Genetic Diversity of Moringa (*Moringa Oleifera* Lam.)

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Abstract

Moringa (*Moringa oleifera* Lam.) is a widespread multipurpose tree with great potential as a high-value crop for its nutritive, therapeutic and prophylactic properties with several industrial applications. Almost every part of the tree is useful with varied end uses. It is an interesting plant owed to its bioactive compounds. *Moringa* species have been widely spread across the tropical and subtropical regions of the world and a total of 13 species of the genus *Moringa* are known. Moringa, being a cross-pollinated tree, high heterogeneity in many character forms is observed resulting in vast genetic diversity in the natural and cultivated accessions and substantial variation in quantitatively inherited traits has been documented which needs to be exploited for concentrated research towards its crop improvement. In-depth knowledge and understanding of the gene flow pattern and population genetic structure in moringa through molecular genetic diversity and population structure of worldwide collections is of great promise. In spite of its varied uses and morphological diversification, the number of accessions collected as germplasm and their conservation in gene banks is very meagre. Documentation of genetic diversity and conservation of germplasm is a necessity for strategic research as well as breeding programmes to develop elite varieties. This chapter highlights the genetic diversity in moringa, its significance in the contemporary nutritional security scenario and its exploitation for future programs.

7.1 Introduction

Moringa (*Moringa oleifera* Lam.) is a widespread multipurpose tree known for its nutritive, therapeutic and prophylactic properties with numerous industrial applications. It is an ancient seldom utilized tree but gaining importance recently due to its exceptional uses. It is being studied for nutritional attributes and as a livestock fodder crop. The fast-growing ability of the crop helps to withstand both severe drought and mild frost conditions and has made moringa, a hardy tree crop cultivated wildly across tropics and subtropics (Csurhes and Navie 2016). Globally, moringa is being promoted as a nutritious tree crop in marginal areas with less rainfall as well as homestead gardens (FAO 2020). This

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tree has the prospective to increase nutrition, food security and boost rural development (Hsu et al. 2006). The long, slender, triangular seed-pod properties earned it the vernacular name 'drumstick tree' and from the horseradish taste of the roots 'horseradish tree' besides, ben oil tree or benzolive tree (Paliwal et al. 2011). It is popularly called as 'mother's best friend' in the Philippines, as it is consumed to increase a woman's milk production as well as for the treatment of anaemia (Estrella et al. 2000; Siddhuraju and Becker 2003). Recently this species has been dubbed as 'miracle tree' or 'natural gift' or 'mother's best friend' (Leone et al. 2015; Pirro et al. 2019).

Almost every part of the tree is useful either for its nutritional value or for commercial purposes, hence, it is also known as the 'natural nutrition of the tropics'. The leaves, flowers, fruits and immature pods are highly nutritious and consumed in Asian, African and American countries such as India, Pakistan, Sri Lanka, Burma, Indonesia, Philippines, Hawaii (Anwar and Bhanger 2003). The extracts from moringa leaves are used to treat malnutrition, augment breast milk in lactating mothers as they are rich in minerals, vitamins and other essential phytochemicals. It has the potential to use as an antioxidant, anticancer, anti-inflammatory, antidiabetic and antimicrobial agent. Moringa seed is extensively used in the purification of drinking water as it acts as a natural coagulant (Gopalakrishnan et al. 2016; Falowo et al. 2018). In various forms, this tree is used to treat turbid water in many countries (Suarez et al. 2003; Bhatia et al. 2007). In addition to food uses, moringa is being used for animal feed (Sánchez et al. 2006). When supplemented to the diet of dairy animals, moringa leaves improve dry matter intake, digestion and milk production, but without affecting the smell, taste or colour of milk (Sánchez et al. 2006). Further, leaf and seed powders are a good source of phytomedical compounds (Anwar et al. 2007). It is also a promising source for bioenergy (Adu-Dapaah et al. 2015).

7.2 Origin and Distribution

Moringa species have been widely spread across the tropical and subtropical countries of the world (Csurhes and Navie 2016). Moringa is thought to originate from northern India, specifically around Agra and Oudh and South of the Himalayan Mountains (Vogt 1996). It spread to eastern Africa during the beginning of the twentieth century (Mallenakuppe et al. 2015). The species is either grown commercially or present in wild across Asian, African, North American, Central American, the Caribbean, South American and Oceania counties (Acevedo-Rodríguez and Strong 2012). In recent times, Moringa is being grown throughout the Middle East, almost the whole tropical belt and is expanding to a diverse environment of tropical and subtropical regions in Africa and America (Pandey et al. 2011; Chang et al. 2019).

