PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol x(x): xx–xx
https://doi.org/10.14719/pst.4241

HORIZON
e-Publishing Group
HePG

RESEARCH ARTICLE

M_4 generation of moringa (*Moringa oleifera* Lam.) mutants revealing genetic diversity for leaf traits based on SSR markers

Bharathi S[1], Nageswari K[1*], Rajesh S[2], Anand G[3], Geetharani P[4], Rajangam J[5] & Williams M[2]

[1]Department of Vegetable Science, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Periyakulam, Theni 625 604, Tamil Nadu, India

[2]Department of Plant Biotechnology, Centre for Plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University, Coimbatore 641 003, Tamil Nadu, India

[3]Department of Plant Breeding and Genetics, Krishi Vigyan Kendra, Agricultural College and Research Institute, Tamil Nadu Agricultural University, Madurai 625 104, Tamil Nadu, India

[4]Department of Seed Science and Technology, Agricultural Research Station, Vaigai Dam, Theni 625 512, Tamil Nadu Agricultural University, India

[5]Horticultural College and Research Institute, Tamil Nadu Agricultural University, Periyakulam, Theni 625 604, Tamil Nadu, India

*Email: nageswarihort@yahoo.co.in

OPEN ACCESS

ARTICLE HISTORY
Received: 03 July 2024
Accepted: 02 November 2024
Available online
Version 1.0 : 04 March 2025

Check for updates

Additional information
Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

Reprints & permissions information is available at https://horizonepublishing.com/journals/index.php/PST/open_access_policy

Publisher's Note: Horizon e-Publishing Group remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etc
See https://horizonepublishing.com/journals/index.php/PST/indexing_abstracting

CITE THIS ARTICLE

Bharathi S, Nageswari K, Rajesh S, Anand S, Geetharani P, Rajangam J, Williams P. M_4 generation of moringa (*Moringa oleifera* Lam.) mutants revealing genetic diversity for leaf traits based on SSR markers. Plant Science Today (Early Access). https:/doi.org/10.14719/pst.4241

Abstract

This study was conducted to develop a variety suitable for high leaf production in moringa. Initially, seeds of PKM 1 variety of moringa were treated with gamma irradiation of 100 Gy (Gray), 200 Gy and 300 Gy at Bhabha Atomic Research Centre (BARC), Mumbai and raised as M_1 generation. The best-performing mutants were selected and forwarded to further generations (M_1 to M_4) based on leaf-related characters. A total of 10 Indian drumsticks (*Moringa oleifera* Lam.) mutants (derived from 200 Gy of gamma irradiation) from M_4 generation with PKM 1 variety were evaluated for morphological and molecular diversity. To evaluate morphological diversity, 4 features (leaf length, leaf breadth, fresh weight and dry weight) were studied under field conditions. Ten sets of markers for simple sequence repeats (SSR), i.e. GenicSSR1081, SSR25987, SSR2927, GenicSSR859, GenicSSR796, SSR6561, GenicSSR890, GenicSSR1204, GenicSSR983 and GenicSSR778; that generated distinct and repeatable bands; were chosen for analyzing molecular diversity. A total of 24 bands were amplified, of which 17 were polymorphic. With an average of 0.391, the polymorphic information content (PIC) values for SSR markers varied from 0.124 for marker SSR2927 to 0.719 for marker GenicSSR778. Based on the cluster analysis with SSR markers, the mutants are divided into 2 groups. The variety PKM 1 was separated by a group whereas; all the other mutants fell into a single group indicating the diversity of mutants from the control. The diverse mutants (15-1-09-70 and 15-1-09-53) based on both morphological and molecular observations were selected and suggested for evaluation through subsequent generations that have the potential to be released as a variety for high leaf production. By increasing the production of highly nutritious moringa leaf, self-sufficiency and nutritional security can be obtained as well as it could benefit the lifestyle improvement of farmers through export to countries where the crop demand is high.

