.307**	-0.789**	1.000										
	P	0.837**	0.351**	-0.205*	0.258**	-0.704**	1.000										
N	G	1.000**	0.398**	-0.368**	0.249*	-0.693**	0.920**	1.000									
	P	1.000**	0.383**	-0.355**	0.238*	-0.693**	0.837**	1.000									
K	G	0.339**	0.391**	-0.394**	0.177 ^{NS}	-0.090 ^{NS}	0.289**	0.339**	1.000								
	P	0.263**	0.277**	-0.310**	0.113 ^{NS}	-0.051 ^{NS}	0.263**	0.263**	1.000								
Mg	G	0.391**	0.904**	-0.953**	0.277**	0.206*	0.270**	0.391**	0.491**	1.000							
	P	0.364**	0.809**	-0.843**	0.233*	0.155 ^{NS}	0.200*	0.364**	0.328**	1.000							
S	G	-0.272**	-0.694**	0.822**	-0.244*	-0.263**	-0.141 ^{NS}	-0.272**	-0.260**	-0.808**	1.000						
	P	-0.269**	-0.676**	0.803**	-0.238*	-0.242*	-0.126 ^{NS}	-0.269**	-0.209*	-0.705**	1.000						
P	G	0.501**	0.866**	-0.887**	0.363**	0.031	0.388**	0.501**	0.423**	0.983**	-0.718**	1.000					
	P	0.482**	0.848**	-0.869**	0.345**	0.030	0.342**	0.482**	0.330**	0.871**	-0.697**	1.000					
Ca	G	-0.342**	-0.388**	0.476*	-0.156	0.001	-0.247*	-0.342**	-0.998**	-0.500**	0.355**	-0.449**	1.000				
	P	-0.280**	-0.308**	0.387**	-0.117	0.001	-0.237*	-0.280**	-0.857**	-0.365**	0.288**	-0.383**	1.000				
Zn	G	-0.392**	-0.850**	0.997**	-0.266**	-0.286**	-0.259**	-0.392**	-0.431**	-0.982**	0.866**	-0.917**	0.515**	1.000			
	P	-0.371**	-0.800**	0.946**	-0.246*	-0.251**	-0.216*	-0.371**	-0.343**	-0.841**	0.822**	-0.856**	0.429**	1.000			
Fe	G	0.403**	0.009	-0.207*	0.246*	-0.215*	0.254**	0.403**	0.185	0.164	-0.173	0.274**	-0.218*	-0.256**	1.000		
	P	0.375**	0.003	-0.200*	0.225*	-0.191	0.250*	0.375**	0.157	0.145	-0.155	0.266**	-0.200*	-0.233*	1.000		
Cu	G	-0.268**	-0.331**	0.410**	-0.152	-0.016	-0.184	-0.268**	-0.998**	-0.469**	0.345**	-0.409**	0.932**	0.458**	-0.158	1.000	
	P	-0.243*	-0.317**	0.389**	-0.142	-0.019	-0.176	-0.243*	-0.819**	-0.381**	0.329**	-0.382**	0.877**	0.427**	-0.141	1.000	
Mn	G	0.470**	0.913**	-0.969**	0.254**	0.162	0.308**	0.470**	0.493**	0.865**	-0.779**	0.813**	-0.516**	-0.806**	0.243*	-0.484**	1.000
	P	0.367**	0.696**	-0.781**	0.211*	0.142	0.290**	0.367**	0.348**	0.771**	-0.617**	0.788**	-0.402**	-0.765**	0.202*	-0.389**	1.000

** Significant at 1% level * Significant at 5% level; G = genotypic correlation, P = phenotypic correlation

Where, CP is crude protein, EE is ether extract, CF is crude fiber, AC is ash content, NFE is nitrogen free extract, TAA is total amino acids, N is nitrogen, K is potassium, Mg is magnesium, S is sulphur, P is phosphorus, Ca is calcium, Zn is zinc, Fe is iron, Cu is copper and Mn is manganese

Table: 24. Principal component analysis of pod and germination parameters of *Moringa oleifera* Lam. progenies

	PC 1	PC 2	PC 3	PC 4
PL	0.268	-0.065	0.071	0.633
PB	-0.239	0.094	0.172	-0.374
SP	0.191	-0.105	0.136	0.467
SWW	-0.349	0.226	0.332	0.229
SW	-0.346	0.245	0.328	0.229
SK	-0.346	0.258	0.287	0.064
SL	0.076	0.441	-0.263	-0.127
SB	0.123	0.443	0.011	0.079
SKL	0.051	0.469	-0.280	0.088
SKB	0.104	0.357	-0.370	0.141
GP	0.367	0.142	0.383	-0.189
GI	0.384	0.146	0.343	-0.172
GV	0.396	0.140	0.312	-0.128
Eigen value	4.70	3.10	1.63	1.14
Variance (%)	36.12	23.82	12.51	8.78

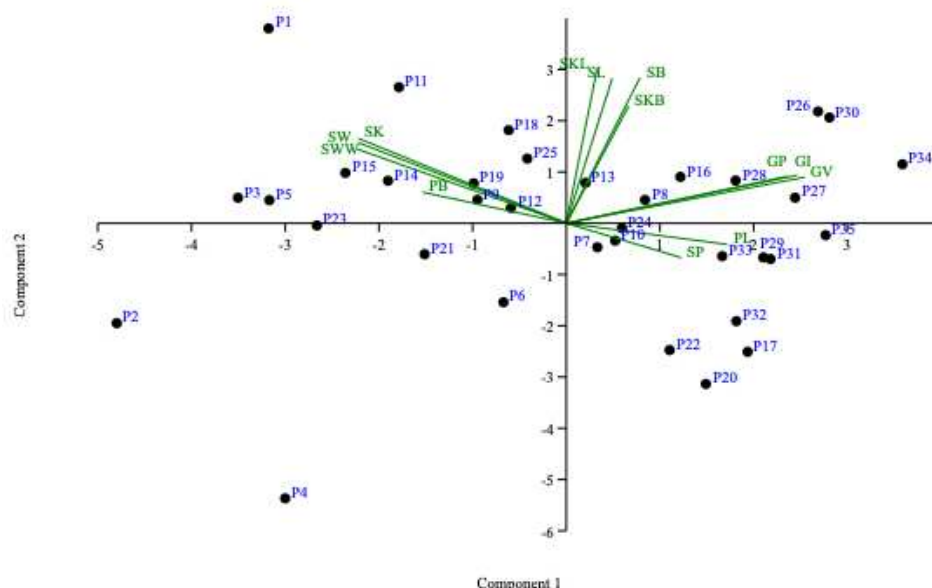
Where: PL is pod length, PB is pod breadth, S/P is seeds per pod, SWW is 100 seed weight with wings, SW is 100 seed weight without wings, SK is 100 seed kernel weight, SL is seed length, SB is seed breadth, SKL is seed kernel length, SKB is seed kernel breadth, GI is germination index and GV is germination value.

Component matrix revealed that PC1 showed high positive loading for germination value (0.396), germination index (0.384), germination percentage (0.367) and pod length (0.268) whereas it showed high negative loading for 100 seed weight with wings (-0.349), 100 seeds weight without wings (-0.346), 100 seed kernel weight (-0.346) and pod breadth (-0.239). PC2 enabled high positive loading for seed kernel length (0.469), seed breadth (0.443), seed length (0.441) and seed kernel breadth (0.357). PC3 exhibited high positive loading for germination percentage (0.383), germination index (0.343), 100 seed weight with wings (0.332), 100 seed weight without wings (0.328) and germination value (0.312) whereas, high negative loading for seed kernel breadth (-0.370), seed kernel length (-0.280) and seed length (-0.263). Principal component 4 had high positive loading for pod length (0.633) and seeds per pod (0.467) while had high negative loading for pod breadth (-0.374). The prominent traits contributing maximum variability and desegregating in different principal components have the tendency to remain together which may be kept into consideration during utilization of these characters in crop improvement programme as a donor for the associated traits.

The biplot showed the observations as points in the plane formed by two principal components. The contribution of first two Principal components to the total variability was maximum (59.938 per cent), thus these two were plotted to reveal the relationship between them. Progenies P1, P3, P5, P8, P9, P11, P12, P13, P14, P15, P16, P18, P19, P25, P26, P27, P28, P30 and P34 clustered towards better side of Principal Component 1. Progenies P7, P8,

P10, P13, P16, P17, P20, P22, P24, P26, P27, P28, P29, P30, P31, P32, P33, P34 and P35 clustered towards better side of Principal component 2 (Figure 2.). Since these two principal components contribute more than 50 per cent of the total variability along with high positive loading of yield attributing traits, it can be concluded that progenies, viz., P8, P13, P16, P26, P27, P28, P30 and P34 which clustered towards the better side of both Principal Component 1 and Principal Component 2, were superior collectively for pod, germination parameters and their attributing traits among all the lines studied.

Figure: 2. Distribution and grouping of *Moringa oleifera* Lam. progenies across first two principal components for pod, seed and germination parameters using scatter plot



The result of this PCA explained the genetic diversity in morphological parameters of *Moringa oleifera* Lam. progenies. In this PCA, first three principal components had eigen value greater than one and they cumulatively explained 72.10 per cent of the total variation present in the original data set where PC1 and PC2 combinedly accounted for more than half of the variation (61.78 %). Contribution of variation by PC1, PC2 and PC3 was 49.51 per cent, 12.27 per cent and 10.32 per cent, respectively. The respective eigen values for PC1, PC2 and PC3 were 6.44, 1.60 and 1.34 (Table 25.).

Component matrix revealed that PC1 showed high positive loading for number of branches (0.360), fresh shoot weight (0.349), plant height (0.345) collar diameter (0.340) and petiole length (0.338) whereas negative loading observed only for fresh root: shoot ratio (-0.066). PC2 exhibited high positive loading for dry root: shoot ratio (0.695), dry root weight

(0.207) and internodal length (0.160). In addition, PC2 showed high negative loading for dry shoot weight (-0.458), fresh root: shoot ratio (-0.362) and leaf area (-0.237). PC3 revealed high positive loading for fresh root: shoot ratio (0.728), fresh root weight (0.483) and dry root: shoot ratio (0.300) whereas negative loading was observed for leaf angle (-0.241) (Table 25.).

The contribution of first two Principal components to the total variability was maximum (61.78 per cent), thus these two were plotted to reveal the relationship between them for morphological characters. Progenies P2, P3, P10, P12, P13, P14, P16, P17, P19, P20, P24, P25, P27, P28, P29, P31, P32 and P34 clustered towards better side of Principal Component 1. Progenies P14, P22, P23, P24, P25, P27, P28, P29, P30, P31, P32, P34, P33 and P35 clustered towards better side of Principal component 2 (Figure 3.). Since these two principal components contribute more than 50 per cent of the total variability along with high positive loading of yield attributing traits, it can be concluded that progenies, viz., P14, P24, P25, P27, P28, P29, P31, P32 and P34 which clustered towards the better side of both Principal Component 1 and Principal Component 2, were superior collectively for morphological characters and their attributing traits among all the lines studied. The results are in line with the findings of Thakur and Thakur (2016) for *Ulmus villosa* Brandis.

Figure: 3. Distribution and grouping of progenies of *Moringa oleifera* Lam. across first two principal components for morphological and biomass parameters using scatter plot

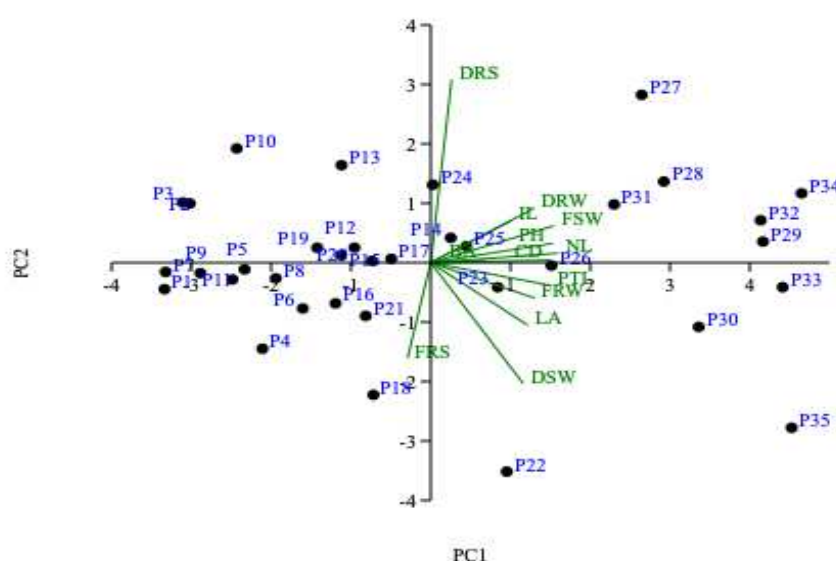


Table: 25. Principal component analysis of morphological parameters of *Moringa oleifera* Lam. progenies

	PC 1	PC 2	PC 3
PH	0.345	0.0730	0.006
CD	0.340	0.010	0.033
IL	0.229	0.160	0.089
PTL	0.338	-0.086	0.030
BA	0.154	0.007	-0.241
NL	0.360	0.036	-0.066
LA	0.274	-0.237	-0.178
FSW	0.349	0.140	-0.114
FRW	0.294	-0.134	0.483
DSW	0.261	-0.458	-0.140
DRW	0.293	0.207	0.102
FRS	-0.066	-0.362	0.728
DRS	0.059	0.695	0.300
Eigen value	6.44	1.60	1.34
Variance (%)	49.51	12.27	10.32

Where, PH is plant height, CD is collar diameter, IL is internodal length, PTL is petiole length, BA is leaf angle, NL is number of branches, LA is leaf area, FSW is fresh shoot weight, FRW is fresh root weight, DSW is dry shoot weight, DRW is dry root weight, FSR fresh root: shoot ratio and DRS is dry root: shoot ratio.

The result of this PCA (Table 26.) explained the genetic diversity in leaf proximate principles, leaf biochemical estimates and leaf mineral contents of *Moringa oleifera* Lam. progenies. In this PCA, first four principal components had eigen value greater than one where PC1 contributed to 47.73 per cent variation with 7.64 eigen value, PC2 contributed to 22.42 per cent variation with 3.59 eigen value, PC3 contributed to 13.00 per cent variation with 2.08 eigen value and PC4 contributed to 6.46 per cent variation with 1.03 eigen value. These four principal components contributed to total of 89.61 per cent variation where PC1 and PC2 combinedly contributed to 70.15 per cent variation.

Component matrix revealed that PC1 showed high positive loading for phosphorus content (0.334), magnesium content (0.331), manganese content (0.331) and ether extract (0.312) whereas showed high negative loading for zinc content (-0.335), crude fiber (-0.326) and sulphur content (0.276). PC2 manifested high positive loading for total amino acids (0.443), crude protein (0.442) and nitrogen content (0.442) while showed negative loading for nitrogen free extract (-0.509). PC3 exhibited high positive loading for copper content (0.536)

and calcium content (0.518) whereas potassium content showed high negative loading (-0.529) under PC3. PC4 revealed high positive loading for iron content (0.788) and total ash content (0.514) while ether extract showed high negative loading (-0.244) under it.