7.3 Genetic Resources of Moringa

Moringa belongs to the order Brassicales and the monogeneric family Moringaceae, which consists of about 33 species (Arora et al. 2013). Among these, 13 species, viz., *M. arborea, M. longituba, M. pygmaea, M. concanensis, M. borziana, M. hildebrandtii, M. stenopetala M. oleifera, M. ovalifolia, M. peregrina, M. rivae, M. drouhardi, M. ruspoliana* are found worldwide (Stephenson and Fahey 2004). Origin of moringa species could be traced back to different countries: *M. arborea*, native to Kenya; *M. borziana* from Somalia and Kenya; *M. rivae and M. stenopetala* originated from Kenya and Ethiopia; *M. pygmaea* native to Somalia; *M. longituba* from Ethiopia and Somalia*; M ruspoliana* native to Ethiopia; *M. ovalifolia* from Namibia and Angola; *M. drouhardii* and *M. hildebrandi* from Madagascar; *M. peregrine* from Red Sea and Horn of Africa; *M. concanensis* and *Moringa oleifera* indigenous to sub-Himalayan tracts of northern India, among which *Moringa oleifera* has been the most well-studied

and used species for human consumption (Paliwal et al. 2011). Further, *M. arborea, M. ruspoliana, M. longituba, M. stenopetala M. rivae,* and *M. borziana* are in danger of extinction (Stephenson and Fahey 2004). Only *M. oleifera* is cultivated (Sánchez et al. 2006). Moringa cytological studies revealed that *Moringa oleifera* has 2c genome size of 1.2 pico gram (pg) (Ohri and Kumar 1986). The diploid chromosome number of this species is 2n = 28 (PROTA 2017).

Distribution of several ecotypes can be seen across India with several vernacular names, namely; Jaffna and Chavakacheri murungai (soft and taste fruits), Chemmurungai (red-tipped fruits), Kadumurungai with small and inferior fruits, Palmurungai and Punamurungai having a bitter taste, Kodikalmurungai with short fruit, wild Kadumurunga and Kodikkal Murungai (Kumar Ganesan et al. 2014). Two varieties viz., PKM-1; PKM-2 from Tamil Nadu and one variety, Bhagya from Karnataka, India, have been developed higher pod yield, pod quality and nutritional parameters. Several moringa varieties with desirable attributes also developed from Kerala, India (Kumar Ganesan et al. 2014). Even though vast variability is present as cultivated accessions and natural accessions, systematic studies towards the collection of wild and local forms as well as cultivated accessions across the globe and genetic diversity quantification are meagre. In spite of its varied uses and morphological diversification, the number of accessions collected as germplasm and their conservation in germplasm banks are just emerging across the world. The gap between the inherent genetic variability available and its poor representation in gene banks is a hindrance for crop improvement programs that needs to be addressed. So, gene/germplasm banks covering the entire genetic variability in Moringa is a necessity for concentrated research as well as breeding programmes to develop elite varieties.

Most of the genetic variation of *M. oleifera* is reported in India (Kumar Ganesan et al. 2014; PROTA 2017). The species has various cultivars, including some cultivated as annuals in temperate climates. DNA barcode details are available at the Barcode of Life Data Systems (Godino et al. 2015). Research centers concentrated on *Moringa oleifera* improvement across the globe are AVRDC (Taiwan), Moringa Philippines Foundation (Philippines), Moringa community (Zambia) and Rural development initiative (Zambia). Germplasm collections are stored at various facilities worldwide (Patricio and Palada 2015). The World Vegetable Center at Taiwan maintains collections of four moringa species, the majority of which are *M. oleifera* (Palada et al. 2017). Further, moringa collections are also maintained at the International Moringa Germplasm Collection in Mexico and Burkina Faso and the Philippines (IMGC 2017; PROTA 2017). The International Moringa Germplasm Collection houses living material of 12 of the 13 *Moringa* species at the Instituto de Biología, Universidad Nacional Autónoma de México (UNAM), Mexico (PROTA 2017).

7.4 Genetic Diversity in *Moringa Oleifera*

Genetic diversity is an essential pre-requisite in crop improvement. The chances of obtaining desirable transgressive segregants depend on the extent of parental genetic diversity: the greater the genetic diversity, the higher the prospects of desirable segregants. Diverse agro-ecological conditions, migration of genetic material due to genetic drift, gene flow, introduction/exchange of genetic stocks at national and international levels, along with adoptive as well as intensive artificial selection are responsible for the diversification of drumstick plants.