Keywords

diversity; moringa; mutants; selection; SSR

Introduction

India has been using *Moringa oleifera* as a medicine since the 18th century BC, with various plant parts used as traditional remedies. The plant's root, bark, gum, leaves, fruit, flowers, seeds and seed oil have been used to treat various illnesses, including gout and anemia, skin infections edema, bronchitis, asthma, diarrhea, headaches, joint discomfort and rheumatism (1-5). Traditional medical systems also treat measles, smallpox, conjunctivitis, hemorrhoids, goiter, earache, fevers, digestive disorders, wounds and diabetes (6, 7). When added to food products, *M. oleifera* increases its nutritional value and can be used to produce nutrient-enriched bakery goods that are well-received by consumers and have good storage quality. This is an intriguing strategy to improve people's health and nutritional status, particularly in areas where food is scarce or extreme poverty exists (8). Mutagenesis, the process of introducing mutations into an organism's genome, has been used in plant breeding. With induced mutagenesis and related breeding techniques, various crops can have their quantitative and qualitative characteristics improved in a shorter period of time than it takes with traditional breeding (9). The advantages of mutation breeding as a flexible and useful method for any crop is highlighted by the global impact of agricultural varieties developed from it (10). SSR are areas between microsatellites, which are amplified using primer set called SSR for molecular analysis. With a small proportion of co-dominant template DNA, SSR analysis are highly polymorphic and repeatable, especially when examining the distribution of repeat sequence abundance across genomes (11, 12). The first SSR marker unique to *M. oleifera* was developed in the year 2010 and they have since been suggested as helpful markers for in-depth genetic population research (13). Over time, SSRs have proven useful for classifying underused agricultural germplasm and evaluating molecular diversity (12, 14). Therefore, it is believed that using molecular markers in conjunction with mutation breeding is a good way to increase the frequency and efficiency of genetic variation. In particular, SSR are breeder-friendly markers that simplify counting of the genetic variations arising due to mutagenesis (15). Through this, morphologically and genetically diverse mutants with high leaf yield can be identified and selected, which might be used to attain self-sufficiency in nutrition-rich food.

Materials and Methods

Mutation treatment

At Bhabha Atomic Research Centre (BARC), Mumbai, the seeds of the PKM 1 moringa variety were subjected to varying doses of gamma irradiation (100 Gy, 200 Gy, 300 Gy) based on the source of irradiation at appropriate times at the rate of 400 seeds per treatment. The seeds were raised and observed for leaf and leaf-attributing traits. The selected best-performing progenies were selfed and forwarded to the M_2 generation (16).

Selection

Mutants exhibiting higher yields and yield-related traits were then advanced to the third generation (M_3). From the M_3 generation, top-performing mutants were chosen and progressed to the M_4 generation (17). Among these selected plants, the mutant family 15-1-09, derived from PKM 1 moringa irradiated with 200 Gy gamma radiations, underwent selection from the M_1 to the M_3 generation and raised in the M_4 generation. All seeds were grown as individual plants without replication. The best progenies from the M_4 generation were selected based on leaf yield and related characteristics and were subjected to further molecular characterization. In this study, the mutants that are the derivative of 200 Gy of gamma irradiation were taken for morphological and molecular characterization.

Leaf traits

Leaf length (cm) and leaf breadth (cm) were observed in the third rachis from the top of each plant. Leaf fresh weight (g) and dry weight (g) was calculated by harvesting on 45th day after transplanting.