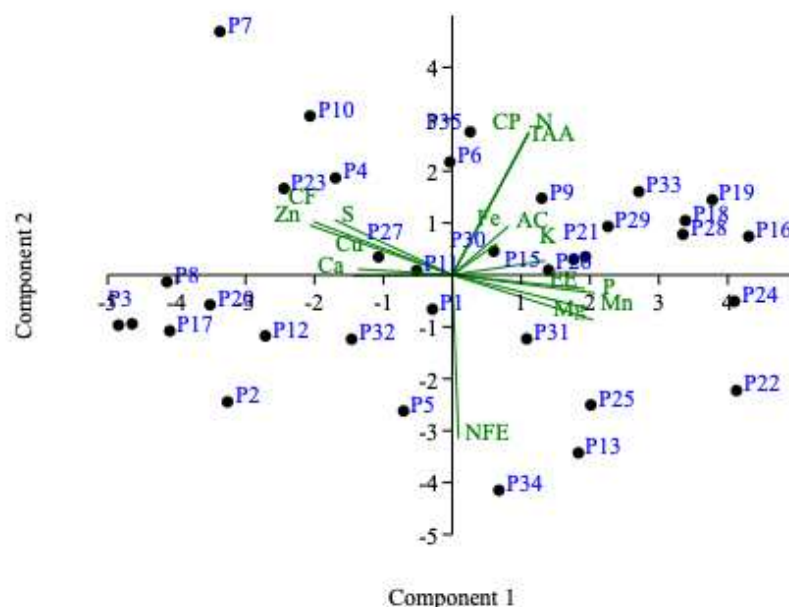
The contribution of first two Principal Components to the total variability was maximum (70.147 per cent), thus these two were plotted to reveal the relationship between them for morphological characters. Progenies P1, P4, P6, P7, P9, P10, P15, P16, P18, P19, P21, P23, P26, P27, P28, P29, P30, P33 and P35 clustered towards better side of Principal Component 1. Progenies P9, P13, P15, P16, P18, P19, P21, P22, P24, P25, P26, P28, P29, P30, P31, P33, P34 and P35 clustered towards better side of Principal Component 2 (Figure 4.). Since these two principal components contribute more than 50 per cent of the total variability along with high positive loading of yield attributing traits, it can be concluded that progenies, viz., P9, P15, P16, P18, P19, P21, P26, P28, P29, P30, P33 and P35 which clustered towards the better side of both Principal Component 1 and Principal Component 2, were superior collectively for morphological characters and their attributing traits among all the lines studied.

Table: 26. Principal component analysis of leaf proximate principles, leaf biochemical estimates and leaf mineral contents of *Moringa oleifera* Lam. progenies

	PC 1	PC 2	PC 3	PC 4
CP	0.180	0.442	0.077	-0.119
EE	0.312	-0.043	0.186	-0.244
CF	-0.326	0.166	-0.137	-0.001
AC	0.133	0.153	0.088	0.514
NFE	0.015	-0.509	-0.024	0.005
TAA	0.179	0.443	0.079	-0.119
N	0.180	0.442	0.078	-0.119
P	0.334	-0.057	0.152	0.039
K	0.219	0.044	-0.529	-0.052
Ca	-0.233	-0.003	0.518	0.011
Mg	0.331	-0.141	0.118	-0.059
Zn	-0.335	0.155	-0.113	-0.029
S	-0.276	0.171	-0.155	-0.052
Fe	0.106	0.098	-0.033	0.788
Cu	-0.222	0.018	0.536	0.025
Mn	0.331	-0.109	0.101	-0.031
Eigen value	7.64	3.59	2.08	1.03
Variance (%)	47.73	22.42	13.00	6.46

Where, CP is crude protein, EE is ether extract, CF is crude fiber, AC is ash content, NFE is nitrogen free extract, TAA is total amino acids, N is nitrogen, K is potassium, Mg is magnesium, S is sulphur, P is phosphorus, Ca is calcium, Zn is zinc, Fe is iron, Cu is copper and Mn is manganese.

Figure: 4. Distribution and grouping of progenies of *Moringa oleifera* Lam. across first two principal components for leaf proximate principles, leaf biochemical estimates and leaf mineral contents using scatter plot



The results are in accordance with the findings of Raphael *et al.*, (2021) for seed characters in *Moringa oleifera* Lam. Karunakar *et al.*, (2022) had similar findings for seed and plant morphological characters of *Moringa oleifera* Lam. Popoola *et al.*, (2016) found similar findings with pod and seed characteristics and germination parameters of *Moringa oleifera* Lam. Udensi and Edu (2015) found similar results for leaf proximate principles and leaf mineral contents in *Vigna unguiculata*.

4.9 Path coefficient analysis

Data related to path coefficient analysis of pod, seed and germination characters is presented in the Table 27. The component characters of germination percentage expressing genotypic correlation were partitioned into direct and indirect effects and these effects are presented in Table. Here germination percentage was taken as dependent trait.

Seed length exerted negative direct effect on germination percentage (-0.5251). The positive indirect effects were observed through pod length, 100 seed with wings, 100 seed without wings, seed breadth, seed kernel length, seed kernel breadth, germination index and germination value while negative indirect effects were observed through pod breadth, seeds per pod and 100 seed kernel weight.

Seed breadth exerted positive direct effect on germination percentage (0.4192). The positive indirect effects were observed through pod breadth, seeds per pod, 100 seed weight without wings, seed kernel length, seed kernel breadth, germination index and germination value while negative indirect effects were observed through pod length, 100 seed weight with wings and 100 seed kernel weight.

Seed kernel length exerted positive direct effect on germination percentage (0.0855). The positive indirect effects were observed through pod length, pod breadth, 100 seed weight without wings, seed breadth, seed kernel breadth, germination index and germination value while negative indirect effects were observed through seeds per pod, 100 seed weight with wings, 100 seed kernel weight and seed length.

Seed kernel breadth exerted positive direct effect on germination percentage (0.1507). The positive indirect effects were observed through pod breadth, 100 seed weight with wings, 100 seed kernel weight, seed breadth, seed kernel length, germination index and germination value while negative indirect effects were observed through pod length, seeds per pod, 100 seed weight without wings and seed length.

The pod length exerted negative direct effect on germination percentage (-0.5189). The positive indirect effects were observed through pod breadth, seeds per pod, 100 seed weight with wings, 100 seed kernel weight, seed length, seed breadth, seed kernel breadth, germination index and germination value while negative indirect effects were observed through 100 seed kernel weight and seed kernel length.

Pod breadth exerted negative direct effect on germination percentage (-0.1124). The positive indirect effects were observed through pod length and 100 seed weight without wings while negative indirect effects were observed through seeds per pod, 100 seed weight with wings, 100 seed kernel weight, seed length, seed breadth, seed kernel length, seed kernel breadth, germination index and germination value.

Seeds per pod exerted positive direct effect on germination percentage (0.3027). The positive indirect effects were observed through pod breadth, 100 seed weight with wings, 100 seed kernel weight, seed length, seed breadth, germination index and germination value while negative indirect effects were observed through pod length, 100 seed weight without wings, seed kernel length and seed kernel breadth.

100 seed weight with wings exerted negative direct effect on germination percentage (-0.9045). The positive indirect effects were observed through pod length, 100 seed weight without wings, seed length, seed breadth and seed kernel length while negative indirect effects were observed through pod breadth, seeds per pod, 100 seed kernel weight, seed kernel breadth, germination index and germination value.

100 seed weight without wings exerted positive direct effect on germination percentage (-0.9045). The positive indirect effects were observed pod length, seed breadth and seed kernel length while negative indirect effects were observed through pod breadth, seeds per pod, 100 seed weight without wings, 100 seed kernel weight, seed length, seed kernel breadth, germination index and germination value.

100 seed kernel weight exerted negative direct effect on germination percentage (-0.1617). The positive indirect effects were observed through pod length, 100 seed weight with wings, seed breadth and seed kernel length while negative indirect effects were observed through pod breadth, seeds per pod, 100 seed weight with wings, seed length, seed kernel breadth, germination index and germination value.

Germination index exerted positive direct effect on germination percentage (0.8278). The positive indirect effects were observed through pod breadth, seeds per pod, 100 seed weight with wings, 100 seed kernel weight, seed breadth, seed kernel length, seed kernel breadth and germination value while negative indirect effects were observed through pod length, 100 seed weight without wings and seed length.

Germination value exerted positive direct effect on germination percentage (0.2361). The positive indirect effects were observed through pod breadth, seeds per pod, 100 seed weight with wings, 100 seed kernel weight, seed breadth, seed kernel length, seed kernel breadth and germination index while negative indirect effects were observed through pod length, 100 seed weight without wings and seed length.

It is evident from the Table 28. that plant height exerted positive direct effect on total fresh biomass (0.0555). The positive indirect effects were observed through petiole length, fresh shoot weight, fresh root weight, dry shoot weight and dry root: shoot ratio while negative indirect effects were observed through collar diameter, internodal length, leaf angle, number of branches, leaf area, dry root shoot weight and fresh root: shoot ratio.

Table: 27. Path coefficient analysis showing direct and indirect effects of seed and germination parameters on germination percentage of *Moringa oleifera* Lam.

	PL	PB	SP	SWW	SW	SK	SL	SB	SKL	SKB	GI	GV
PL	-0.5189	0.0518	0.1350	0.2535	-0.2519	0.0705	0.0916	0.0461	-0.0030	0.0267	0.3464	0.1032
PB	0.2394	-0.1124	-0.0516	-0.3612	0.3529	-0.0727	-0.0097	-0.0114	-0.0025	-0.0084	-0.1039	-0.0707
SP	-0.2313	0.0192	0.3027	0.2434	-0.2200	0.0476	0.0230	0.0141	-0.0068	-0.0197	0.0131	0.0680
SWW	0.1454	-0.0449	-0.0814	-0.9045	0.8779	-0.1452	0.0098	0.0621	0.0101	-0.0086	-0.1703	-0.1022
SW	0.1493	-0.0453	-0.0761	-0.9068	0.8757	-0.1497	-0.0100	0.0638	0.0136	-0.0056	-0.1470	-0.1002
SK	0.2262	-0.0505	-0.0890	-0.8119	0.8103	-0.1617	-0.0582	0.0329	0.0193	-0.0044	-0.1305	-0.0965
SL	0.0906	-0.0021	-0.0133	0.0168	0.0167	-0.0179	-0.5251	0.3146	0.0609	0.0729	0.1230	0.0404
SB	-0.0571	0.0031	0.0102	-0.1341	0.1332	-0.0127	-0.3940	0.4192	0.0464	0.0589	0.1766	0.0888
SKL	0.0182	0.0033	-0.0241	-0.1067	0.1390	-0.0364	-0.3738	0.2273	0.0855	0.1133	0.0377	0.0426
SKB	-0.0918	0.0062	-0.0396	0.0516	-0.0325	0.0047	-0.2539	0.1638	0.0643	0.1507	0.0547	0.0474
GI	-0.2171	0.0141	0.0048	0.1861	-0.1555	0.0255	-0.0780	0.0894	0.0039	0.0100	0.8278	0.1868
GV	-0.2270	0.0336	0.0872	0.3914	-0.3717	0.0661	-0.0899	0.1577	0.0154	0.0303	0.6549	0.2361

Residual are: -0.04497

Where: PL is pod length, PB is pod breadth, S/P is seeds per pod, SWW is 100 seed weight with wings, SW is 100 seed weight without wings, SK is 100 seed kernel weight, SL is seed length, SB is seed breadth, SKL is seed kernel length, SKB is seed kernel breadth, GI is germination index and GV is germination value.

Collar diameter exerted negative direct effect on total fresh biomass (-0.0062). The positive indirect effects were observed through plant height, petiole length, fresh shoot biomass, fresh root biomass, dry shoot biomass and dry root: shoot ratio while negative indirect effects were observed through internodal length, leaf angle, number of branches, leaf area, dry root weight and fresh root: shoot ratio.

Internodal length exerted negative direct effect on total fresh biomass (-0.0285). The positive indirect effects were observed through plant height, petiole length, fresh shoot weight, fresh root weight, dry shoot weight and dry root: shoot ratio while negative indirect effects were observed through collar diameter, leaf angle, number of branches, leaf area, dry root weight and fresh root: shoot ratio.

Petiole length exerted positive direct effect on total fresh biomass (0.0395). The positive indirect effects were observed through plant height, fresh shoot weight, fresh root weight, dry shoot weight and dry root: shoot ratio while negative indirect effects were observed through collar diameter internodal length, leaf angle, number of branches, leaf area, dry root weight and fresh root: shoot ratio.

Leaf angle exerted negative direct effect on total fresh biomass (-0.0161). The positive indirect effects were observed through plant height, petiole length, fresh shoot weight, fresh root weight and dry shoot weight while negative indirect effects were observed through collar diameter, internodal length, number of branches, leaf area, dry root weight, fresh root: shoot ratio and dry root: shoot ratio.

Number of branches exerted negative direct effect on total fresh biomass (-0.0970). The positive indirect effects were observed through plant height, petiole length, fresh shoot weight, fresh root weight, dry shoot weight and dry root: shoot ratio while negative indirect effects were observed through collar diameter, internodal length, leaf angle, leaf area, dry root weight and fresh root: shoot ratio.

Leaf area exerted negative direct effect on total fresh biomass (-0.0054). The positive indirect effects were observed through plant height, petiole length, fresh shoot weight, fresh root weight and dry shoot weight while negative indirect effects were observed through collar diameter, internodal length, leaf angle, number of branches, dry root weight and fresh root: shoot ratio.

Fresh shoot weight exerted positive direct effect on total fresh biomass (0.8576). The positive indirect effects were observed through plant height, petiole length, fresh root weight, dry shoot weight and dry root: shoot ratio while negative indirect effects were observed through collar diameter, internodal length, leaf angle, number of branches, leaf area, dry root weight and fresh root: shoot ratio.

Fresh root weight exerted positive direct effect on total fresh biomass (0.2217). The positive indirect effects were observed through plant height, petiole length, fresh shoot weight, dry shoot weight, fresh root: shoot ratio and dry root: shoot ratio while negative indirect effects were observed through collar diameter, internodal length, leaf angle, number of branches, leaf area and dry root weight.

Dry shoot weight exerted positive direct effect on total fresh biomass (0.1684). The positive indirect effects were observed through plant height, petiole length, fresh shoot weight and fresh root weight while negative indirect effects were observed through collar diameter, internodal length, leaf angle, number of branches, leaf area, dry root weight fresh root: shoot ratio and dry root: shoot ratio.

Dry root weight exerted negative direct effect on total fresh biomass (-0.1437). The positive indirect effects were observed through plant height, petiole length, fresh shoot weight, fresh root weight, dry shoot weight and dry root: shoot ratio while negative indirect effects were observed through collar diameter, internodal length, leaf angle, number of branches, leaf area and fresh root: shoot ratio.

Fresh root: shoot ratio exerted positive direct effect on total fresh biomass (0.0411). The positive indirect effects were observed through collar diameter, internodal length, leaf angle, number of branches, leaf area, fresh root weight and dry root weight while negative indirect effects were observed through plant height, petiole length, fresh shoot weight, dry shoot weight and dry root: shoot ratio.