Moringa, being an outbreeding tree, high heterogeneity with high diversification in many characters are observed. The crop shows extensive diversity of morphological and biochemical properties, which serve as a resource for its genetic improvement (Godino et al. 2015). Wide variability was revealed like tree habit from deciduous to evergreen; shape of the tree varying from semi spreading to upright; flowering time wherein some trees flower throughout the year and some flower in two distinct seasons

(Ramachandran et al. 1980; Raja et al. 2013) and resistance to hairy caterpillar (Mgendi et al. 2010; Raja et al. 2013).

Thirty-six genetically diverse genotypes of drumstick were clustered into five clusters based on the content of vitamin C, protein, nitrogen, phosphorous, potassium, calcium, iron and magnesium. The analysis used measures the forces of differentiation at two levels, namely intra-cluster and inter-cluster levels, and thus help in the selection of genetically divergent parents for exploitation in hybridization programmes (Tak and Maurya 2015). Genetic diversity, population structure and correlation in Indian populations of *Moringa oleifera* studied by Rajalakshmi et al. (2019) revealed the high variability existent at an intra-population level.

7.5 Nutritional Diversity in Moringa

Moringa is one of the most useful trees that can be used for food, nutraceutical value and industrial purposes (Khalafalla et al. 2010). People use its leaves, flowers and fresh pods as vegetables, while others use it as livestock feed (Anjorin et al. 2010). The significance of moringa to address nutritional deficiencies is based on the abundance of vitamins, minerals and protein found in the leaves and pods. These include vital nutrients such as beta-carotene, iron, zinc, vitamin C and all the essential amino acids (Moyo et al. 2011). The plant is rich in other bioactive and anti-inflammatory compounds like phenolics and isothiocyanates, while being relatively low in anti-nutrients (Falowo et al. 2018; Waterman et al. 2014). Different parts of the plant are differing with respect to their nutritional value. 100 g of pod yields 26 cal of energy; 2.5 g protein; 0.1 g fat; 3.7 g carbohydrates; 4.8 g fibre; 0.05 mg vitamin B1; 0.07 mg vitamin B2; 0.2 mg vitamin B3; 120 mg vitamin C; 30 mg calcium; 24 mg magnesium; 110 mg phosphorous; 259 mg potassium; 3.1 mg copper; 5.3 mg iron and 137 mg sulphur. Whereas, fresh leaves yield about 92 cal of energy and 6.7 g protein; 1.7 g fat; 12.5 g carbohydrates; 0.9 g fibre; 0.06 mg vitamin B1; 0.05 mg vitamin B2; 0.8 mg vitamin B3; 220 mg vitamin C; 448 mg vitamin A; 440 mg calcium; 42 mg magnesium; 70 mg phosphorous; 259 mg potassium; 0.07 mg copper and 0.85 mg iron (Gopalakrishnan et al. 2016). The leaves are considered as a better source of protein, Ca and Fe while the pods are rich in P, Na and Mg. Both the edible parts (leaves and pods) more specifically leaves have the potential to be used as micronutrient supplements for food products (Mallenakuppe et al. 2015).

The presence of wide nutritional variation in Moringa could be accredited to varying genetic backgrounds of the plant, in terms of ecotype, cultivar as well as environmental factors (Sánchez-Machado et al. 2010). Nutritional composition of four species; *M. oleifera, M. peregrine, M. stenopetala, M. drouhardii* indicated high overall nutritive value, antioxidants and glucosinolates, with low oxalate content, whereas *M. oleifera* contained the highest amounts of β-carotene, ascorbate, α-tocopherol, iron and protein content and *M. peregrina* was the uppermost for antioxidants (Yang et al. 2006). Substantial variation for chlorophyll, protein, macronutrient and micronutrient content in the leaves of different genotypes of *M. oleifera* and *M. peregrina* was revealed, which indicated higher chlorophyll in *M. peregrine* and higher protein content in *M. oleifera* (19.1–32.9%) than *M. peregrine* (12.5–24.6%).

Similarly, Fe and Zn contents varied between 250–490 ppm and 26.7–58.3 ppm respectively in *M. oleifera* versus 285–403 ppm and 15.7–38.9 ppm in *M. peregrine*. Mp genotypes were mostly intermediate in K content compared to Mo genotypes (1.3–2.0%). Calcium content in both species ranged from 0.03 to 1.3%. The highest Mg content was found in Mp genotypes (1.0%). Genotypes also varied in their content in Zn, Cu, and Mn for both species (Hassanein 2018). A similar trend of macronutrients was reported on *M. oleifera* genotypes grown in the Philippines (Magat et al. 2009). Further in Ghana, different genotypes of *M. oleifera* varied in protein and Ca contents with a wider range of

protein, 26–27% in Ghana and significantly differed in their contents of all studied nutrients (Asante et al. 2014). Saudi Arabia *M. oleifera* genotypes were reported to be more diverse than those of other regions for their nutrient contents (Nouman et al. 2016).