Genetic diversity with SSR

The plant DNA sample was extracted by the hexadecyltrimethylammonium bromide (CTAB) method of isolation. The leaf sample was ground with CTAB and a pinch of polyvinyl pyrrolidone and macerated, then placed in Eppendorf centrifuge tubes filled with β-mercaptoethanol (β-ME). The tubes were incubated at 65 °C for 45 min in the water bath, then cooled for 15 min. A mixture of isoamyl alcohol and chloroform was added, mixed by inversion, centrifuged and the supernatant (aqueous phase) was extracted. Ice-cold isopropanol was added to the supernatant and the mixture was then stored at 4 °C or -80 °C for 2-3 hr. The tubes were centrifuged at 4 °C, 7200 rpm for 15 mins and the supernatant was discarded. The pellets were then added to 70% ethanol, centrifuged again and air-dried for 1-2 hr. The pellets were then dispersed in nuclease-free water and stored at -30 °C (18). The genomic DNA isolated from selected mutated lines and control plants was quantified using a Nanodrop Spectrophotometer (Genova Nano 69357). DNA integrity was evaluated through 0.8% (w/v) agarose gel electrophoresis. All DNA samples were uniformly diluted to 50 ng/µL with sterile water for use as templates in PCR amplification with selected SSR markers. For PCR amplification, 10 SSR markers (SSR2927, GenicSSR1204, GenicSSR1081, GenicSSR983, GenicSSR796, GenicSSR859, GenicSSR890, GenicSSR778, SSR6561 and SSR25987) were utilized with a slight modification using smART Prime master mix instead of individual PCR chemical components (19). The primer (1 µL) was combined with DNA template (2 µL), 1X master mix (3 µL) and water (4 µL) to make up a reaction mixture. The amplification was performed using a thermal cycler (Veriti™, Thermo Fisher Scientific), with varying annealing temperatures depending on the melting temperature of the primer pair. The custom oligos were from M/s Bioserve Biotechnologies Pvt Ltd, Hyderabad, India. The amplified products were separated using an ethidium bromide stained 3% (w/v) agarose gel at 120 V for 1.5-2 hr. The gel image was viewed and captured

by UV transillumination using a gel documentation unit (BIO-RAD, Gel Doc XR). The list of SSR markers used in this study is provided in Table 1.

Polymorphism information content (PIC) values

The equation for calculating PIC with the use of a codominant marker is given below:

$$PIC_j = 1 - \sum_{i=1}^{n} Pi^2 \qquad \text{(Eqn.1)}$$

where, *i* is *i*-th allele of the *j*-th marker, n is the number of the *j*-th marker's alleles and P is allele frequency (20-22).

Statistical analysis

The PIC values were calculated using Microsoft Excel. The morphological data were analyzed with progeny-wise analysis for un-replicated data using the TNAUSTAT application. The study used a binary data matrix to analyze genetic diversity, utilizing discrete variables and un-weighed Pair Group Method of Arithmetic Means (UPGMA) with arithmetic averages. The NTSYS-2.02i software was used for cluster analysis and estimating genetic diversity.

Results and Discussion

While there are many breeding techniques available to develop new plant variants, mutation breeding is particularly effective because it can generate new variants randomly without altering the entire genotype. Its ability to preserve recessive alleles enables breeders to create new varieties more quickly. This research aimed to develop new types of leafy biomass in moringa through physically induced mutagenesis (using Gamma rays) and to analyze the resulting morphological and genetic variations to identify desirable mutants.

Performance of SSR markers

Among the 10 SSR markers (GenicSSR1081, SSR25987, SSR2927, GenicSSR859, GenicSSR796, SSR6561, GenicSSR890, GenicSSR1204, GenicSSR983 and GenicSSR778) used, 8 markers showed polymorphism except GenicSSR1204 which was monomorphic. The marker SSR25987 showed no amplification. The PIC value was highest in the marker GenicSSR778 (0.719), followed by GenicSSR859 (0.669) and GenicSSR890 (0.595). PIC values ranged from 0.719 to 0.124. A total of 24 alleles were covered by the 9 markers, among which 17 were polymorphic. 1 monomorphic allele was seen when the markers SSR2927, GeneicSSR859 and GenicSSR778 were used, which showed a total of 3 different bands among the 11 samples analyzed. Also, the markers GeneicSSR890 and SSR6561 showed 1 monomorphic allele out of four and 2 alleles produced by each respectively. All the other markers *viz.,* GenicSSR983, GenicSSR1081 and GenicSSR796 showed 100% polymorphism. Similarly, among the generated primer pairs of 22336 genomic and genic SSR markers, 117 SSR markerr pairs were chosen at random and tested in 4 typical moringa ecotypes and cultivars. Out of the examined moringa accessions, 94.44% exhibited a high degree of DNA polymorphism, producing one to 4 alleles per SSR marker (average of 1.75). The maximum PIC value of these accessions was 0.95 (23). The performance of different markers based on PIC value is given in Table 2.