Dry root: shoot ratio exerted positive direct effect on total fresh biomass (0.1436). The positive indirect effects were observed through plant height, petiole length, leaf angle, leaf area, fresh shoot weight and fresh root weight, while negative indirect effects were observed through collar diameter, internodal length, number of branches, dry shoot weight, dry root weight and fresh root: shoot weight. The results are in line with the findings of Bhagta *et al.*, (2019) in *Grewia optiva* Drummond.

Table: 28. Path coefficient analysis showing direct and indirect effects of nursery morphological and biomass parameters on total fresh biomass of *Moringa oleifera* Lam. progenies

	PH	CD	IL	PL	BA	NB	LA	FSW	FRW	DSW	DRW	FR	DR
PH	0.0555	-0.0048	-0.0184	0.0263	-0.0073	-0.0807	-0.0030	0.6750	0.1508	0.0744	-0.0793	-0.0066	0.0224
CD	0.0430	-0.0062	-0.0150	0.0302	-0.0050	-0.0783	-0.0028	0.6200	0.1378	0.0978	-0.0972	-0.0058	0.0193
IL	0.0357	-0.0032	-0.0285	0.0141	-0.0032	-0.0364	-0.0015	0.4646	0.0956	0.0484	-0.0606	-0.0053	0.0227
PL	0.0369	-0.0047	-0.0102	0.0395	-0.0047	-0.0875	-0.0032	0.6959	0.1621	0.1023	-0.0909	-0.0041	0.0071
BA	0.0251	-0.0019	-0.0057	0.0116	-0.0161	-0.0390	-0.0011	0.2412	0.0386	0.0408	-0.0198	-0.0069	-0.0008
NB	0.0462	-0.0050	-0.0107	0.0357	-0.0065	-0.0970	-0.0035	0.7519	0.1533	0.1014	-0.0996	-0.0110	0.0203
LA	0.0310	-0.0031	-0.0081	0.0232	-0.0032	-0.0627	-0.0054	0.5071	0.1039	0.1084	-0.0708	-0.0063	-0.0230
FSW	0.0437	-0.0045	-0.0155	0.0321	-0.0045	-0.0851	-0.0032	0.8576	0.1590	0.0760	-0.0879	-0.0159	0.0246
FRW	0.0378	-0.0038	-0.0123	0.0289	-0.0028	-0.0671	-0.0026	0.6150	0.2217	0.0744	-0.0757	0.0146	0.0229
DSW	0.0245	-0.0036	-0.0082	0.0240	-0.0039	-0.0584	-0.0035	0.3871	0.0980	0.1684	-0.0856	-0.0004	-0.0612
DRW	0.0307	-0.0042	-0.0120	0.0250	-0.0022	-0.0672	-0.0027	0.5250	0.1168	0.1003	-0.1437	-0.0059	0.0652
FRS	-0.0089	0.0009	0.0037	-0.0040	0.0027	0.0260	0.0008	-0.3326	0.0786	-0.0017	0.0208	0.0411	-0.0096
DRS	0.0087	-0.0008	-0.0045	0.0020	0.0001	-0.0137	0.0009	0.1468	0.0353	-0.0717	-0.0652	-0.0027	0.1436

Residual: 0.00002

Where, PH is plant height, CD is collar diameter, IL is internodal length, PTL is petiole length, BA is leaf angle, NB is number of branches, LA is leaf area, FSW is fresh shoot weight, FRW is fresh root weight, DSW is dry shoot weight, DRW is dry root weight, FRS fresh root: shoot ratio and DRS is dry root: shoot ratio.

Data presented in the Table 29. revealed that crude protein exerted positive direct effect on total fresh biomass (0.3282). The positive indirect effects were observed through ether extract, crude fiber, total amino acids, phosphorus content, calcium content, iron content and copper content whereas negative indirect effects were observed through ash content, nitrogen free extract, nitrogen content, potassium content, magnesium content, sulphur content, zinc content and manganese content.

Ether extract exerted positive direct effect on total fresh biomass (0.3871). The positive indirect effects were observed through crude protein, crude fiber, nitrogen free extract, total amino acids, phosphorus content, calcium content, iron content and copper content whereas negative indirect effects were observed through total ash content, nitrogen content, potassium content, magnesium content, sulphur content, zinc content and manganese content.

Crude fiber exerted negative direct effect on total fresh biomass (-0.0547). The positive indirect effects were observed through total ash content, nitrogen content, potassium content, magnesium content, sulphur content, zinc content and manganese content while negative indirect effects were observed through crude protein, ether extract, nitrogen free extract, total amino acids, phosphorus content, calcium content, iron content and copper content.

Total ash content exerted negative direct effect on total fresh biomass (-0.0315). The positive indirect effects were observed through crude protein, ether extract, crude fiber, total amino acids, phosphorus content, calcium content, iron content and copper content whereas negative indirect effects were observed through nitrogen free extract, nitrogen content, potassium content, magnesium content, sulphur content, zinc content and manganese content.

Nitrogen free extract exerted positive direct effect on total fresh biomass (0.3219). The positive indirect effects were observed through ether extract, crude fiber, total ash content, nitrogen content, potassium content, phosphorus content and copper content while negative indirect effects were observed through crude protein, total ash content, magnesium content, sulphur content, calcium content, zinc content, iron content and manganese content.

Total amino acids exerted positive direct effect on total fresh biomass (0.1713). The positive indirect effects were observed through crude protein, ether extract, crude fiber, phosphorus content, calcium content, iron content and copper content while negative direct

effects were observed through total ash content, nitrogen free extract, nitrogen content, potassium content, magnesium content, sulphur content, zinc content and manganese content.

Nitrogen exerted negative direct effect on total fresh biomass (-0.6271). The positive indirect effects were observed through crude protein, ether extract, crude fiber, total amino acids, phosphorus content, calcium content, iron content and copper content while negative indirect effects were observed through total ash content, nitrogen free extract, potassium content, magnesium content, sulphur content, zinc content and manganese content.

Potassium content exerted negative direct effect on total fresh biomass (-0.2804). The positive indirect effects were observed through crude protein, ether extract, crude fiber, total amino acids, phosphorus content, calcium content, iron content and copper content while negative indirect effects were observed through total ash content, nitrogen free extract, nitrogen content, magnesium content, sulphur content, zinc content and manganese content.

Magnesium content exerted negative direct effect on total fresh biomass (-0.0511). The positive indirect effects were observed through crude protein, ether extract, crude fiber, nitrogen free extract, total amino acids, phosphorus content, calcium content, iron content and copper content while negative indirect effects were observed through total ash content, nitrogen content, potassium content, sulphur content, zinc content and manganese content.

Sulphur content exerted positive direct effect on total fresh biomass (0.2718). The positive indirect effects were observed through total ash content, nitrogen content, potassium content, magnesium content, zinc content and manganese content, whereas negative indirect effects were observed through crude fiber, ether extract, crude fiber, nitrogen free extract, total amino acids, phosphorus content, calcium content, iron content and copper content.

Phosphorus content exerted positive direct effect on total fresh biomass (0.6359). The positive indirect effects were observed through crude protein, ether extract, crude fiber, nitrogen free extract, total amino acids, calcium content, iron content and copper content while negative indirect effects were observed through total ash content, nitrogen content, potassium content, magnesium content, sulphur content, zinc content and manganese content.

Calcium content exerted negative direct effect on total fresh biomass (-0.5614). The positive indirect effects were observed through total ash content, nitrogen free extract, nitrogen content, potassium content, magnesium content, sulphur content, zinc content and

manganese content while negative indirect effects were observed through crude protein, ether extract, crude fiber, total amino acids, phosphorus content, iron content and copper content.

Zinc content exerted positive direct effect on total fresh biomass (0.7590). The positive indirect effects were observed through total ash content, nitrogen content, potassium content, magnesium content, sulphur content and manganese content while negative indirect effects were observed through crude protein, ether extract, crude fiber, nitrogen free extract, total amino acids, phosphorus content, calcium content, iron content and copper content.

Iron content exerted positive direct effect on total fresh biomass (0.5490). The positive indirect effects were observed through crude protein, ether extract, crude fiber, total amino acids, phosphorus content, calcium content and copper content while negative indirect effects were observed through total ash content, nitrogen free extract, nitrogen content, potassium content, magnesium content, sulphur content, zinc content and manganese content.

Copper content exerted negative direct effect on total fresh biomass (-0.2694). The positive indirect effects were observed through total ash content, nitrogen content, potassium content, magnesium content, sulphur content, zinc content and manganese content while negative indirect effects were observed through crude protein, ether extract, crude fiber, nitrogen free extract, total amino acids, phosphorus content, calcium content and iron content.

Manganese content exerted negative direct effect on total fresh biomass (-0.1461). The positive indirect effects were observed through crude protein, ether extract, crude fiber, nitrogen free extract, total amino acids, phosphorus content, calcium content, iron content and copper content while negative indirect effects were observed through total ash content, nitrogen content, potassium content, magnesium content, sulphur content and zinc content. Bhagta *et al.*, (2019) had similar findings for progeny morphological and leaf proximate principles on total fresh biomass of *Grewia optiva*. Pavithra *et al.*, (2013) had findings for pod and seed characteristics on seed oil content in *Pongamia pinnata*. Similarly, the findings of Selvakumari (2013) showed the direct and indirect effects of plant height, leaf area, pod length, pod breadth, number of seeds per pod and 100 seed weight on oil seed content in *Moringa oleifera* Lam.

Table: 29. Path coefficient analysis showing direct and indirect effects of leaf proximate principles, leaf biochemical estimates and leaf mineral contents on total fresh biomass of *Moringa oleifera* Lam. progenies

	CP	EE	CF	AC	NFE	TAA	N	K	Mg	S	P	Ca	Zn	Fe	Cu	Mn
CP	0.3282	0.1540	0.0202	-0.0078	-0.2229	0.1576	-0.6271	-0.0950	-0.0200	-0.0740	0.3187	0.1920	-0.2973	0.2212	0.0722	-0.0687
EE	0.1305	0.3871	0.0465	-0.0085	0.0203	0.0695	-0.2494	-0.1096	-0.0462	-0.1887	0.5509	0.2176	-0.6450	0.0049	0.0890	-0.1334
CF	-0.1209	-0.3288	-0.0547	0.0082	-0.0966	-0.0397	0.2310	0.1106	0.0486	0.2233	-0.5638	-0.2671	0.7643	-0.1136	-0.1104	0.1415
AC	0.0816	0.1042	0.0143	-0.0315	-0.1292	0.0525	-0.1559	-0.0495	-0.0142	-0.0663	0.2310	0.0874	-0.2016	0.1349	0.0410	-0.0371
NFE	-0.2273	0.0245	0.0164	0.0127	0.3219	-0.1352	0.4343	0.0251	-0.0105	-0.0714	0.0200	-0.0004	-0.2170	-0.1181	0.0043	-0.0237
TAA	0.3020	0.1570	0.0127	-0.0097	-0.2541	0.1713	-0.5770	-0.0809	-0.0138	-0.0385	0.2467	0.1385	-0.1969	0.1394	0.0497	-0.0450
N	0.3282	0.1540	0.0202	-0.0078	-0.2229	0.1576	-0.6271	-0.0950	-0.0200	-0.0740	0.3187	0.1920	-0.2973	0.2212	0.0722	-0.0687
K	0.1112	0.1513	0.0216	-0.0056	-0.0288	0.0494	-0.2125	-0.2804	-0.0251	-0.0707	0.2692	0.5601	-0.3275	0.1015	0.2768	-0.0720
Mg	0.1285	0.3500	0.0521	-0.0087	0.0663	0.0462	-0.2455	-0.1378	-0.0511	-0.2196	0.6248	0.2808	-0.7456	0.0901	0.1263	-0.1556
S	-0.0893	-0.2688	-0.0450	0.0077	-0.0845	-0.0242	0.1706	0.0729	0.0413	0.2718	-0.4566	-0.1996	0.6570	-0.0950	-0.0930	0.1139
P	0.1645	0.3354	0.0485	-0.0115	0.0101	0.0665	-0.3143	-0.1187	-0.0502	-0.1952	0.6359	0.2520	-0.6962	0.1504	0.1101	-0.1480
Ca	-0.1122	-0.1500	-0.0260	0.0049	0.0002	-0.0423	0.2144	0.2798	0.0255	0.0966	-0.2854	-0.5614	0.3908	-0.1197	-0.2779	0.0754
Zn	-0.1286	-0.3290	-0.0551	0.0084	-0.0920	-0.0444	0.2456	0.1210	0.0502	0.2353	-0.5832	-0.2890	0.7590	-0.1405	-0.1235	0.1470
Fe	0.1322	0.0035	0.0113	-0.0077	-0.0693	0.0435	-0.2526	-0.0518	-0.0084	-0.0470	0.1743	0.1224	-0.1943	0.5490	0.0425	-0.0355
Cu	-0.0880	-0.1280	-0.0224	0.0048	-0.0052	-0.0316	0.1681	0.2882	0.0239	0.0938	-0.2599	-0.5793	0.3480	-0.0867	-0.2694	0.0707
Mn	0.1544	0.3536	0.0530	-0.0080	0.0522	0.0528	-0.2950	-0.1383	-0.0544	-0.2119	0.6440	0.2898	-0.7638	0.1334	0.1303	-0.1461

Residue: 0.52147

Where, CP is crude protein, EE is ether extract, CF is crude fiber, AC is ash content, NFE is nitrogen free extract, TAA is total amino acids, N is nitrogen, K is potassium, Mg is magnesium, S is sulphur, P is phosphorus, Ca is calcium, Zn is zinc, Fe is iron, Cu is copper and Mn is manganese

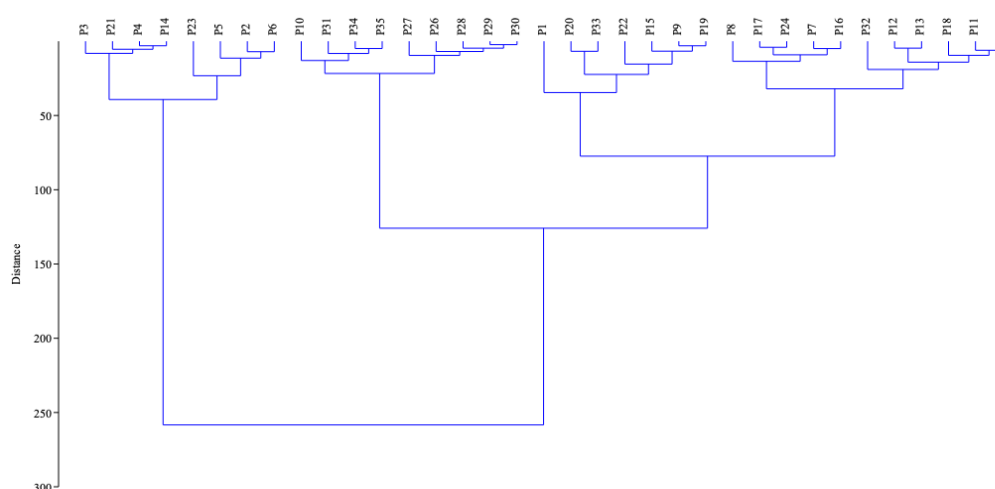
4.10 Cluster analysis

The cluster analysis of pod and germination characters of moringa progenies based on Ward's method suggested the formation of two clusters (Figure 5.). Among the two clusters, cluster I comprised of 8 progenies whereas cluster II was the largest with 27 progenies.