Various accessions of moringa from different agro-ecological regions of Rajasthan, Uttarakhand, Uttar Pradesh, Madhya Pradesh and Goa have revealed high variability in the quantity of phytochemicals (Nouman et al. 2016). Two cultivars from the two ecological regions Chapai Nawabganj (L1) and Pabna (L2) of Bangladesh indicated a wide variation in nutritional value for protein and mineral nutrients viz., Ca, Fe, P and K. Ten moringa accessions, selected from a survey of 60 moringa accessions revealed variation for nutrient contents (Islam et al. 2020). Polyphenolics composition, antioxidant activity and contents of selected nutrients in the leaves from seven cultivars of *M. oleifera* ('Tumu', 'Sunyaw', 'Kumasi', 'Techiman', 'China', 'Pakistan Black' and 'Pakistan White') in Pakistan revealed variation among cultivars (Nouman et al. 2016).

7.6 Polymorphism and Allelic Frequency

Molecular diversity among seven populations of *M. oleifera* from Africa was explored and molecular variance of 59.15% between populations was inferred. When compared with the Indian population, African population showed 18.59% of variation existed between the two populations (Muluvi et al. 1999). These seven populations also revealed a total of 236 amplifications, of which 157 (66.5%) were found to be polymorphic between or within the populations (Muluvi et al. 1999). The microsatellite marker analysis of 24 germplasm of moringa exhibited an average of two to six alleles per locus. The expected and observed heterozygosity varied from 0.361 to 0.761 and from 0.01 to 0.875, respectively (Wu et al. 2010). Taxonomic analysis of 75 accessions of Moringa from Nigeria revealed a high level of polymorphism between the samples from south-central and northern parts of the country (Abubakar et al. 2011).

The high degree of polymorphism (74%) with respect to the genetic relationship was reported between the accessions from Brazil (da Silva et al. 2012). 161 accessions of *M. oleifera* from Asia, Africa, South and North America, and the Caribbean revealed allelic diversity of 8.3 alleles per SSR marker with a total of 158 alleles in 131 wild accessions collected from Pakistan and 30 accessions obtained from Florida (Shahzad et al. 2013).

Further, the genetic diversity in these 12 Indian populations using SSR primers elucidated a total of 35 bands of which 29 bands exhibited polymorphism (82.86%) with 1.84 bands /primer. Analysis of molecular variance (AMOVA) revealed 2% diversity which could be attributed to differences among regions, 3% at the level of populations and 95% contributed by the genotypes within the populations (Kumar Ganesan et al. 2014). A total of 74 alleles with a range of 4 to 15 were detected in 70 accessions of *M. oleifera* with an average of 7.4 alleles per locus. 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 (Popoola et al. 2017). The genotypes collected from different countries revealed the presence of a higher genetic variance of 1.80 and 0.13 for the Malaysian population and 0.30 and 0.19 for the international population, respectively (Rufai et al. 2013).

Three molecular marker techniques, *i.e.* random amplified polymorphic DNA (RAPD), inter simple sequence repeat (ISSR) and cytochrome P450 gene-based markers in eight Indian cultivars of *M. oleifera*, revealed 48.68, 48.57 and 40.00% polymorphisms, respectively (Saini et al. 2013). Genetic variability analysis in 97 *M. oleifera* accessions indicated an average of 59.6% polymorphism through ISSR and 70.13% with SRAP markers. The mean gene