Gamma rays on leaf characters of M_4 mutants

The mutant 15-1-09-70 exhibited a larger leaf breadth (1.96 cm), followed by 15-1-09-39 (1.92 cm). Also, the mutants 15-1-09-44 (1.81 cm), 15-1-09-16 (1.72 cm), 15-1-09-21 (1.71 cm) and 15-1-09-64 (1.62 cm) showed broader leaf comparing the control PKM 1 (1.65 cm). In the case of leaf length, the mutant 15-1-09-70 (2.83 cm) recorded the longest leaf length, followed by 15-1-09-44 (2.82 cm) and 15-1-09-64 (2.81 cm) than the control (2.75 cm) (Fig. 1). Similarly, a

Table 1. SSR markers used in this study and their primer sequence

SSR marker	Forward	Reverse	Annealing temperature (°C)
SSR2927	CCAGGAGGCAATGCTTAGC	TTTTGCTCAATGGCACCTGC	55
GenicSSR1204	CATGGCCTCTTCCTGACTCC	AAATCACACGCTTTTGCCGG	55
GenicSSR1081	GACAGACCTAGACCCACTGG	GGAGTTCTTCGAGGCCTACG	59
GenicSSR983	TGGGCATCATCTTGTTGGGC	TGTGAAATTCAACTTGCCTGAGG	57
GenicSSR859	TCCGTCCCATAGGTACCTCC	CCCGATGCAGAAGGAGATGG	59
GenicSSR890	ACTTGCCAGGGTTATGGTGG	ACTCCACCTTGCAAAATGGC	55
GenicSSR778	CGTCAACTACCATTGAATCGGC	GGCAATTCATGAAGTCGGAGC	57
GenicSSR796	TCTTAACTTCCTCGCCTGCC	AGAAAGGCATAGAGGTCGCG	57
SSR6561	AGAAAGCATGCAAGTGTGGC	CAAAACGACAACACCACCCC	55
SSR25987	GGAGTATAGGGTTTAGGGCTAAGC	TAGATTCAGCCTGGCCTTGC	56

Table 2. Performance of different markers on PIC value

Primer	No. of alleles	No. of polymorphic alleles	Polymorphism percentage	PIC
GeneicSSR1081	2	2	100	0.396
SSR25987	-	No amplification	-	-
SSR2927	3	2	66.67	0.124
GenicSSR859	3	2	66.67	0.669
GenicSSR796	2	2	100	0.165
SSR6561	4	3	75	0.330
GenicSSR890	2	1	50	0.595
GenicSSR1204	1	0	Monomorphic	-
GenicSSR983	3	3	100	0.132
GenicSSR778	3	2	66.67	0.719