Cluster I had two sub-clusters (Ia and Ib) where each sub-cluster comprised of four progenies. Progenies P3, P21, P4 and P14 were under first sub-cluster while the second sub-cluster comprised of progenies P23, P5, P2 and P6. Cluster II composed of two major clusters (IIa and IIb). The first major cluster (IIa) comprised two minor clusters. The first minor cluster (IIaa) comprised of four progenies (P10, P31, P34 and P35) while second minor cluster (IIab) comprised of five progenies (P27, P26, P28, P29 and P30). The second major cluster (IIb) comprised three minor clusters. The first minor cluster comprised of 7 progenies (P1, P20, P33, P22, P15, P9 and P19), second minor cluster comprised of five progenies (P8, P17, P24, P7, P16) while third minor cluster comprised of six progenies (P32, P12, P13, P18, P11 and P25).

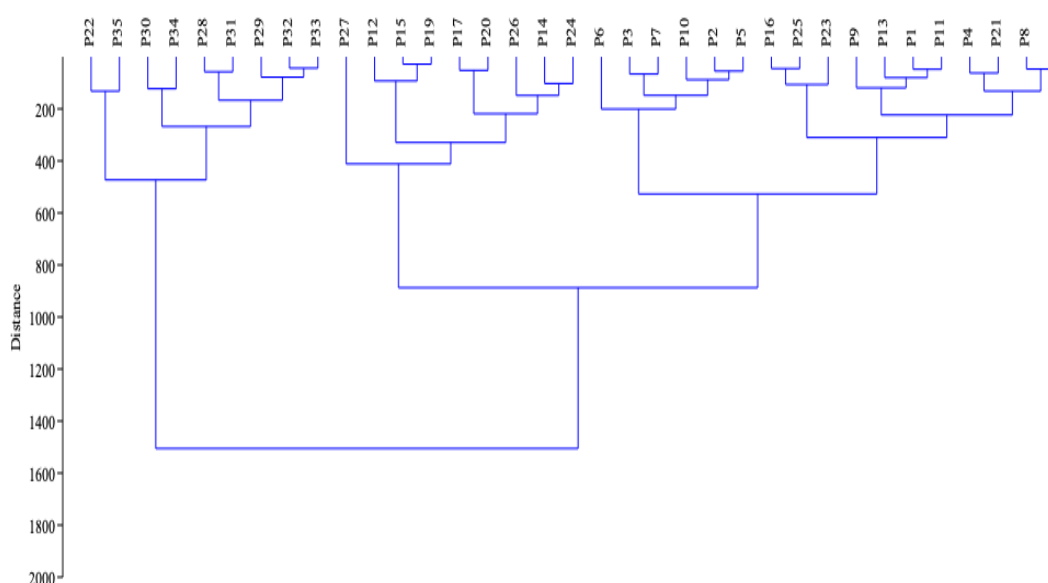
P4 and P14, P2 and P6, P34 and P35, P29 and P30, P20 and P33, P9 and P19, P17 and P24, P7 and P16, P12 and P13 and P11 and P25 were closest to each other independently while P3 and P25 were widest apart. The result supports the findings of Raphael *et al.*, (2021), Popoola *et al.*, (2016), Zhigila *et al.*, (2015) and Chan *et al.*, (2018) in *Moringa oleifera* Lam. Tak (2012) also found similar results for pod, seed and leaf mineral characters in *Moringa oleifera* Lam.

Figure: 5. Cluster analysis of *Moringa oleifera* Lam. on the basis of pod and germination parameters



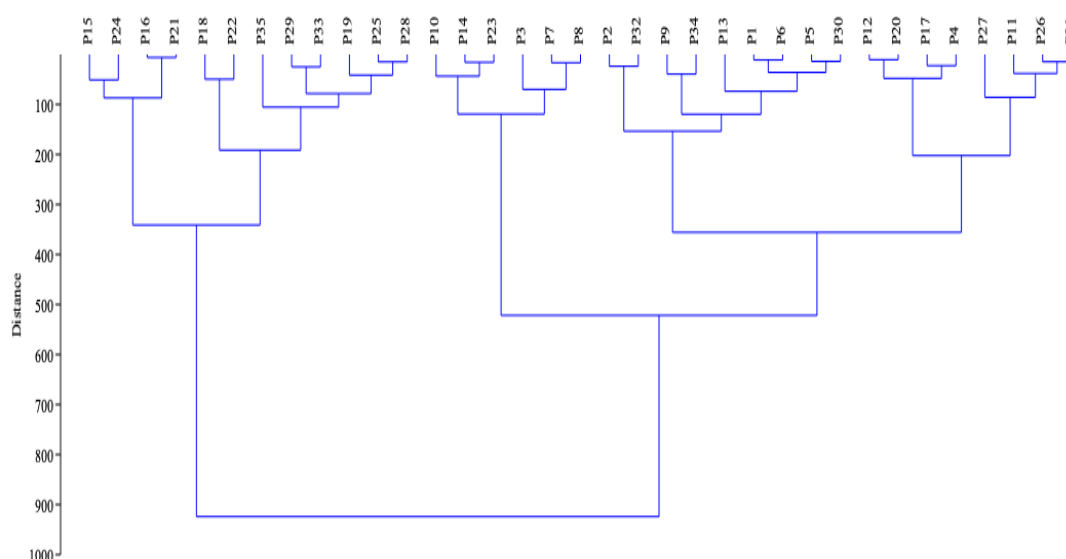
The cluster analysis of morphological characters of *Moringa* progenies based on Ward's method suggested the formation of two clusters (Figure 6.). Among the two clusters, cluster I comprised of 9 progenies while cluster II was comprised of 26 progenies. Cluster I further divided into two major clusters (Ia and Ib) where first major cluster (Ia) comprised of two progenies (P22 and P35) whereas second major cluster (Ib) further divides into two minor clusters (Iba and Ibb). First minor cluster (Iba) comprised of two progenies (P30 and P34) while second major (Ibb) comprised of three progenies (P28, P31, P29, P32 and P33). Cluster II divided into two major clusters (IIa and IIb). First major cluster (IIa) comprised of three minor clusters in which, first minor cluster (IIaa) comprised of one progeny (P27), second minor cluster (IIab) comprised of three progenies (P12, P15 and P19) and third minor (IIac) comprised of five progenies (P17, P20, P26, P14 and P24). Second major cluster (IIb) comprised of two minor clusters where first minor cluster (IIba) comprised of six progenies (P6, P3, P7, P10, P2 and P5) and second minor cluster comprised of eleven progenies (P16, P25, P23, P9, P13, P1, P11, P4, P21, P8 and P18). Progenies P32 and P33, P15 and P19, P16 and P25 and P1 and P11 were closest to each other individually while P22 and P18 were widest apart. The present results were supported by the findings by Wani (2012) for *Albizia lebbbeck* Benth and Yadav *et al.*, (2019) in *Melia dubia*.

Figure: 6. Cluster analysis of *Moringa oleifera* Lam. progenies on the basis of morphological and biomass characters



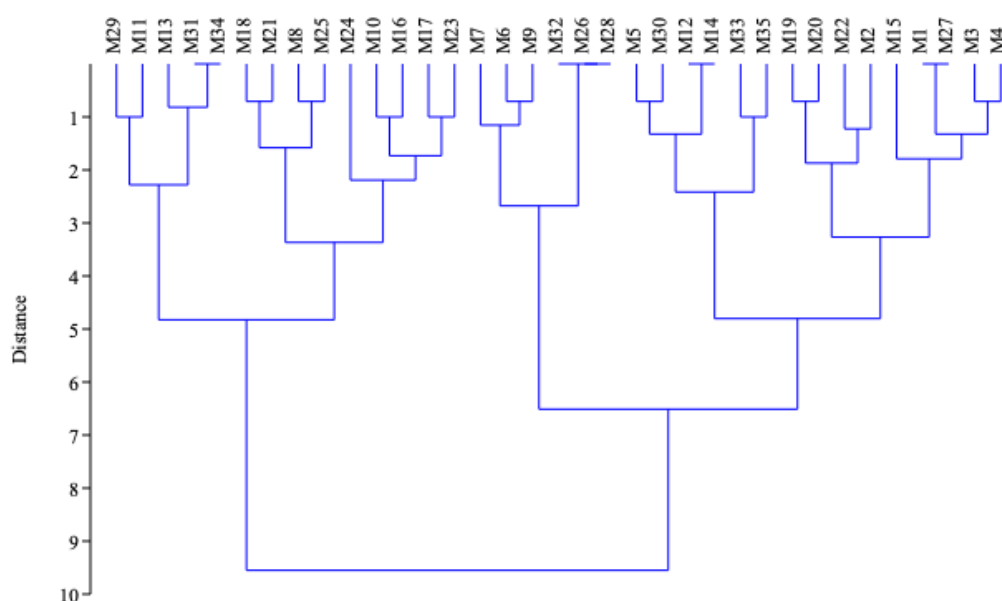
The cluster analysis of leaf proximate principles, leaf biochemical estimates and leaf mineral contents of *Moringa* progenies based on Ward's method suggested the formation of two clusters (Figure 7.). Among the two clusters, cluster I comprised of 12 progenies while cluster II comprised of 23 progenies. Cluster I divided into two major clusters (Ia and Ib). Major cluster Ia comprised of two minor clusters having two progenies (P15 and P24) and (P16 and P21) each. Major cluster Ib also comprised of two minor clusters where first minor cluster (Iba) comprised of two progenies (P18 and P22) while second minor cluster (Ibb) comprised of six progenies (P35, P29, P33, P19, P25 and P28). Cluster II also divided into two major clusters (IIa and IIb) which further comprised two minor clusters each. Both minor clusters (IIaa and IIab) of first major cluster (IIa) comprised of three progenies each, viz., P10, P14 and P23; P3, P7 and P8, respectively. In major cluster IIb, first minor cluster (IIba) comprised of nine progenies (P2, P32, P9, P34, P13, P1, P6, P5 and P30) while second minor cluster (IIbb) comprised of eight progenies (P12, P20, P17, P4, P27, P11, P26 and P31). The Progenies 16 and 21, 14 and 23, 1 and 6, 5 and 30, 26 and 31 were closest to each other individually while P15 and P31 were widest apart. Similar findings were reported by Anand *et al.*, (2013) for leaf dry matter and proximate principles in *Bauhinia variegata* Linn.

Figure: 7. Cluster analysis of *Moringa oleifera* Lam. progenies on the basis of leaf proximate principles, leaf biochemical estimates and leaf mineral contents



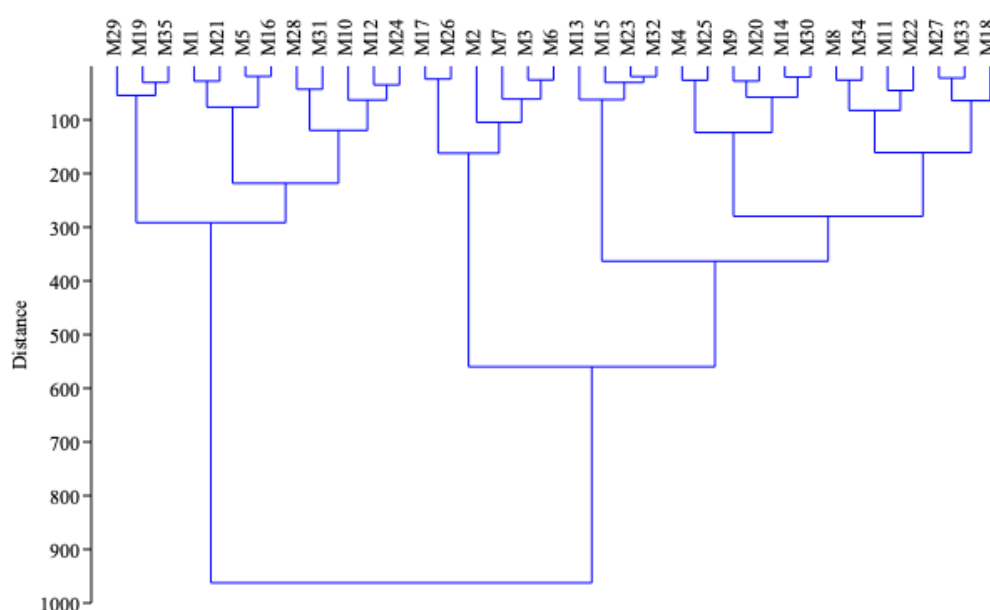
The cluster analysis of qualitative characters of *Moringa* mother trees based on Ward's method suggested the formation of two clusters (Figure 8.). Among the two clusters, cluster I comprised of 14 mother trees while cluster II comprised of 21 mother trees. Cluster I divided into two major clusters (Ia and Ib). major cluster Ia further consisted of two minor clusters (Iaa and Iab) which comprised of two mother trees (M29 and M11) and three mother trees (M13, M31 and M34), respectively. Major cluster Ib also consisted of two minor clusters which comprised of four mother trees (M18, M21, M8 and M25) and five mother trees (M24, M10, M16, M17 and M23), respectively. Cluster II also comprised of two major clusters (IIa and IIb). First major cluster (IIa) consisted of two minor clusters (IIaa and IIab) having three mother trees each. First minor cluster (IIaa) consisted of M7, M6 and M9 whereas second minor cluster consisted of M32, M26 and M28. Second major cluster (IIb) consisted of three minor clusters (IIba, IIbb and IIbc). First minor cluster consisted of six mother trees (M5, M30, M12 M14, M33 and M35), second minor cluster consisted of four mother trees (M19, M20, M22 and M2) and third minor cluster consisted of five mother trees (M15, M1, M27, M3 and M4). Mother trees M31 and M34, M32, M26 and M28, M12 and M14, M1 and M27 were closest to each other individually while M29 and M4 were widest apart. Similar findings were reported by N'Djolosse *et al.*, (2019) in *Anacardium occidentale* L.

Figure: 8. Cluster analysis of qualitative characters of *Moringa oleifera* Lam. mother trees



The cluster analysis of leaf proximate principles, leaf biochemical estimates and leaf mineral contents of *Moringa* mother trees based on Ward's method suggested the formation of two clusters (Figure 9.). Among the two clusters, cluster I comprised of 12 mother trees while cluster II comprised of 23 mother trees. Cluster I divided into two major clusters (Ia and Ib). First major cluster (Ia) comprised of three mother trees (M29, M19 and M35). Second major cluster was composed of two minor clusters (Iba and Ibb) comprising of four mother trees (M1, M21, M5 and M16) and five mother trees (M28, M31, M19, M12 and M24), respectively. Cluster II also comprised of two major clusters (IIa and IIb). Major cluster IIa further comprised of two minor clusters (IIaa and IIab) which included two mother trees (M17 and M26) and four mother trees (M2, M7, M3 and M6), respectively. On the other hand, major cluster IIb comprised of three minor clusters (IIba, IIbb and IIbc) which included four mother trees (M13, M15, M23 and M32), six mother trees (M4, M25, M9, M20, M14 and M30) and seven mother trees (M8, M34, M11, M22, M27, M33 and M18), respectively. Mother trees M17 and M26, M3 and M6, M23 and M32, M4 and M25, M9 and M20, M14 and M30, M8 and M34, M27 and M33 were closest to each other individually while M29 and M18 were widest apart. The results are supported by the findings of Tak (2012) and Selvakumari (2013) in *Moringa oleifera* Lam.