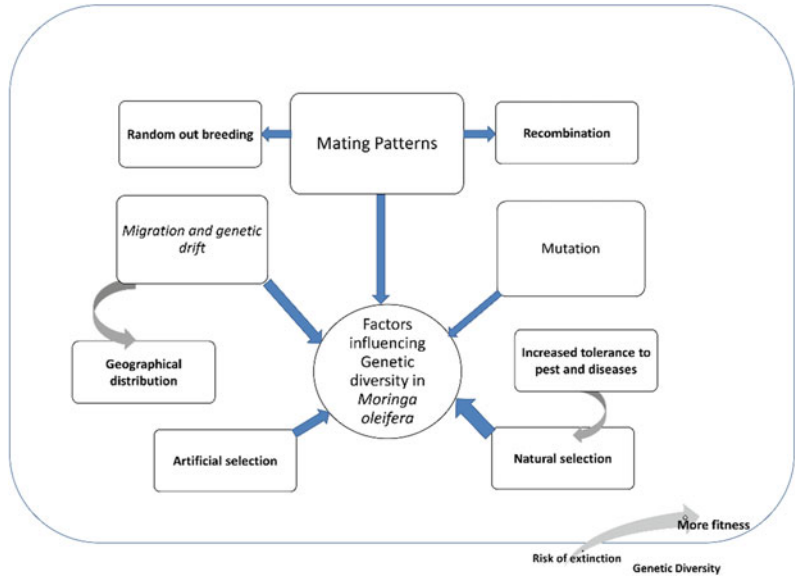

Fig. 7.1 Factors influencing genetic diversity in *Moringa oleifera*

diversity h (0.2), gene flow N_m (2.86) and a PhiPT value (0.14) estimated for the markers indicate the high variability existent at an intra-population level. Population structure results and cluster tree divided the samples into two groups: One group consists of Tamil Nadu accessions and the other comprises Andhra Pradesh and Odisha accessions (Rajalakshmi et al 2019). Figure 7.1 describes different factors that influence the Moringa genetic diversity.

Figure 7.1 Multiple factors affecting genetic diversity in a population of drumstick: Being a cross-pollinated crop, the genetic variability within and among the populations of moringa is derived from a wide assortment of genes and alleles which is affected by various factors. Mating patterns such as non-random outbreeding is common in cross-pollinated species. Random forces, such as population bottleneck causes changes in allelic frequency, which leads to genetic drift. Individuals within a population are subjected to spontaneous mutation, which evolves the species. Movement of individuals into or out of a defined location causes the migration of a set of alleles within a population. Natural disasters and competition for food and other resources favour certain individuals, naturally selecting them for further generations.

7.7 Principal Coordinate Analysis

Principal coordinate analysis (PCA) clusters and relates individuals or populations based on genetic distances. PCA of 20 genotypes of *M. oleifera* from different countries classified the twenty genotypes of *Moringa* into five major groups; two genotypes from Malaysia in one group. Dimension one of the PCA ranged from 0.49 to 0.92, while dimension two varied from −0.65 to 0.26 and dimension three varied from −0.65 to 0.68 (Rufai et al. 2013). PCA of 12 Indian populations of *M. oleifera* revealed very high genetic diversity in the Indian drumstick collection (Kumar Ganesan et al. 2014).

Phenotypic intraspecific variations among 40 accessions of *M. oleifera* collected from different agro-ecological zones of Nigeria revealed a high degree of intraspecific variability for reproductive characters. The first five principal component axes explained 61.40% of the overall variation with PC1 (23.92%) and PC2 (14.19%) contributing 38.11% of the total variation (Popoola et al. 2016). Commercially grown *Moringa oleifera* cultivars from India resulted in PCA plots where all the cultivars were assembled into four major clusters and genotypes belonging

to a particular sub-cluster were grouped together (Saini et al. 2013).

7.8 Phylogenetic and Population Structure

In-depth knowledge and understanding of the detailed gene flow pattern and population genetic structure in Moringa is very meagre (Muluvi et al. 2004). Thus, the development of efficient molecular markers for *M. oleifera* is needed. The genetic diversity and population structure of worldwide collections of *M. oleifera* were investigated using 19 simple sequence repeat (SSR) markers along with a partial sequence of the chloroplast gene *atpB* which demonstrated large genetic diversity present in wild collections from Pakistan (Shahzad et al. 2013).

Parsimony analyses to infer the phylogenetic relationships of all 13 species of the genus *Moringa* revealed the four bottle trees in a basal paraphyletic assemblage, with the three species of slender trees, including the economically important *M. oleifera* forming a clade that is sister to a clade of the six species of tuberous shrubs and trees of northeast Africa (Olson 2002). 20 M. *oleifera* populations from Nigeria, using amplified fragment length polymorphism (AFLP) primer pairs clustered accessions along eco-geographical lines while others grouped disparately (Popoola et al. 2019).

Model-based population structure of 12 Indian *M. oleifera* populations using *K* values from 1 to 20 resulted in five clear populations instead of 12 natural populations. Cluster analysis and structure-based population study showed that no geographical isolation exists between genotypes collected from the southern and northern parts of India (Kumar Ganesan et al. 2014). Population structure analysis of 161 M. *oleifera* accessions collected from nine other countries of the world was grouped into three clusters and there was a sharp decrease in delta-K values from K2 to K3, with a plateau at K4 (Shahzad et al. 2013). Evolutionary studies of the *M. oleifera* genome revealed a recent surge of plastid to nucleus gene duplications that led to massive amounts of plastid DNA in the *Moringa* nuclear genome, representing 4.71%, the largest reported so far (Ojeda-López et al. 2020).