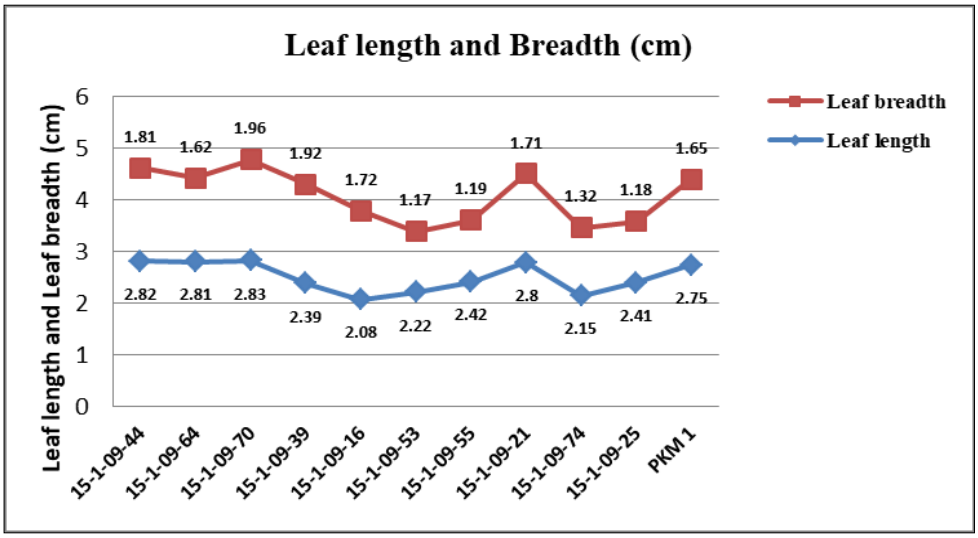

Fig. 1. Performance of mutants on leaf length and breadth.

study revealed that considerable differences were observed in leaf length and leaf breadth among the 19 mutant lines of *Amaranthus cruentus* comparing the control that were subjected to gamma irradiation (24).

For fresh weight of the leaf, the mutant 15-1-09-70 recorded the highest of 1022.14 g, followed by 15-1-09-53 (723.4 g) as compared to the control (595. 63 g). Similarly, dry leaf weight was the highest in 15-1-09-70 (424.52 g), followed by 15-1-09-53 (238.58 g) in comparison with the PKM 1 (158.72 g) (Fig. 2). Similarly, fresh and dry weight of the moringa leaf was increased in the first generation when gamma irradiation of 40 Gy and 60 Gy was used (25). Also, in Amaranthus, leaf dry matter production was increased when treated with different doses of gamma irradiation among which 850 Gy was found to be the better dose (26). The other mutants showed a decreasing trend with the fresh and dry weight of the leaves than the wild type PKM 1. The increase and decrease of performance among the mutants may be due to the direct or indirect effect of biotic and abiotic factors prevailing throughout the cropping period. As there is a difference among each mutant in the given characters, molecular characterization was carried out to identify the genetic variability among them.

Genetic variability with SSR markers

To comprehensively understand the phenotypic variation in the selected lines, it is essential to verify the existence of molecular-level variation. Although various molecular markers are available for analyzing genetic variation across the entire genome, SSR markers are recommended due to their user-friendly nature for breeders (15). This study's genetic variability analysis using SSR markers revealed that all the ten markers used were polymorphic when comparing ten selected mutant lines (15-1-09-44, 15-1-09-70, 15-1-09-39, 15-1-09-16, 15-1-09-53, 15-1-09-55, 15-1-09-21, 15-1-09-33, 15-1-09-74 and 15-1-09-25) with the wild type, PKM 1. Also, it was reported that these polymorphic markers are linked to specific leaf traits: GenicSSR778 (longer leaf length), GenicSSR796 (leaf length and width), GenicSSR983 (fresh weight), GenicSSR859 (longer leaf length) and GenicSSR890 (leaf morphology) (23, 27). Similarly, genetic characterization of moringa for nutritional characters was done using 9 SSR primers that revealed a strong relationship between nutrition and classification of the genotypes (28). The observed phenotypic variation and polymorphic information on these functional markers demonstrate that the mutant 15-1-09-70 exhibited an increase in leaf length, width, fresh weight and dry weight compared to PKM 1 and it differed