Figure: 9. Cluster analysis of quantitative characters of *Moringa oleifera* Lam. mother trees



4.11 Diversity analysis using SSR markers

Twenty SSR primer pairs were used for genetic diversity analysis, out of which 16 showed PCR amplification. These 16 amplified primers generated a total of 60 bands. All these primers showed 100 per cent polymorphism except MO1. The number of bands per primer ranged from 2 to 6 with an average of 3.75 alleles per primer. The band size ranged between 100-500 bp (Table 30.).

It is evident from Table 30. that the primers i.e., MO1 (Plate 7.), MO44, MO45 and MO46 (Plate 8.) amplified 2 bands each; MO41 and MO56 (Plate 9.) amplified 3 bands each; MO13, MO18, MO48, MO55 and MO58 amplified 4 bands each; MO6, MO8, MO10 and MO12 amplified 5 bands each whereas MO15 amplified 6 bands, respectively.

The present results are in accordance with the findings of Popoola *et al.*, (2017), Rajalakshmi *et al.*, (2017), Ganeshan *et al.*, (2014), Selvakumari (2013) and Shahzad *et al.*, (2013) with respect to the number of bands amplified by the same set of primers in *Moringa oleifera* Lam. The present results corroborate well with the respective authors for the number of bands amplified i.e., 2, 4, 5 and 6 with the following primers: 2 bands by MO1, MO44, MO45, MO46 (Ganeshan *et al.*, 2014) and MO46 (Selvakumari (2013); 4 bands by MO18 (Popoola *et al.*, 2017) and MO55 (Rajalakshmi *et al.*, 2017); 5 bands by MO6 (Rajalakshmi *et al.*, 2017) and 6 bands by MO15 (Rajalakshmi *et al.*, 2017), in particular study.

Table: 30. Number of bands and per cent polymorphism in *Moringa oleifera* Lam. genotypes using SSR markers

Primer	Total number of bands	Number of monomorphic bands	Number of polymorphic bands	Per cent polymorphism (%)	Band size range (bp)
MO1	2	1	1	50	200-250
MO6	5	0	5	100	200-250
MO8	5	0	5	100	250-300
MO10	5	0	5	100	250-300
MO12	5	0	5	100	250-500
MO13	4	0	4	100	100-250
MO15	6	0	6	100	100-250
MO18	4	0	4	100	100-200
MO41	3	0	3	100	100-200
MO44	2	0	2	100	250-450
MO45	2	0	2	100	150-250
MO46	2	0	2	100	100-250
MO48	4	0	4	100	100-250
MO55	4	0	4	100	100-300
MO56	3	0	3	100	150-250
MO58	4	0	4	100	100-200
Total	60	1	59	96.88	

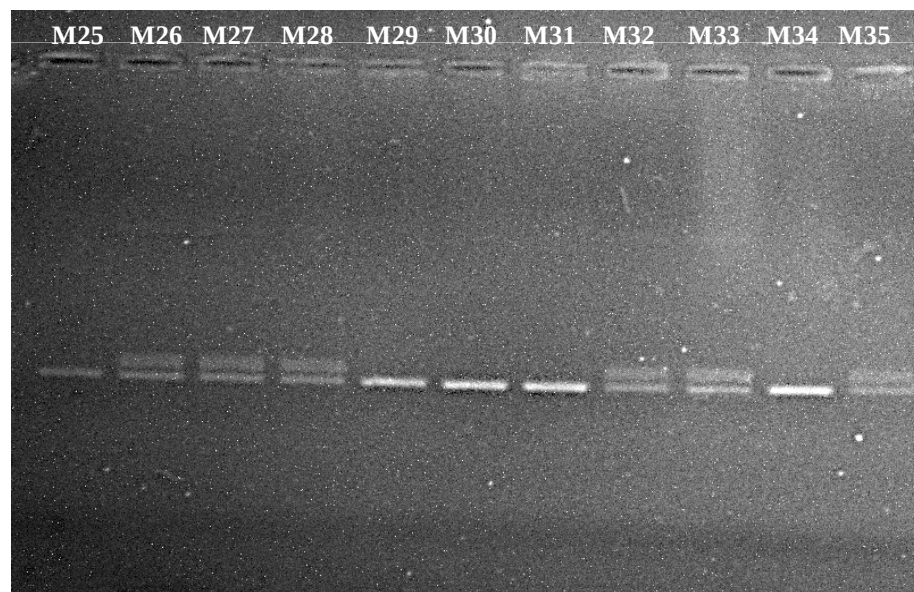
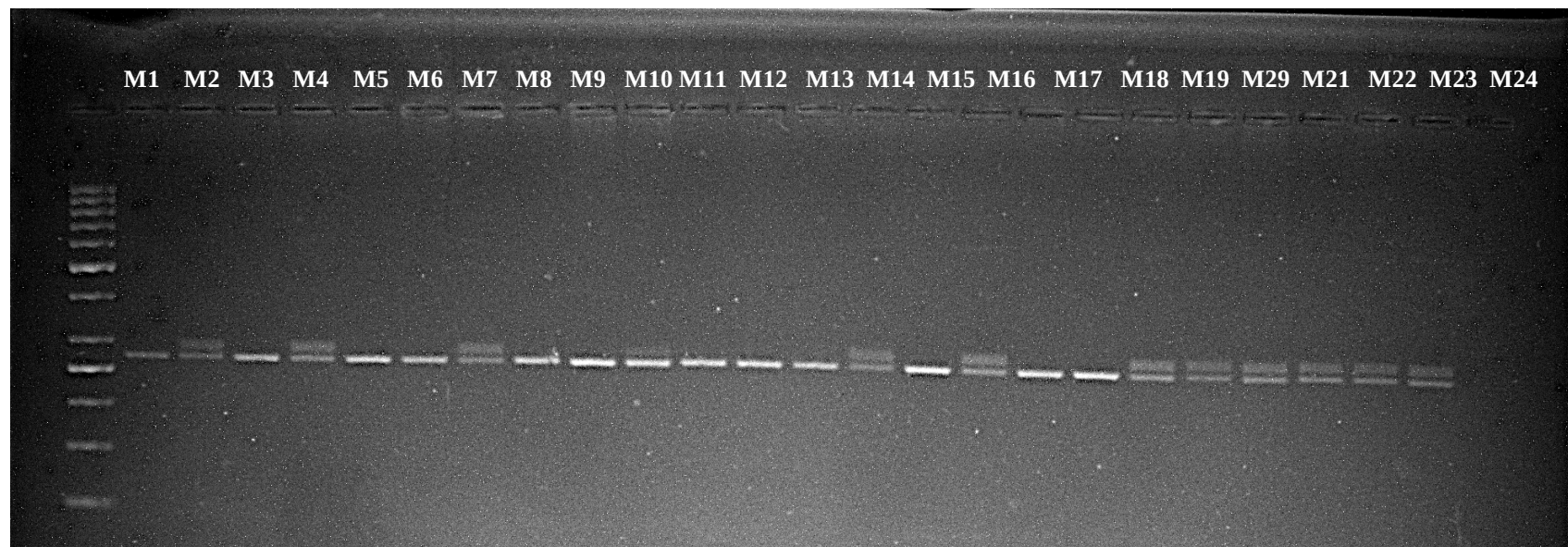


Plate: 7. PCR amplification of *Moringa oleifera* Lam. mother trees using SSR primer MO 1

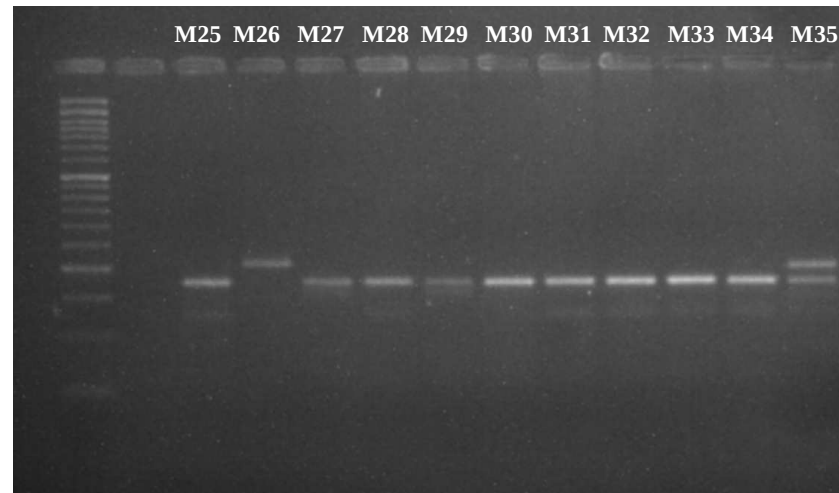
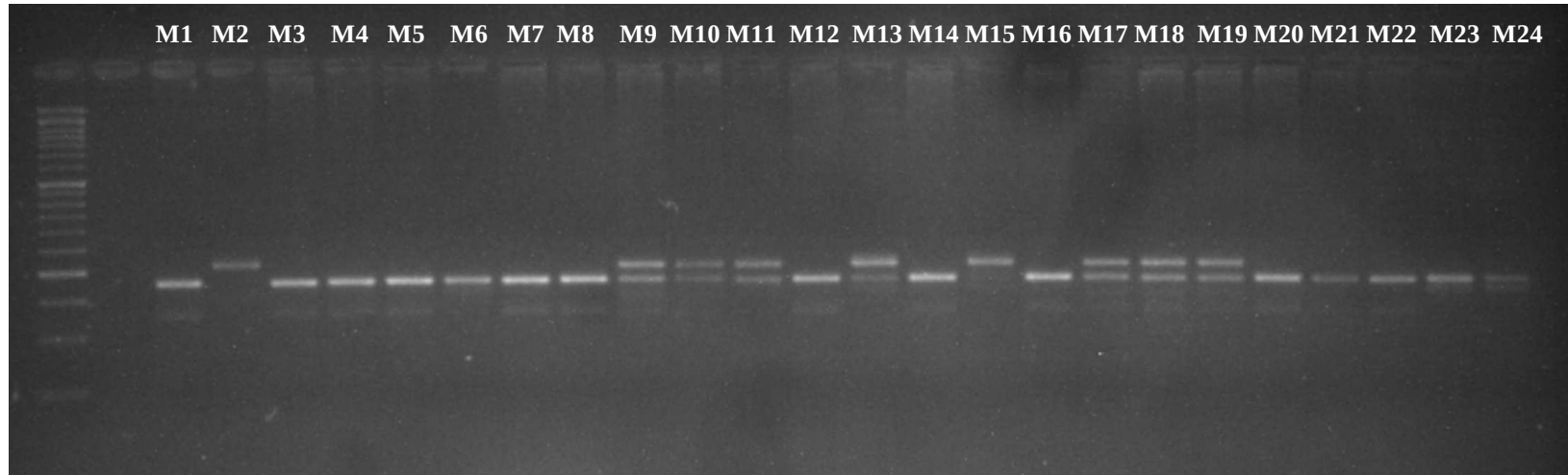


Plate: 8. PCR amplification of *Moringa oleifera* Lam. mother trees using SSR primer MO 46

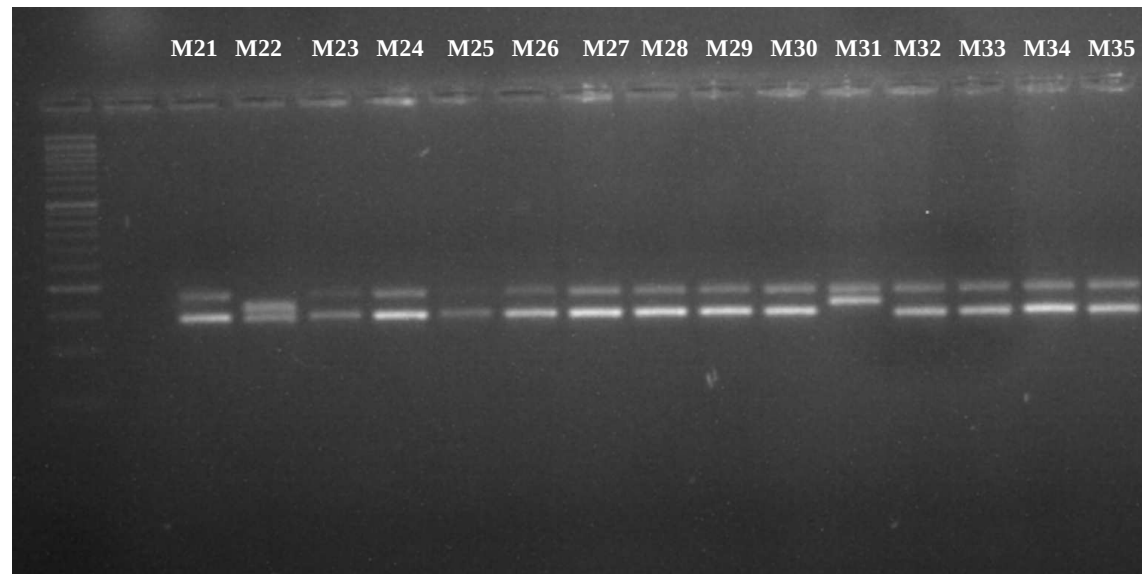
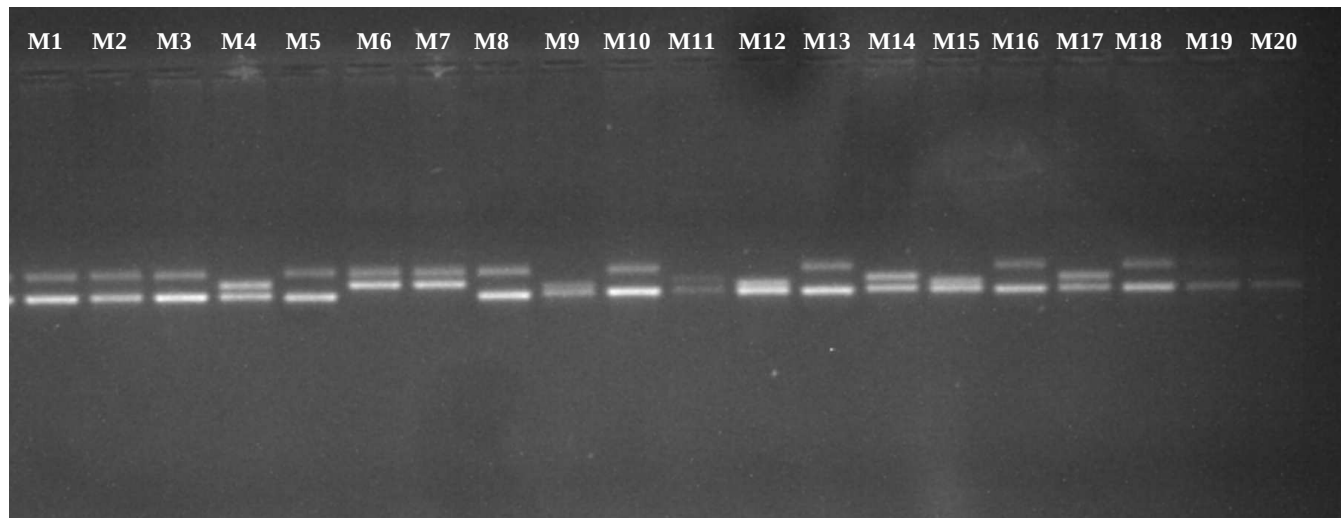
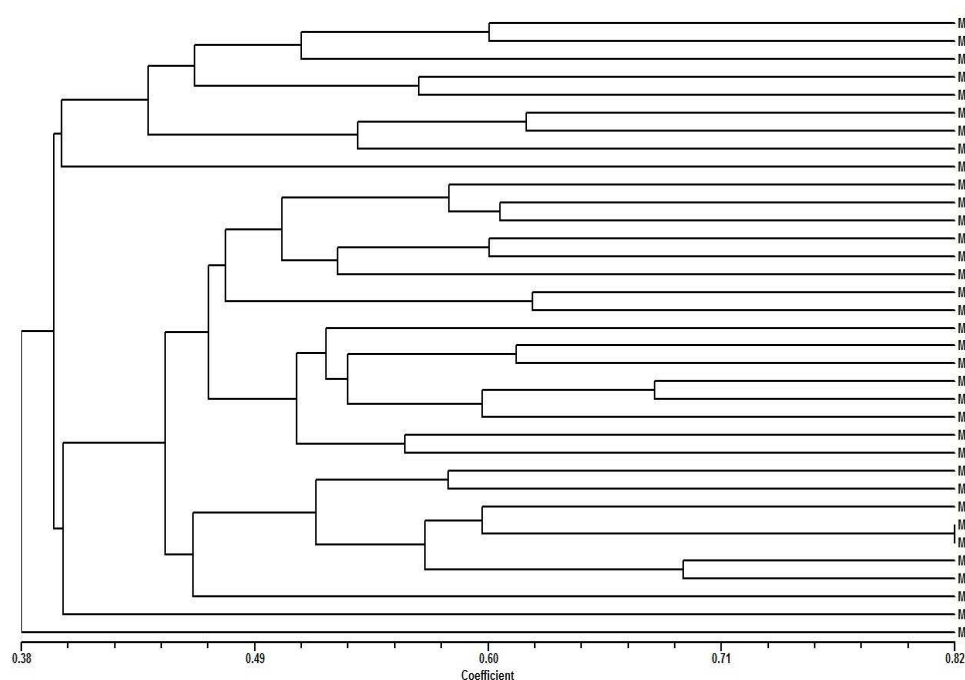