7.9 Conclusion

Moringa oleifera natural populations are broadly distributed throughout Asian tropical and subtropical regions, with rich genetic diversity in their morphology, nutritional and other useful traits. Geographic populations of *Moringa* species have varied levels of genetic relatedness and distinct genetic parameters. There is a need for concentrated biological, phylogenetic, genomic and biochemical studies on this species. Conservation efforts of Moringa as a genetic resource should be initiated across Asia to prevent extinction. The study of polymorphism and allelic frequencies revealed significantly observed and expected heterozygosity among the sampled populations across the globe, which indicated significant genetic diversity. The combined results of the phylogenetic tree, population structure analysis, and PCA indicated significant clustering across the populations of *Moringa oleifera* from different regions of the world. However, there is a need for further studies with larger collections to assess genetic diversity and eventually conserve the Moringa populations.

References

Abubakar BY, MuArsquo S, Khan AU, Adamu AK (2011) Morpho-anatomical variation in some accessions of *Moringa oleifera* Lam. from Northern Nigeria. Afr J Plant Sci 5(12):742–748

Acevedo-Rodríguez P, Strong MT (2012) Catalogue of seed plants of the West Indies. Smithsonian Contributions to Botany

Adu-Dapaah H, Osei-Bonsu I, Oduro I, Asiedu J (2015) Recent advances in production, processing and utilization of Moringa oleifera in Ghana. In: International symposium on Moringa, vol 1158, pp 179–186

Anjorin TS, Ikokoh P, Okolo S (2010) Mineral composition of *Moringa oleifera* leaves, pods and seeds from two regions in Abuja, Nigeria. Int J Agric Biol 12:431–434

Anwar F, Bhanger MI (2003) Analytical characterization of *Moringa oleifera* seed oil grown in temperate