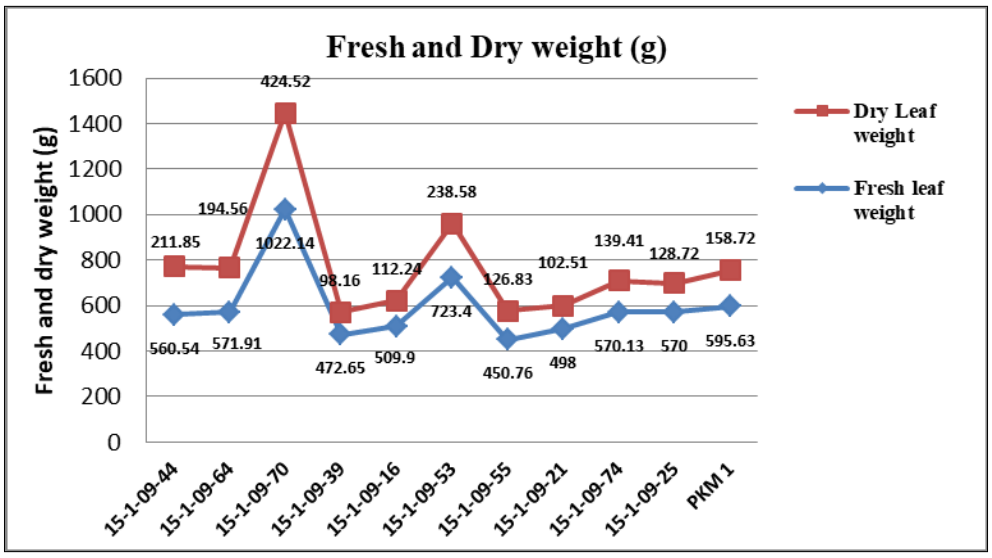

Fig. 2. Performance of mutants on fresh and dry weight.

from the wild type at the molecular level for the specified SSR motifs. Therefore, it was concluded that mutant 15-1-09-70 could be valuable for further screening in successive generations to inherit these desirable leafy traits and as potential donor in various breeding techniques and crop improvement programs. Also, evaluation through subsequent generations and selection can aid in identifying stable performing mutants for leaf yield based on the performance with relation to length, breadth, fresh weight and dry weight of the leaf. This results in high leaf production which has a greater demand in the market that ultimately results in improving the economic status of farmers as well as to combat food and nutrition deficiency.

Cluster analysis of the mutants

Based on the presence and absence of bands with the 10 SSR markers, the 10 selected mutants along with the PKM 1 variety were divided into 2 major clusters. The variety PKM 1 was separated into a single cluster with 6 other mutants (15-1-09-75, 15-1-09-55, 15-1-09-74, 15-1-09-64, 15-1-09-16, 15-1-09-70 and the control PKM 1), whereas the other 4 mutants (15-1-09-44, 15-1-09-53, 15-1-09-21 and 15-1-09-39) fell into a cluster together. Hence, based on genotypic evaluation, the influence of gamma irradiation on the leaf characters can be observed. In-depth analysis revealed further distribution of the members of the clusters, for example, the plants, 15-1-09-44 and 15-1-09-53, were closely related to each other. Similarly, the mutants 15-1-09-55 with 15-1-09-74 and the control PKM 1 with 15-1-09-70 showed a similar trend of relatedness (Fig. 3). In corroboration to this, an earlier study conducted with 32 genotypes of moringa, that were examined using the D^2 method of variability, revealed that the genotypes CBE MO 13, CBE MO 15, CBE MO 19, CBE MO 28, CBE MO 29 and CBE MO 31 were considered promising with all the necessary qualities for a future breeding program, these genotypes can therefore be further explored (29). Similar to this, in chilli germplasms, genetic diversity was revealed by SSR markers. The study divided the plants into four major clusters based on a similarity matrix utilizing the UPGMA dendrogram (30).

Conclusion

Mutation breeding has gained popularity over the past few years and has been embraced by various nations. With the development of diverse plant breeding techniques, genetics and biotechnological instruments, mutation breeding helps to boost global food and agricultural production, which eventually helps to end world hunger and enhance worldwide nutritional status. Through this study, it was evident that all the mutants are diverse from control in one or the other way. The mutants, 15-1-09-70 and 15-1-09-53, were identified as diverse in leaf-related characteristics; both through morphological and molecular diversity analysis which can be forwarded to the next generation. These mutants have the potential to be released as a variety through further evaluation. However, selfing followed by selection in the next generation can result in stability of its performance. To increase the accuracy of selection in a breeding program, molecular markers that are closely linked to the genes that control the particular traits might be employed. For highly heritable, marker-assisted selection (MAS) works particularly well. Also, further research in this field could benefit from the investigation of other molecular markers or the integration of genetic information with phenotypic characteristics.

Acknowledgements

The authors would like to acknowledge the staff of Horticultural College and Research Institute, Periyakulam for their support and guidance resulting in the successful conducting of the experiment.