Plate: 9. PCR amplification of *Moringa oleifera* Lam. mother trees using SSR primer MO 56

The dendrogram constructed through NTSYS software grouped all the mother trees into two major clusters, viz., I and II (Figure 10). Cluster I is further sub-grouped into two clusters: Ia and Ib. Cluster Ia comprised of mother trees M1, M5, M6, M2, M8, M4, M9, M12 and M11, respectively. While the rest of mother trees were in cluster Ib. On the other hand, cluster II had only one mother tree i.e., M3. M3 was found to be highly distinct from rest of the mother trees studied in the present investigation. Likewise, M1 and M3 were found to be distantly related. It can be inferred that M34 and M35 were found to be closely related to each other. Besides this, the similarity coefficient of 0.82 (Figure 10.) was observed among the mother trees used in the present study.

Based on the results obtained in the present study using morphological and SSR markers, it can be concluded that phenotypic characters have a limited importance for diversity analysis since they are generally influenced by environmental factors and developmental stage of the plant. On the contrary, the SSR markers used in this study were highly found to be polymorphic and efficient in revealing the level of genetic diversity present in the *Moringa* populations. Besides, SSR markers are randomly distributed throughout the genome and in majority of cases most regions of the genome (nearly 90 %) are not expressed at phenotypic level (Dahlberg, 2000). So, it is very difficult to find out similarity between grouping based on SSR and morphological traits.

Figure: 10. Cluster analysis of *Moringa oleifera* Lam. mother trees using molecular markers



Furthermore, the small number of genomic SSR primers i.e., 16 used in the present study cannot be effectively correlated with the findings inferred from the morphological and qualitative characters to much extent. However, the information generated from the molecular analysis would be valuable for efficient management of germplasm and for effective utilization of materials in breeding programmes to produce hybrids of desirable characteristics. Therefore, the application of morphological descriptors in management of germplasm should be validated using molecular markers.

Chapter-5

SUMMARY AND CONCLUSION

The present studies entitled “Genetic diversity in wild populations of *Moringa oleifera* Lam. (Drumstick tree) in north-west India” were carried out in the Department of Tree Improvement and Genetic Resources of Dr. Yashwant Singh Parmar University of Horticulture and Forestry Nauni, Solan (H.P.) during 2020-23. The area having populations of *M. oleifera* Lam. in North-west India were surveyed and seven seed sources namely Samba (Samba, Jammu and Kashmir), Dunera (Pathankot, Punjab), Vikasnagar (Dehradun, Uttarakhand), Lamlehri (Una, Himachal Pradesh) Tirla (Solan, Himachal Pradesh) Siswa Dam (Sahibzada Ajit Singh Nagar, Punjab) and Morni Hills (Panchkula, Haryana) were finalized. These seed sources were distantly located along geographic stretch of foothills of Himalayas, varying across the altitudinal ranges approximately from 351 m to 549 m amsl. The study included assessment of the variability in *Moringa* at qualitative, quantitative as well as at molecular level.

The study was divided into four experiments, viz., survey and selection of plus (mother) trees, variation studies in morphological and biomass characters in half-sib progenies at nursery stage, nutritional evaluation of progenies and mother trees and assessment of genetic diversity of mother trees using SSR markers. Plus tree selection was done by base line methodology. Five mother trees per location were selected. Data were recorded on tree height (m), DBH (cm), crown spread (m²) and total leaf biomass (kg) along with qualitative characteristics of mother trees. Variability with respect to seed and seedling traits was conducted by sowing the seeds collected from plus trees. Significant variation was recorded for all the parameters except survival percentage and leaf dry matter content of both mother trees as well as progeny.

The qualitative characters showed high grade variation in mother trees. Additionally, tree height of selected mother trees ranged between 9.01-13.41 m, DBH ranged between 25.19-33.97 cm, crown spread ranged between 56.00-76.80 m² and total leaf biomass ranged between 35.21-61.14 kg.

In mother trees, highest crude protein was observed in M2 (24.94 %), highest ether extract was observed in M19 (6.29 %), highest crude fiber was observed in M28 (15.32 %),

total ash content was observed in M7 (11.86 %), highest nitrogen free extract was observed in M11 (65.63 %) and highest amino acid content was observed in M2 (16.30 %).

Highest nitrogen content was observed in M2 (3990.42 mg/100 g), phosphorus content in M19 (81.85 mg/100 g), potassium content in M7 (1861.33 mg/100 g), calcium content in M18 (2.10 mg/100 g), magnesium content in M20 (772.00 mg/100 g), sulphur content in M7 (1421.67 mg/100 g), iron content in M27 (43.67 mg/100 g), zinc content M7 (3.25 mg/100 g), copper content M6 (0.87 mg/100 g) and manganese content M18 (4.62 mg/100 g) in mother trees.

In progenies, highest crude protein was observed in P7 (27.49 %), highest ether extract was observed in P19 (7.07 %), highest crude fiber was observed in P3 (13.60 %), total ash content was observed in P22 (10.39 %), highest nitrogen free extract was observed in P34 (63.51 %) and highest amino acid content was observed in M7 (19.12 %).

Highest nitrogen content was observed in P7 (4397.87 mg/100 g), phosphorus content in P22 (74.68 mg/100 g), potassium content in P29 (1761.02 mg/100 g), calcium content in P20 (1.89 mg/100 g), magnesium content in P16 (777.88 mg/100 g), sulphur content in P7 (1279.19 mg/100 g), iron content in P18 (49.60 mg/100 g), zinc content P7 (3.87 mg/100 g), copper content P14 and P18 (0.78 mg/100 g) and manganese content P22 (4.82 mg/100 g) in Moringa progenies.

Significant variation were observed for morphological and biomass characteristics of Moringa progenies. Highest plant height was observed in P35 (234.44 cm), collar diameter in P34 (34.85 mm), number of leaves in P35 (43.20), leaf area in P30 (1014.09 cm²), fresh shoot weight in P30 (1509.01 g), fresh root weight in P35 (634.41 g), total fresh biomass in P29 (2029.43 g), dry shoot weight in P35 (412.44 g) and dry root weight in P29 (160.35 g).

PCV was slightly higher than GCV for every parameter showing a slight effect of environment in variability of expression of traits. High heritability with high genetic gain was observed for pod breadth, seeds per pod, 100 seed weight with wings, 100 seed weight without wings, 100 seed kernel weight, germination percentage, germination index and germination value; plant height and total fresh biomass; ether extract content, crude fiber content, phosphorus content, zinc content, sulphur content, iron content and manganese content indicating that these traits can be improved through selection.

Germination percentage, germination index and germination value showed significant positive correlation among themselves. In addition to this, pod length, pod breadth, seeds per pod and seed breadth had significantly positive correlation with germination percentage.

Plant height, collar diameter, internodal length, petiole length, leaf angle, number of leaves, leaf area and fresh and dry biomass characters showed significantly positive correlation with total fresh biomass of *Moringa* progenies.

Ether extract showed significantly positive correlation with ash content, total amino acids, nitrogen content, potassium content, magnesium content, phosphorus content and manganese content. Crude fiber showed significantly positive correlation with sulphur content, calcium content and copper content. Sulphur content showed significantly positive correlation with calcium content, zinc content and copper content. Phosphorus content showed significantly positive correlation with iron and manganese. Zinc content showed significantly positive correlation with copper content while iron content showed significantly positive correlation with manganese content.

Principal Component Analysis transformed pod, seed and germination parameters into four major principal components having Eigen value of more than 1 thereby, indicating that these components are responsible for higher magnitude of variance in the population (81.23 %). PCA biplot revealed that progenies, viz., P8, P13, P16, P26, P27, P28, P30 and P34 were found to be superior performers for desirable traits and can be used as parents in hybridization programme.

Principal Component Analysis transformed morphological characteristics of *Moringa* progenies into three major principal components having Eigen value of more than 1 thereby, indicating that these components are responsible for higher magnitude of variance in the population (72.10 %). PCA biplot revealed that progenies, viz., P14, P24, P25, P27, P28, P29, P31, P32 and P34 were found to be lying in better side of both PC1 and PC2. These progenies could be used for further breeding programmes.

Principal Component Analysis transformed leaf proximate principles, leaf biochemical estimates and leaf mineral contents of *Moringa* progenies into four major principal components having Eigen value of more than 1 thereby, indicating that these components are responsible for higher magnitude of variance in the population (89.61 %). PCA biplot revealed that progenies, viz., P9, P15, P16, P18, P19, P21, P26, P28, P29, P30,

P33 and P35 were found to be superior performers for desirable leaf proximate principles and leaf mineral contents and can be used as parents in hybridization programme.

Study on path coefficient analysis revealed high positive direct effect of seeds per pod, 100 seed weight without wings, seed breadth, germination index and germination value on germination percentage. Total fresh biomass had high positive direct effects of fresh shoot weight, dry shoot weight, fresh root weight and dry root: shoot ratio on it. On the other hand, high and positive direct effects of crude protein, ether extract, nitrogen free extract, sulphur content, phosphorus content, zinc and iron content was seen on total fresh biomass.

Cluster analysis by Ward's method analysis grouped the progenies into two clusters in respect of pod, seed and germination parameters. Cluster I and II included 8 and 27 progenies, respectively.

Cluster analysis on morphological characteristics; leaf proximate principles, leaf biochemical estimates and leaf mineral contents divided both the dendrograms into two clusters having 9, 26 and 12, 23 progenies, respectively.

Similarly, cluster analysis on qualitative along with quantitative characteristics divided both the dendrograms into two clusters having 14, 21 and 12, 23 mother trees, respectively.

Genetic diversity of thirty five mother trees of *Moringa oleifera* Lam. was analysed at molecular level by using twenty SSR markers. Sixteen markers showed amplification out of which all sixteen markers showed polymorphism (total 100 %). On an average, 3.75 bands per primer were produced by SSR primers. Two distinct clusters and a level of genetic dissimilarity between qualitative characters and molecular analysis was estimated from the respective dendrograms.

Conclusion

The present investigations carried out for the evaluation of Drumstick (*Moringa oleifera* Lam.) on the basis of morphological, nutritional and molecular levels have revealed the following conclusions:

1. A huge amount of variation was observed for qualitative, quantitative and molecular aspects of mother trees.
2. Phenotypic characters have a limited importance for diversity analysis since they are generally influenced by environmental factors and developmental stage of the plant.

On the contrary, the SSR markers used in this study were highly found to be polymorphic and efficient in revealing the level of genetic diversity present in the Moringa populations.

3. The extent of variation among progenies in respect of seed traits, germination parameters, seedling growth and biomass traits and nutritional evaluation parameters was very large.
4. Total fresh biomass is the most important character of economic importance of this species. The genotypes from Siswa Dam and Morni Hills were proven to be highly superior for selection of biomass characters.
5. Characters having high heritability in addition to high genetic gain should be exploited along with highly positively correlated characters so as to develop genotypes of higher morphological as well as nutritive values.
6. The progenies proved to be the best ones found during the study for various economic traits in Moringa based on their *per se* performance were: P3 for crude fiber content, P7 for number of leaves, crude protein content, total amino acids, nitrogen content, sulphur content and zinc content, P10 for germination percentage, P16 for magnesium content, P18 for iron content, P19 for ether extract, P20 for calcium content, P22 for total ash content, phosphorus content and manganese content, P29 for potassium content, P30 for leaf area and fresh shoot weight, P32 for pod length, P34 for collar diameter and nitrogen free extract and P35 for seeds per pod, plant height and fresh root weight.
7. These progenies could further be exploited at field conditions and multilocation trails could be conducted for evaluation of genetic superiority of these progenies for further selections.

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APPENDIX- I

Analysis of variance (ANOVA)

1. ANOVA for variation in pod, seed and germination parameters of *Moringa oleifera* Lam.

a. Analysis of Variance for Pod length.

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	1,865.85	54.878	49.825
Error	68	74.897	1.101	
Total	104	1945.97		

b. Analysis of Variance for Pod breadth.

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	13.332	0.392	213.174
Error	68	0.125	0.002	
Total	104	13.462		

c. Analysis of Variance for Seeds per pod.

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	954.528	28.074	122.377
Error	68	15.6	0.229	
Total	104	970.846		

d. Analysis of Variance for 100 seed weight with wings.

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	336.743	9.904	99.371
Error	68	6.778	0.1	
Total	104	343.545		

e. Analysis of Variance for 100 seed weight without wings.

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	318.143	9.357	118.607
Error	68	5.365	0.079	
Total	104	323.732		

f. Analysis of Variance for 100 seed kernel weight.

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	324.212	9.536	159.376
Error	68	4.069	0.06	
Total	104	328.331		

g. Analysis of Variance for seed length.