regions of Pakistan. J Agric Food Chem 51(22):6558–6563
Anwar F, Latif S, Ashraf M, Gilani AH (2007) *Moringa oleifera*: a food plant with multiple medicinal uses. Phytother Res 21(1):17–25
Arora DS, Onsare JG, Kaur H (2013) Bioprospecting of Moringa (Moringaceae): microbiological perspective. J Pharmacogn Phytochem 1(6):193–215
Asante WJ, Nasare IL, Tom-Dery D, Ochire-Boadu K, Kentil KB (2014) Nutrient composition of Moringa oleifera leaves from two agro ecological zones in Ghana. Afr J Plant Sci 8(1):65–71
Bhatia S, Othman Z, Ahmad AL (2007) Pretreatment of palm oil mill effluent (POME) using *Moringa oleifera* seeds as a natural coagulant. J Hazard Mater 145(1–2):120–126
Chang Y, Liu H, Liu M, Liao X, Sahu SK, Fu Y, Song B, Cheng S, Kariba R, Muthemba S, Hendre PS (2019) The draft genomes of five agriculturally important African orphan crops. Gigacience 8(3):giy152
Csurhes S, Navie S (2016) Horseradish tree: *Moringa oleifera*. Queensland Government, Brisbane, AUS.
da Silva AV, dos Santos AR, da Silva LA, Feitosa RB, Almeida CS, da Silva GM, Rangel MS (2012) Moringa genetic diversity from germplasm bank using RAPD markers. Trop Subtrop Agroecosystems 15 (1):31–39
Estrella MC, Jacinto Bias III, V, David GZ, Taup MA, (2000) A double-blind, randomized controlled trial on the use of malunggay (*Moringa oleifera*) for augmentation of the volume of breastmilk among non-nursing mothers of preterm infants. Phillipp J Pediatr 49(1):3–6
Falowo AB, Mukumbo FE, Idamokoro EM, Lorenzo JM, Afolayan AJ, Muchenje V (2018) Multi-functional application of *Moringa oleifera* Lam. in nutrition and animal food products: a review. Food Res Int 106:317–334
FAO (2020). http://www.fao.org/traditional-crops/moringa/en/
Godino M, Arias C, Izquierdo MI (2015) Moringa oleifera: potential areas of cultivation on the Iberian Peninsula. In: International symposium on Moringa, vol 1158, pp 405–412
Gopalakrishnan L, Doriya K, Kumar DS (2016) *Moringa oleifera*: a review on nutritive importance and its medicinal application. Food Sci Hum Well 5(2):49–56
Hassanein AM (2018) Nutritional, chemical and molecular characterisation of *Moringa oleifera* Lam. and *Moringa peregrina* (Forssk.) Fiori genotypes. J Hortic Sci Biotechnol 93(5), 500–5009
Hsu R, Midcap S, Arbainsyah DW (2006) *Moringa oleifera* medicinal and Economic uses. International course on Economic botany, National Herbarium, Leiden, The Netherlands
IMGC (2017) International Moringa Germplasm Collection. https://moringaceae.org/index.html
Islam MA, Sheikh A, Waterman C, Hosenuzzaman M (2020) Morphology, pod yield and nutritional quality of two cultivars of Moringa (*Moringa oleifera*) in Bangladesh. Indian J Sci Technol 13(37):3893–3903
Khalafalla MM, Abdellatef E, Dafalla HM, Nassrallah AA, Aboul-Enein KM, Lightfoot DA, El-Deeb FE, El-Shemy HA (2010) Active principle from *Moringa oleifera* Lam leaves effective against two leukemias and a hepatocarcinoma. Afr J Biotechnol 9(49):8467–8471
Kumar Ganesan S, Singh R, Choudhury DR, Bharadwaj J, Gupta V, Singode A (2014) Genetic diversity and population structure study of drumstick (Moringa oleifera Lam.) using morphological and SSR markers. Ind Crop Prod 60:316–325
Leone A, Spada A, Battezzati A, Schiraldi A, Aristil J, Bertoli S (2015) Cultivation, genetic, ethnopharmacology, phytochemistry and pharmacology of *Moringa oleifera* leaves: an overview. Int J Mol Sci 16 (6):12791–12835
Magat SS, Raquepo MC, Pabustan CD (2009) Mineral macro-nutrients, micro-nutrients and other elements in leaves of malunggay plant (*Moringa oleifera*) sampled in some locations in The Philippines. Technology-Advisory Notes
Mallenakuppe R, Homabalegowda H, Gouri MD, Basavaraju PS, Chandrashekharaiah UB (2015) History, taxonomy and propagation of *Moringa oleifera*-a review. Crops J 3(3.28):3–15
Mgendi MG, Manoko MK, Nyomora AM (2010) Genetic diversity between cultivated and non-cultivated *Moringa oleifera* Lam. provenances assessed by RAPD markers/ *Moringa oleifera*'nin ekili olan ve olmayan kökenlerinin genetik çesitliliginin RAPD markörleri ile degerlendirilmesi. Mol Cell Biol 8(2):95
Moyo B, Masika PJ, Hugo A, Muchenje V (2011) Nutritional characterization of Moringa (*Moringa oleifera* Lam.) leaves. Afr J Biotechnol 10 (60):12925–12933
Muluvi GM, Sprent JI, Soranzo N, Provan J, Odee D, Folkard G, McNicol JW, Powell W (1999) Amplified fragment length polymorphism (AFLP) analysis of genetic variation in *Moringa oleifera* Lam. Mol Ecol 8 (3):463–470
Muluvi GM, Sprent JI, Odee D, Powell W (2004) Estimates of outcrossing rates in *Moringa oleifera* using Amplified fragment length polymorphism (AFLP). Afr J Biotechnol 3(2):145–151
Nouman W, Anwar F, Gull T, Newton A, Rosa E, Domínguez-Perles R (2016) Profiling of polyphenolics, nutrients and antioxidant potential of germplasm's leaves from seven cultivars of *Moringa oleifera* Lam. Ind Crop Prod 83:166–176
Ohri D, Kumar A (1986) Nuclear DNA amounts in some tropical hardwoods. Caryologia 39(3–4):303–307
Ojeda-López J, Marczuk-Rojas JP, Polushkina OA, Purucker D, Salinas M, Carretero-Paulet L (2020) Evolutionary analysis of the *Moringa oleifera* genome reveals a recent burst of plastid to nucleus gene duplications. Sci Rep 10(1):1–5
Olson ME (2002) Combining data from DNA sequences and morphology for a phylogeny of Moringaceae (Brassicales). Syst Syst Bot 27(1):55–73

Palada MC, Ebert AW, Yang RY, Chang LC, Chang J, Wu DL (2017) Progress in research and development of Moringa at the World Vegetable Center

Paliwal R, Sharma V, Sharma S (2011) Elucidation of free radical scavenging and antioxidant activity of aqueous and hydro-ethanolic extracts of *Moringa oleifera* pods. Res J Pharm Tech 4(4):566–571

Pandey A, Pradheep K, Gupta R, Nayar ER, Bhandari DC (2011) 'Drumstick tree'(*Moringa oleifera* Lam.): a multipurpose potential species in India. Genet Resour Crop Evol 58(3):453–460

Patricio HG, Palada MC (2015) Adaptability and horticultural characterization of different Moringa accessions in Central Philippines. In: International symposium on Moringa, vol 1158, pp 45–54