Authors' contributions

BS carried out the molecular genetic studies and drafted the manuscript. NK, RS AG, GP and RJ designed, corrected and improved the manuscript. WM improved the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

References

1. Suzana D, Suyatna FD, Andrajati R, Sari SP, Mun'im A. Effect of *Moringa oleifera* leaves extract against hematology and blood biochemical value of patients with iron deficiency anemia. J of Young Pharmacists. 2017;9(1):79. https://doi.org/10.5530jyp.2017.1s.20
2. Cretella ABM, da Silva SB, Pawloski PL, Ruziska RM, Scharf DR, Ascari J, et al. Expanding the anti-inflammatory potential of *Moringa oleifera*: topical effect of seed oil on skin inflammation and hyperproliferation. J Ethnopharmacol. 2020;254:112708. https://doi.org/10.1016/j.jep.2020.112708
3. Mehta A, Agrawal B. Investigation into the mechanism of action of *Moringa oleifera* for its anti-asthmatic activity. Orient Pharm Exp Med. 2008;8(1):24–31. https://doi.org/10.3742/OPEM.2008.8.1.024
4. Popoola JO, Obembe OO. Local knowledge, use pattern and geographical distribution of *Moringa oleifera* Lam. (Moringaceae) in Nigeria. J Ethnopharmacol. 2013;150(2):682–91. https://doi.org/10.1016/j.jep.2013.09.043
5. Saleem A, Saleem M, Akhtar MF. Antioxidant, anti-inflammatory and antiarthritic potential of *Moringa oleifera* Lam: An ethnomedicinal plant of Moringaceae family. South African J Bot. 2020;128:246–56. https://doi.org/10.1016/j.sajb.2019.11.023
6. Abdulrahman MD, Hamad SW. Traditional methods for treatment and management of measles in northern Nigeria: Medicinal plants and their molecular docking. Ethnobot Res and Appl. 2022;23. http://eprints.tiu.edu.iq/id/eprint/991
7. Sanjay P, Dwivedi K, Shigru. (*Moringa oleifera* Lam.): A critical review. Int J Ayu Pharm Chem. 2015;3.
8. Milla PG, Penalver R, Nieto G. Health benefits of uses and applications of *Moringa oleifera* in bakery products. Plants. 2021;10(2):318. https://doi.org/10.3390/plants10020318
9. Pandit R, Bhusal B, Regmi R, Neupane P, Bhattarai K, Maharjan B, et al. Mutation breeding for crop improvement: A review. Rev