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	1.356	0.04	48.069
Error	68	0.056	0.001	
Total	104	1.4169		

h. Analysis of Variance for seed breadth.

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	1.012	0.03	95.563
Error	68	0.021	0.00031	
Total	104	1.038		

i. Analysis of Variance for seed kernel length.

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	0.355	0.0104	28.892
Error	68	0.025	0.000368	
Total	104	0.38021		

j. Analysis of Variance for seed kernel breadth.

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	0.25	0.00735	52.08
Error	68	0.01	0.000147	
Total	104	0.261		

k. Analysis of Variance for germination index.

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	1,09,286.25	3,214.30	15.401
Error	68	14,191.70	208.701	
Total	104	1,23,992.921		

l. Analysis of Variance for germination value.

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	48,387.70	1,423.17	8.8
Error	68	10,997.30	161.725	
Total	104	59,764.348		

m. Analysis of Variance for germination percentage.

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	38,741.43	1,139.45	13.847
Error	68	5,595.71	82.29	
Total	104	44,974.759		

m. Analysis of Variance for survival percentage.

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	4472.43	131.54	0.97
Error	68	9252.71	136.069	
Total	104	14379.55		

2. ANOVA for variation in morphological characteristics of *Moringa oleifera* Lam. progenies

a. Analysis of Variance for plant height:

i. after 2 months

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	714.301	21.009	34.935
Error	68	40.893	0.601	
Total	104	757.228		

ii. after 4 months

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	5,390.27	158.537	62.978
Error	68	171.179	2.517	
Total	104	5,566.64		

iii. after 6 months

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	16,538.01	486.412	132.422
Error	68	249.777	3.673	
Total	104	16,801.65		

iv. after 8 months

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	31,125.29	915.45	102.109
Error	68	609.646	8.965	
Total	104	31,754.99		

v. after 10 months

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	52,297.76	1,538.17	107.884
Error	68	969.522	14.258	
Total	104	53,289.74		

vi. after 12 months

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	1,00,214.73	2,947.49	63.172
Error	68	3,172.76	46.658	
Total	104	1,12,005.70		

b. Analysis of Variance for collar diameter:

i. after 2 months

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	26.591	0.782	18.716
Error	68	2.842	0.042	
Total	104	29.549		

ii. after 4 months

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	185.238	5.448	18.176
Error	68	20.383	0.3	
Total	104	208.741		

iii. after 6 months

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	380.594	11.194	15.904
Error	68	47.863	0.704	
Total	104	437.975		

iv. after 8 months

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	781.245	22.978	14.975
Error	68	104.339	1.534	
Total	104	922.817		

v. after 10 months

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	1,210.06	35.59	12.682
Error	68	190.838	2.806	
Total	104	1,402.79		

vi. after 12 months

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	1,968.86	57.908	12.652
Error	68	311.242	4.577	
Total	104	2,421.01		

c. Analysis of Variance for internodal length:

i. after 2 months

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	12.471	0.367	7.193
Error	68	3.468	0.051	
Total	104	16.207		

ii. after 4 months

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	6.973	0.205	1.297
Error	68	10.752	0.158	
Total	104	17.813		

iii. after 6 months

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	14.088	0.414	7.453
Error	68	3.781	0.056	
Total	104	18.13		

iv. after 8 months

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	13.214	0.389	7.169
Error	68	3.686	0.054	
Total	104	17.271		

v. after 10 months

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	13.181	0.388	7.051
Error	68	3.739	0.055	
Total	104	18.266		

vi. after 12 months

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	4.324	0.127	22.878
Error	68	0.378	0.006	
Total	104	5.729		

d. Analysis of Variance for petiole length:

i. after 2 months

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	8.503	0.25	26.01
Error	68	0.654	0.01	
Total	104	9.213		

ii. after 4 months

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	40.757	1.199	22.6
Error	68	3.607	0.053	
Total	104	115.512		

iii. after 6 months

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	59.341	1.745	22.642
Error	68	5.242	0.077	
Total	104	152.308		

iv. after 8 months

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	93.341	2.745	22.571
Error	68	8.271	0.122	
Total	104	200.808		

v. after 10 months

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	67.249	1.978	19.247
Error	68	6.988	0.103	
Total	104	200.4		

vi. after 12 months

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	79.248	2.331	19.186
Error	68	8.261	0.121	
Total	104	333.417		

e. Analysis of Variance for leaf angle:

i. after 2 months

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	2,202.79	64.788	10.13
Error	68	434.889	6.395	
Total	104	2,637.91		

ii. after 4 months

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	2,212.49	65.073	13.355
Error	68	331.333	4.873	
Total	104	2,545.95		

iii. after 6 months

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	2,429.86	71.467	11.128
Error	68	436.719	6.422	
Total	104	2,870.04		

iv. after 8 months

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	3,922.92	115.38	13.544
Error	68	579.292	8.519	
Total	104	4,517.29		

v. after 10 months

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	3,960.82	116.495	17.684
Error	68	447.943	6.587	
Total	104	4,426.10		

vi. after 12 months

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	3,922.91	115.38	45.061
Error	68	174.114	2.56	
Total	104	4,517.277		

f. Analysis of Variance for number of leaves:

i. after 2 months

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	6.993	0.206	2.628
Error	68	5.322	0.078	
Total	104	13.047		

ii. after 4 months

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	169.572	4.987	51.686
Error	68	6.562	0.096	
Total	104	176.351		

iii. after 6 months

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	571.881	16.82	71.865
Error	68	15.915	0.234	
Total	104	588.684		

iv. after 8 months

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	1,214.82	35.73	114.064
Error	68	21.301	0.313	
Total	104	1,237.22		

v. after 10 months

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	1,640.56	48.252	132.028
Error	68	24.852	0.365	
Total	104	1,665.55		

vi. after 12 months

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	2,702.02	79.471	33.884
Error	68	159.484	2.345	
Total	104	3234.665		

g. Analysis of Variance for leaf area:

i. after 2 months

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	7,488.63	220.254	28.742
Error	68	521.102	7.663	
Total	104	8,025.31		

ii. after 4 months

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	35,610.43	1,047.37	33.835
Error	68	2,104.95	30.955	
Total	104	39,637.28		

iii. after 6 months

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	81,019.74	2,382.93	37.069
Error	68	4,371.26	64.283	
Total	104	89,031.01		

iv. after 8 months

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	4,98,357.61	14,657.58	74.147
Error	68	13,442.42	197.683	
Total	104	5,14,233.10		

v. after 10 months

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	8,19,442.87	24,101.26	77.436
Error	68	21,164.50	311.243	
Total	104	8,43,947.90		

vi. after 12 months

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	12,04,960.98	35,440.03	116.86
Error	68	20,622.54	303.27	
Total	104	12,38,957.92		

3. ANOVA for variation in biomass characteristics of *Moringa oleifera* Lam. progenies.

i. Fresh shoot weight

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	43,45,937.07	1,27,821.68	22.535
Error	68	3,85,698.03	5,672.03	
Total	104	52,95,163.67		

ii. Fresh root weight

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	7,71,029.70	22,677.34	11.567
Error	68	1,33,313.83	1,960.50	
Total	104			

iii. Dry shoot weight

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	3,13,910.09	9,232.65	19.694
Error	68	31,879.51	468.816	
Total	104	4,06,598.79		

iv. Dry root weight

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	56,143.74	1,651.29	11.483
Error	68	9,778.44	143.801	
Total	104	84,679.11		

v. Fresh root: shoot ratio

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	0.425	0.012	5.796
Error	68	0.147	0.002	
Total	104	0.589		

vi. Fry root: shoot ratio

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	0.566	0.017	7.654
Error	68	0.148	0.002	
Total	104	0.732		

vii. Total fresh biomass

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	75,39,220.66	2,21,741.78	33.13
Error	68	4,55,135.25	6,693.17	
Total	104	94,39,599.18		

4. ANOVA for variation in leaf dry matter content, leaf proximate principles, leaf biochemical estimates and leaf mineral contents of *Moringa oleifera* Lam. mother trees.

i. Leaf dry matter content

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	5.688	0.167	0.796
Error	68	14.298	0.210	
Total	104	20.901		

ii. Crude protein

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	613.74	18.051	51.522
Error	68	23.824	0.35	
Total	104	637.564		

iii. Ether extract

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	123.729	3.639	41.363
Error	68	5.983	0.088	
Total	104	129.712		

iv. Crude fiber

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	1,363.49	40.103	66.453
Error	68	41.037	0.603	
Total	104	1,404.527		

v. Ash content

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	177.229	5.213	30.226
Error	68	11.727	0.172	
Total	104	188.956		

vi. Nitrogen free extract

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	2,070.88	60.908	44.961
Error	68	92.118	1.355	
Total	104	2,162.998		

vii. Total amino acids

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	236.599	6.959	25.369
Error	68	18.652	0.274	
Total	104	255.251		

viii. Nitrogen content

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	1,57,15,057.73	4,62,207.58	51.48
Error	68	6,10,507.86	8,978.06	
Total	104	1,63,25,565.59		

ix. Phosphorus content

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	23,444.83	689.554	108.311
Error	68	432.918	6.366	
Total	104	23,877.748		

x. Potassium content

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	24,72,514.91	72,721.03	11.081
Error	68	4,46,262.06	6,562.68	
Total	104	29,18,776.97		

xi. Calcium content

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	1.247	0.037	45.243
Error	68	0.055	0.001	
Total	104	1.302		

xii. Magnesium content

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	18,16,866.06	53,437.24	66.435
Error	68	54,695.89	804.351	
Total	104	18,71,561.95		

xiii. Sulphur content

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	55,62,073.58	1,63,590.40	155.038
Error	68	71,751.22	1,055.17	
Total	104	56,33,824.80		

xiv. Zinc content

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	9.885	0.291	87.294
Error	68	0.226	0.003	
Total	104	10.111		

xv. Iron content

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	4,183.33	123.039	30.809
Error	68	271.564	3.994	
Total	104	4,454.894		

xvi. Copper content

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	2.367	0.07	90.929
Error	68	0.052	0.001	
Total	104	2.419		

xvii. Manganese content

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	24.236	0.713	24.645
Error	68	1.967	0.029	
Total	104	26.203		

5. ANOVA for variation in leaf dry matter content, leaf proximate principles, leaf biochemical estimates and leaf mineral contents of *Moringa oleifera* Lam. progenies.

i. Leaf dry matter content

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	5.156	0.152	0.762
Error	68	13.531	0.199	
Total	104	18.793		

ii. Crude fiber

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	745.426	21.924	71.843
Error	68	20.752	0.305	
Total	104	766.178		

iii. Ether extract

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	151.651	4.46	71.436
Error	68	4.246	0.062	
Total	104	155.897		

iv. Crude fiber

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	1,112.23	32.712	161.939
Error	68	13.736	0.202	
Total	104	1,125.966		

v. Ash content

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	141.708	4.168	37.47
Error	68	7.564	0.111	
Total	104	149.272		

vi. Nitrogen free extract

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	1,375.01	40.441	56.017
Error	68	49.093	0.722	
Total	104	1,424.103		

vii. Total amino acids

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	355.327	10.451	21.414
Error	68	33.187	0.488	
Total	104	388.514		

viii. Nitrogen content

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	1,90,82,897.65	5,61,261.70	71.843
Error	68	5,31,240.28	7,812.36	
Total	104	1,96,14,137.93		

ix. Phosphorus content

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	22,36,367.71	65,775.52	7.812
Error	68	5,72,545.19	8,419.78	
Total	104	28,08,912.90		

x. Potassium content

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	18,24,805.21	53,670.74	11.355
Error	68	3,21,407.99	4,726.59	
Total	104	21,46,213.20		

xi. Calcium content

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	43,98,784.85	1,29,376.03	113.082
Error	68	77,798.16	1,144.09	
Total	104	44,76,583.01		

xii. Magnesium content

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	19,838.49	583.485	86.86
Error	68	456.791	6.718	
Total	104	20,295.281		

xiii. Sulphur content

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	0.978	0.029	9.997
Error	68	0.196	0.003	
Total	104	1.174		

xiv. Zinc content

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	23.886	0.703	90.842
Error	68	0.526	0.008	
Total	104	24.412		

xv. Iron content

Source of Variation	df	SS	MSS	F _{cal}
Treatment	34	5,686.37	167.246	53.878
Error	68	211.082	3.104	
Total	104			

xvi. Copper content

Source of Variation	df	SS	MSS	F_{cal}
Treatment	34	1.897	0.056	42.009
Error	68	0.09	0.001	
Total	104	1.987		

xvii. Manganese content

Source of Variation	df	SS	MSS	F_{cal}
Treatment	34	29.068	0.855	7.19
Error	68	8.085	0.119	
Total	104	37.153		

APPENDIX- II

Analysis of covariance (ANCOVA)

1. Covariance matrix for variation in pod, seed and germination parameters of *Moringa oleifera* Lam.

	PL	PB	SP	SWW	SW	SK	SL	SB	SKL	SKB	GI	GV	GP
PL	1,865.85	-71.80	583.68	-220.99	-217.56	-334.57	-8.31	4.71	-0.93	3.82	5,257.46	3,994.40	2,926.03
PB	-71.80	13.33	-19.24	26.47	26.10	29.33	0.08	-0.10	-0.06	-0.10	-314.80	-224.58	-145.97
SP	583.68	-19.24	954.53	-150.25	-138.23	-162.40	-1.46	1.07	-1.50	-1.99	2,406.32	1,799.21	1,471.86
SWW	-220.99	26.47	-150.25	336.74	325.04	294.40	-0.40	2.70	1.26	-0.52	-2,287.98	-1,656.91	-1,228.04
SW	-217.56	26.10	-138.23	325.04	318.14	294.87	0.40	2.70	1.70	-0.33	-2,148.90	-1,562.73	-1,142.24
SK	-334.57	29.33	-162.40	294.40	294.87	324.21	2.30	1.43	2.33	-0.27	-2,084.84	-1,505.32	-1,058.07
SL	-8.31	0.08	-1.46	-0.40	0.40	2.30	1.36	0.86	0.48	0.28	69.26	40.53	38.60
SB	4.71	-0.10	1.07	2.70	2.70	1.43	0.86	1.01	0.32	0.20	121.33	79.29	64.56
SKL	-0.93	-0.06	-1.50	1.26	1.70	2.33	0.48	0.32	0.36	0.22	28.39	21.19	13.54
SKB	3.82	-0.10	-1.99	-0.52	-0.33	-0.27	0.28	0.20	0.22	0.25	22.88	20.71	11.27
GI	5,257.46	-314.80	2,406.32	-2,287.98	-2,148.90	-2,084.84	69.26	121.33	28.39	22.88	1,09,286.25	71,753.82	64,197.52
GV	3,994.40	-224.58	1,799.21	-1,656.91	-1,562.73	-1,505.32	40.53	79.29	21.19	20.71	71,753.82	48,387.70	42,055.72
GP	2,926.03	-145.97	1,471.86	-1,228.04	-1,142.24	-1,058.07	38.60	64.56	13.54	11.27	64,197.52	42,055.72	38,741.43