Pirrò S, Minutolo A, Galgani A, Potestà M, Colizzi V, Montesano C (2019) Bioinformatics prediction and experimental validation of MicroRNAs involved in cross-kingdom interaction. J Comput Biol 23 (12):976–989

Popoola JO, Bello OA, Obembe OO (2016) Phenotypic intraspecific variability among some accessions of drumstick (*Moringa oleifera* Lam.). Can J Appl Sci 10 (1):3681–3693

Popoola JO, Bello OA, Olugbuyiro J, Obembe OO (2017) Simple sequence repeats (SSR) analysis of genetic intraspecific relationships of *Moringa oleifera* populations from Nigeria. Sci Int 29(3):645–657

Popoola J, Igwe D, Jegede O, Iwu V, Adegbite A, Omonhinmin C (2019) Agronomic practices, genetic diversity and population structure of *Moringa oleifera* (Lam.) in Nigeria, Preprints.org

PROTA (2017) PROTA4U web database. Wageningen, Netherlands: Plant Resources of Tropical Africa. https://www.prota4u.org/database/

Raja S, Bagle BG, More TA (2013) Drumstick (*Moringa oleifera* Lam.) improvement for semiarid and arid ecosystem: analysis of environmental stability for yield. J Plant Breed Crop Sci 5(8):164–170

Rajalakshmi R, Rajalakshmi S, Parida A (2019) Genetic diversity, population structure and correlation study in *Moringa oleifera* Lam. using ISSR and SRAP markers. Proc Natl Acad Sci India Sect B Biol Sci 89 (4):1361–1371

Ramachandran C, Peter KV, Gopalakrishnan PK (1980) Drumstick (*Moringa oleifera*): a multipurpose Indian vegetable. Econ Bot 1:276–283

Rufai S, Hanafi MM, Rafii MY, Ahmad S, Arolu IW, Ferdous J (2013) Genetic dissection of new genotypes of drumstick tree (*Moringa oleifera* Lam.) using random amplified polymorphic DNA marker. BioMed Res Int

Saini RK, Saad KR, Ravishankar GA, Giridhar P, Shetty NP (2013) Genetic diversity of commercially grown *Moringa oleifera* Lam. cultivars from India by RAPD, ISSR and cytochrome P 450-based markers. Plant Syst Evol 299(7):1205–1213

Sánchez NR, Ledin S, Ledin I (2006) Biomass production and chemical composition of *Moringa oleifera* under different management regimes in Nicaragua. Agrofor Syst 66(3):231–242

Sánchez-Machado DI, Núñez-Gastélum JA, Reyes-Moreno C, Ramírez-Wong B, López-Cervantes J (2010) Nutritional quality of edible parts of *Moringa oleifera*. Food Anal Methods 3(3):175–180

Shahzad U, Khan MA, Jaskani MJ, Khan IA, Korban SS (2013) Genetic diversity and population structure of *Moringa oleifera*. Conserv Genet 14(6):1161–1172

Siddhuraju P, Becker K (2003) Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (*Moringa oleifera* Lam.) leaves. J Agric Food Chem 51(8):2144–2155

Stephenson KK, Fahey JW (2004) Development of tissue culture methods for the rescue and propagation of endangered *Moringa spp.* germplasm. Econ Bot 58(1): S116–124

Suarez M, Entenza JM, Doerries C, Meyer E, Bourquin L, Sutherland J, Marison I, Moreillon P, Mermod N (2003) Expression of a plant-derived peptide harboring water-cleaning and antimicrobial activities. Biotechnol Bioeng 81(1):13–20

Tak S, Maurya IB (2015) Genetic diversity of Moringa oleifera Lam. in Rajasthan, India. In: International symposium on Moringa, vol 1158, pp 71–78

Vogt K (1996) A field worker's guide to the identification, propagation and uses of common trees and shrubs of dryland Sudan

Waterman C, Cheng DM, Rojas-Silva P, Poulev A, Dreifus J, Lila MA, Raskin I (2014) Stable, water extractable isothiocyanates from *Moringa oleifera* leaves attenuate inflammation in vitro. Phytochemistry 03:114–122

Wu JC, Yang J, Gu ZJ, Zhang YP (2010) Isolation and characterization of twenty polymorphic microsatellite loci for *Moringa oleifera* (Moringaceae). HortSciences 45(4):690–692

Yang R Y, Chang LC, Hsu JC, Weng BB, Palada MC, Chadha ML, Levasseur V (2006) Nutritional and functional properties of Moringa leaves–From germplasm, to plant, to food, to health. Moringa leaves: Strategies, standards and markets for a better impact on nutrition in Africa. Moringanews, CDE, CTA, GFU Paris