in Food and Agri. 2021;2(1):31–35. http://doi.org/10.26480/rfna.01.2021.31.35

10. Yali W, Mitiku T. Mutation breeding and its importance in modern plant breeding. J Plant Sci. 2022;10(2):64–70. http://doi.org/10.11648/j.jps.20221002.13
11. He C, Poysa V, Yu K. Development and characterization of simple sequence repeat (SSR) markers and their use in determining relationships among Lycopersicon esculentum cultivars. Theor Appl Genet. 2003;106:363–73. http://doi.org/10.1007/s00122-002-1076-0
12. Park YJ, Lee JK, Kim NS. Simple sequence repeat polymorphisms (SSRPs) for evaluation of molecular diversity and germplasms classification of minor crops. Mol. 2009;14:4546–69. http://doi.org/10.3390/molecules14114546
13. Wu JC, Yang J, Gu ZJ, Zhang YP. Isolation and characterization of twenty polymorphic microsatellite loci for *Moringa oleifera* (Moringaceae). Hort Sci. 2010;45:690–92.
14. Popoola JO, Bello OA, Olugbuyiro JA, Obembe OO. Simple sequence repeats (SSR) analysis of genetic intraspecific relationships of *Moringa oleifera* populations from Nigeria. J critical rev. 2017;9(1): https://doi.org/10.21273/HORTSCI.45.4.690
15. Zheng X, Cheng T, Yang L, Xu J, Tang J, Xie K. Genetic diversity and DNA fingerprints of three important aquatic vegetables by EST-SSR markers. Scientific Reports. 2019;9(1):1–10. https://doi.org/10.1038/s41598-019-50569-3
16. Udhaya KD. Induced polygenic variability in PKM 1 moringa variety for enhanced leaf biomass production. Int J Agri Sci. 2019;975–3710. https://www.bioinfopublication.org/jouarchive.php?opt=&jouid=BPJ0000217
17. Hari K, Nageswari K, Janavi GJ, Geetharani P. Genetic variability, correlation and path analysis of M3 generation mutants in moringa (*Moringa oleifera* L.) for leaf biomass. Biol Forum Int J. 2022;14(3):135–42.
18. Doyle JJ, Doyle JL. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem Bull. 1987;19:11–15.
19. Mgendi MG. Genetic diversity between cultivated and non-cultivated *Moringa oleifera* Lam. Provenances assessed by RAPD markers. J Cell Mol Biol. 2010;8(2):95. http://jcmb.halic.edu.tr
20. Nei M, Li WH. Mathematical model for studying genetic variation in terms of restriction endonucleases. PNAS USA. 1979;76:5269–73. https://doi.org/10.1073/pnas.76.10.526
21. Botstein D, White RL, Skalnick MH, Davies RW. Construction of a genetic linkage map in man using restriction fragment length polymorphism. American J Human Gen. 1980;32:314–31. https://pmc.ncbi.nlm.nih.gov/articles/PMC1686077
22. Chesnokov YV, Artemyeva AM. Evaluation of the measure of polymorphism information of genetic diversity. Agri Biol. 2015;50(5):571–78.
23. Boopathi MN, Williams M, Ranjani VR, Paul AE, Jayakanthan M, Saranya N, et al. Development of novel SSR markers derived from genomic and transcriptomic data of *Moringa oleifera* L. var. PKM 1 and their applicability. The J Horti Sci Biotech. 2022;97(4):487–95. https://doi.org/10.1080/14620316.2021.2014991
24. Kpocheme AOEK, Hotegni NF, Missihoun AA, Gnanvi BN, Atou R, Wouyou A, et al. Morphological characterization of *Amaranthus cruentus* L. mutant lines derived from local and preferred Amaranthus cultivar. Int J Biol Chem Sci. 2022;16(4):1554–69. https://doi.org/10.4314/ijbcs.v16i4.16
25. Mansour HM, Hamideldin N, Abdel-Tawab FM, Fahmy EM, El Demerdash H, Amar MH. A physiological and genetical study for the effect of gamma irradiation on *Moringa olefiera* Lam. Egyptian J Radi Sci Appli. 2018;31(2):125–36.
26. Ashalata VA, Kotasthane AS. Effect of gamma radiation treatment on growth and morphology of red Amaranthus. The Pharma Innov J. 2022;SP-11(9):1469–71. https://doi.org/10.21608/ejrsa.2018.2286.1035
27. Nandhini V, Boopathi NM, Raveendran M, Rajasree V. Enhancing moringa (*Moringa oleifera*) leaf biomass using chemical induced mutagenesis and validating the mutagenesis using simple sequence repeats. Survival. 2022;10:100.
28. Hassanein AMA. Nutritional, chemical and molecular characterisation of *Moringa oleifera* Lam. and *Moringa peregrina* (Forssk.) Fiori genotypes. The J Horti Sci Biotech. 2018;93(5):500–09. https://doi.org/10.1080/14620316.2017.1399834
29. Saraswathi T, Meena P, Pugalendhi L, Boopathi NM. Assessment of genetic divergence and molecular characterization of moringa (*Moringa oleifera* Lam.) genotypes. South African J of Bot. 2023;157:502–07. https://doi.org/10.1016/j.sajb.2023.04.020
30. Haque MI, Ishtiaque S, Islam MM, Mujahidi TA, Rahim MA. Molecular profiling of chilli germplasm by using SSR marker. SAARC J of Agri. 2021;19(1):1–13. https://doi.org/10.3329/sja.v19i1.54774