2. Covariance matrix for variation in morphological and biomass characteristics of *Moringa oleifera* Lam. progenies

	PH	CD	IL	PTL	BA	NB	LA	FSW	FRW	DSW	DRW	FRS	DRS	TFB
PH	1,00,214.73	1,067.03	411.97	1,801.93	8,863.54	13,403.44	1,91,382.33	5,02,706.04	1,81,601.91	77,340.07	39,584.66	-28.17	34.47	6,84,307.82
CD	1,067.03	19.69	4.69	28.80	83.09	179.46	2,406.63	6,474.79	2,307.91	1,411.38	669.42	-0.33	0.39	8,782.70
IL	411.97	4.69	4.32	6.44	25.33	39.25	632.81	2,246.62	762.54	321.48	190.28	-0.14	0.22	3,009.16
PTL	1,801.93	28.80	6.44	79.25	155.75	399.07	5,541.19	14,348.69	5,328.23	2,875.78	1,208.50	-0.52	0.20	19,676.92
BA	8,863.54	83.09	25.33	155.75	3,922.91	1,286.58	13,441.44	34,773.38	9,190.00	8,284.48	1,993.42	-5.91	-0.19	43,963.36
NB	13,403.44	179.46	39.25	399.07	1,286.58	2,702.02	36,180.36	91,342.18	29,930.19	16,785.97	7,965.47	-8.05	4.92	1,21,272.35
LA	1,91,382.33	2,406.63	632.81	5,541.19	13,441.44	36,180.36	12,04,960.98	13,15,519.64	4,25,853.20	3,84,338.36	1,24,090.35	-101.22	-115.73	17,41,372.82
FSW	5,02,706.04	6,474.79	2,246.62	14,348.69	34,773.38	91,342.18	13,15,519.64	43,45,937.07	12,11,127.65	5,02,961.82	2,88,987.60	-539.78	265.16	55,57,064.55
FRW	1,81,601.91	2,307.91	762.54	5,328.23	9,190.00	29,930.19	4,25,853.20	12,11,127.65	7,71,029.70	2,09,073.13	98,687.86	233.94	83.72	19,82,156.82
DSW	77,340.07	1,411.38	321.48	2,875.78	8,284.48	16,785.97	3,84,338.36	5,02,961.82	2,09,073.13	3,13,910.09	74,647.18	2.12	-180.62	7,12,034.83
DRW	39,584.66	669.42	190.28	1,208.50	1,993.42	7,965.47	1,24,090.35	2,88,987.60	98,687.86	74,647.18	56,143.74	-22.99	86.03	3,87,675.47
FRS	-28.17	-0.33	-0.14	-0.52	-5.91	-8.05	-101.22	-539.78	233.94	2.12	-22.99	0.43	-0.05	-305.84
DRS	34.47	0.39	0.22	0.20	-0.19	4.92	-115.73	265.16	83.72	-180.62	86.03	-0.05	0.57	348.87
TFB	6,84,307.82	8,782.70	3,009.16	19,676.92	43,963.36	1,21,272.35	17,41,372.82	55,57,064.55	19,82,156.82	7,12,034.83	3,87,675.47	-305.84	348.87	75,39,220.66

3. Covariance matrix for variation in leaf proximate principles, leaf biochemical estimates and leaf mineral contents of *Moringa oleifera* Lam. progenies.

	CP	EE	CF	AC	NFE	TAA	N	K	Mg	S	P	Ca	Zn	Fe	Cu	Mn
CP	745.43	129.43	-203.07	94.34	-766.13	514.26	119268.11	11437.42	9072.82	-8098.82	1438.00	-6.72	-32.84	518.82	-6.99	44.24
EE	129.43	151.65	-345.86	38.56	26.21	89.74	20709.23	6385.13	14436.37	-17771.43	1492.08	-4.36	-50.09	6.52	-5.47	54.91
CF	-203.07	-345.86	1112.23	-101.40	-461.89	-140.00	-32491.29	-18030.71	-41047.27	57031.03	-4136.41	14.56	160.68	-514.30	18.29	-160.69
AC	94.34	38.56	-101.40	141.71	-173.20	65.10	15093.98	2699.08	4190.18	-6040.39	598.77	-1.71	-14.99	213.91	-2.46	15.23
NFE	-766.13	26.21	-461.89	-173.20	1375.01	-529.09	-122580.03	-2490.92	13347.90	-25120.38	607.57	-1.77	-62.77	-224.95	-3.38	46.33
TAA	514.26	89.74	-140.00	65.10	-529.09	355.33	82281.21	7853.49	6204.83	-5389.11	988.13	-4.59	-22.46	359.08	-4.71	30.62
N	119268.11	20709.23	-32491.29	15093.98	-122580.03	82281.21	19082897.65	1829986.47	1451651.09	-1295811.43	230079.92	-1075.32	-5254.15	83010.97	-1118.29	7077.69
K	11437.42	6385.13	-18030.71	2699.08	-2490.92	7853.49	1829986.47	2236367.71	859078.57	-752296.93	81461.31	-1389.89	-2904.93	19597.12	-1948.02	3476.25
Mg	9072.82	14436.37	-41047.27	4190.18	13347.90	6204.83	1451651.09	859078.57	1824805.21	-2179605.80	178938.57	-591.96	-6133.93	15972.89	-805.54	6876.07
S	-8098.82	-17771.43	57031.03	-6040.39	-25120.38	-5389.11	-1295811.43	-752296.93	-2179605.80	4398784.85	-209971.32	677.77	8718.69	-26385.30	966.83	-8068.85
P	1438.00	1492.08	-4136.41	598.77	607.57	988.13	230079.92	81461.31	178938.57	-209971.32	19838.49	-58.62	-616.49	2879.90	-76.88	701.13
Ca	-6.72	-4.36	14.56	-1.71	-1.77	-4.59	-1075.32	-1389.89	-591.96	677.77	-58.62	0.98	2.32	-15.91	1.32	-2.48
Zn	-32.84	-50.09	160.68	-14.99	-62.77	-22.46	-5254.15	-2904.93	-6133.93	8718.69	-616.49	2.32	23.89	-91.50	2.99	-23.97
Fe	518.82	6.52	-514.30	213.91	-224.95	359.08	83010.97	19597.12	15972.89	-26385.30	2879.90	-15.91	-91.50	5686.37	-15.80	92.07
Cu	-6.99	-5.47	18.29	-2.46	-3.38	-4.71	-1118.29	-1948.02	-805.54	966.83	-76.88	1.32	2.99	-15.80	1.90	-3.28
Mn	44.24	54.91	-160.69	15.23	46.33	30.62	7077.69	3476.25	6876.07	-8068.85	701.13	-2.48	-23.97	92.07	-3.28	29.07

APPENDIX-III

List of single sequence repeats (SSR) markers used under study along with respective annealing temperatures.

S. No.	Primer Name	Primer Sequence (5' to 3')	Annealing temperature (°C)
1	MO1 (F)	TTGTCTGCCTCCTTTTGTCA	58
	MO1 (R)	AACTGTCACCCTCCTATCCA	
2	MO6 (F)	GCATAGCCACCTTTACTCCT	61
	MO6 (R)	GACTTTTGAAGTCCACCACC	
3	MO8 (F)	GTAGATGGTGCAGCTACTCA	58
	MO8 (R)	TGGGGTTCTTGTCTTTATT	
4	MO10 (F)	CTTTACACCTCAGTATCCCT	58
	MO10 (R)	GTTCTGGCTTATGTTCTCGTT	
5	MO12 (F)	ACCGAAGATGATAAGGTGGG	59
	MO12 (R)	CAAAAGGAAGAACGCAAGAG	
6	MO13 (F)	TTTCGGGTTTCTTTACGC	58
	MO13 (R)	AGCTCACTTTCCATCTCCAT	
7	MO15 (F)	CCCCTCTATTTCCATTTTCC	59
	MO15 (R)	GCTCCATAAACCCCTCTTGCT	
8	MO18 (F)	TTTTCTCCCTTATTGTGCC	58
	MO18 (R)	CCGTTGCCCTTTGTGGTTCA	
9	MO41 (F)	TGGGATTAGGGCATTAGAAA	55
	MO41 (R)	TAGTGGGTCCAAGACAAAGC	
10	MO44 (F)	GGCACATAGGCACGCAATAC	59
	MO44 (R)	CAACAAACCCATCCAGAAA	
11	MO45 (F)	CCTTTGAAGTTGAAAATCTC	58
	MO45 (R)	TTCTAGGGTAGTTGAATCCA	
12	MO46 (F)	ACCAAGGGTTTCAACTGCTG	61
	MO46 (R)	CATTTTGCGACGGTCTCACG	
13	MO48 (F)	AGAAGAACCCAACAGAGGAT	58
	MO48 (R)	CTTTTCACTAACCACCACCC	
14	MO55 (F)	ATTACAGAACGATGAAACCA	56
	MO55 (R)	CTCTTTCCCTCCATTCAACC	
15	MO56 (F)	TCAATACGCCAAGTAAGCAA	60
	MO56 (R)	AAGCACTTCACGCATAAAAC	
16	MO58 (F)	TGGATTTCTTCTCCTGCTAT	58
	MO58 (R)	CACAGTTCTTATTGTATTGG	
17	MO61 (F)	TGTGGGTCTCCTGCCTTTTCTC	60
	MO61 (R)	CTTCTGTCTTTCTTCCTGCT	
18	MO62 (F)	AAACATAGCAACTGTGAGAT	55
	MO62 (R)	CTCCAACAACATACAAAATC	
19	MO64 (F)	TCGGCACCTTCTTCCTCTTT	58
	MO64 (R)	AATCCCTTGACGGACACCAG	
20	MO68 (F)	TGCTTCGCTTCCTCTATTCT	56
	MO68 (R)	ACCACAGGCTTGCTTCAGTA	

APPENDIX-IV

Reagents required for DNA extraction

2X CTAB buffer (1 litre)

2% CTAB solution	20 g
1.4M NaCl	81.8 g
100mM Tris (pH 8.0)	100 ml
20mM EDTA	40 ml
2% PVP	5 g
0.4% beta-mercaptoethanol	4 ml

Final volume was made with 1000ml with distilled H₂O

1 M Tris pH 8.0

121.1 g of Tris

700 ml of double distilled water

Dissolve Tris and bring to 900 ml

Adjust pH to 8.0 using concentrated HCl. Make up the volume to 1000 ml using double distilled water Sterilize by autoclaving

0.5 M EDTA pH 8.0

186.12 g of EDTA

750 ml of double distilled water

Add about 20 g of NaOH pellets

Slowly add more NaOH until the pH 8.0

Make up the volume to 100 ml using double distilled water Sterilize by autoclaving

TE Buffer (1 litre)

10 ml of 1 M Tris pH 8.0

2 ml of 0.5 M EDTA pH 8.0 (1mM)

Make up the volume to 1000ml using double distilled water Sterilize by autoclaving

3 M Sodium acetate (1 litre)

408.24 g of Sodium acetate in 800 ml of double distilled water Adjust the pH with glacial acetic acid

Make up the volume to 100ml using double distilled water Sterilize by autoclaving

Chloroform: Isoamyl alcohol (500 ml) (24:1)

480 ml of chloroform

20 ml of Isoamyl alcohol

APPENDIX-V

Agarose gel electrophoresis

Material

a. Loading dye

Bromophenol blue	0.025 g
Sucrose	4 g
TBE	10 ml

b. Agarose

0.8 per cent agarose was used to check DNA quality and 2 per cent for SSR PCR product

c. 10X TBE (Tris Borate EDTA) buffer

Tris base	107.8 g
Boric acid	55.03 g
EDTA 0.5M	10 ml

d. Staining solution

Ethidium bromide of 10mg/ml (10 mg of ethidium bromide was added to 1 ml of sterile water)

e. Electrophoresis unit, power pack, casting tray and comb protocol

1. Gel casting plate and comb were washed with distilled water, wiped with alcohol; the two open ends of the Pyrex gel casting plate were sealed with cello tape and placed on a perfectly horizontal platform.
2. 2 per cent agarose was added to 1X TBE and boiled till agarose was dissolved completely and then cooled to 50 °C to 60 °C. Ethidium bromide was used as staining agent at the final concentration of 1µg/ml.
3. Agarose gel was then poured into the gel-casting tray and the comb was placed properly and allowed to solidify.
4. After solidification of the agarose, the comb and cello tape were removed.

5. The casting gel was placed in the electrophoresis unit with wells towards the cathode and submerged with 1X TBE to a depth of about 1cm.
6. The reaction mixtures (15 μ l) were mixed well with 2 μ l of loading dye and were loaded into the gel wells.
7. The gel was run at 100 volts for 2-3 hours and bands were visualized and documented in the gel documentation system.

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Title of the thesis : **Genetic diversity in wild populations of *Moringa oleifera* Lam. (Drumstick tree) in north-west India**

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Major Advisor : Dr. Hari Paul Sankhyan

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Minor Field : Forest Genetic Resources

Degree Awarded : PhD in Forestry (Forest Genetic Resources)

Year of Award of Degree : 2023

Number of pages in Thesis : 138+xxvii

Number of words in the abstract : 289

ABSTRACT

The present study entitled “Genetic diversity in wild populations of *Moringa oleifera* Lam. (Drumstick tree) in north-west India” was carried out in the Department of Tree Improvement and Genetic Resources of Dr. Yashwant Singh Parmar University of Horticulture and Forestry Nauni, Solan (H.P.) during 2020-23. This investigation was conducted in seven locations in four states/UTs, viz., Samba (Jammu and Kashmir), Dunera (Punjab), Vikasnagar (Uttarakhand), Lamlehri (Himachal Pradesh), Tirla (Himachal Pradesh), Siswa Dam (Punjab) and Morni Hills (Haryana) ranging from 351-549 amsl. Four major experiments were conducted, viz., survey and selection of wild genotypes, morphological variation in selected half-sib progenies, identifying best progenies having superior nutritive values and genetic diversity of mother trees using SSR markers. The mother trees varied significantly in qualitative, morphological, nutritive and molecular levels. Progeny P35 showed highest values for plant height and fresh shoot weight, P7 for number of leaves, total amino acids, N, S and Zn, P30 for leaf area and fresh shoot weight, P19 for ether extract, P3 for crude fiber, P22 for ash content, P and Mn, P34 for collar diameter and nitrogen free extract, P29 for K, P20 for Ca, P16 for Mg, P18 for Fe and P14 for Cu content. High heritability pooled with high genetic gain was observed for germination percentage; plant height and total fresh biomass, ether extract, crude fiber, P, Zn, S, Fe and Mn. Correlation coefficients, path coefficient and principle component analysis exhibited high variability among progenies. Genetic diversity of mother trees was assessed using SSR markers. Sixteen out of 20 SSR markers gave amplification of which all 16 markers gave polymorphism. On average, 3.75 alleles/per SSR were amplified in each mother tree. The overall similarity coefficient was 0.82 where M1 and M3 were distantly related.

Signature of Chairman
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Date:

Signature of Student
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Date:

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Examination Passed	Year of Passing	University/Board	Division	Marks (%)
Matriculation	2011	CBSE	FIRST	8.00
10+2	2013	CBSE	FIRST	69 %
Graduation	2017	Punjab Agricultural University, Ludhiana, Punjab	FIRST	7.03
Post-Graduation	2019	Punjab Agricultural University, Ludhiana, Punjab	FIRST	7.54

(Mohit Kundal)