Scientia Horticulturae 225 (2017) 431–437

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

# The potential of the tropical "miracle tree" *Moringa oleifera* and its desert relative *Moringa peregrina* as edible seed-oil and protein crops under Mediterranean conditions

Yiftach Vaknin*, Adina Mishal

*Department of Natural Resources, Institute of Plant Sciences, Agricultural Research Organization, The Volcani Center, 68 HaMaccabim Road, P.O.B. 15159, Rishon LeZion 7505101, Israel*

ARTICLE INFO

*Keywords:*
Bloom phenology
*Moringa oleifera*
*Moringa peregrina*
Near-infrared reflectance spectroscopy
Seed oil content
Seed protein content

ABSTRACT

The potential of the tropical *Moringa oleifera* Lam. and its desert relative *Moringa peregrina* (Forssk.) Fiori as edible seed-oil and seed-protein crops under Mediterranean conditions was evaluated. Initially, we developed a NIRS (Near Infrared Reflectance Spectroscopy) method for the analysis of seed weight, and seed-oil and protein contents. We found NIRS to be a relatively accurate method for estimating seed traits of both *Moringa* species.

Comparative analysis of bloom phenology and reproductive success between *M. oleifera* and *M. peregrina*, grown under Mediterranean conditions, revealed for *M. oleifera* a shorter juvenility period and a lower variation in bloom and reproductive traits. Both species bloomed in summer and set fruits in autumn. *M. peregrina* also bloomed in late autumn and set fruits in spring the following year. Annually, *M. oleifera* trees produced significantly more flowers and set more fruits. Fruit-set for both species was extremely low (~0.5–1.5%). *M. oleifera* pods were 45% longer, contained 23% more seeds that were 47% lighter with 11% lower oil concentration compared with *M. peregrina*, resulting in approximately six-fold higher oil and protein yields per plant.

In conclusion, *M. oleifera* was better suited for oil and protein production under Mediterranean conditions as it produced more seeds and in a more predictable and uniform manner. However, since *M. peregrina* produced larger seeds with higher oil concentrations and was less susceptible to local diseases, future breeding efforts should be concentrated on producing interspecies hybrids resulting in elevated oil production and reduced susceptibility to diseases.

## 1. Introduction

The Moringaceae is a monogeneric family consisting of 13 species distributed throughout the dry tropics of the old world (Olson and Carlquist, 2001). The most widely distributed and economically important species is *Moringa oleifera* Lam. (*M. oleifera*); a highly utilized tree species, planted as a tree crop throughout the dry tropics in Asia, Africa and America (Ramachandran et al., 1980; Somali et al., 1984) with an unfulfilled potential in the subtropics (Palada, 1996). Also known as the "Miracle Tree", *M. oleifera* is a multi-purpose plant with a tremendous variety of potential uses including seed oil used for food, cosmetics and bioenergy (Sengupta and Gupta, 1970; Ramachandran et al., 1980; Kleiman et al., 2008; Rashid et al., 2008), seed powder used as a natural coagulant for low-cost water purification (Jahn et al., 1986), leaves provide an excellent feedstock for biogas (Sabale et al., 2008) and animal feed (Cohen-Zinder et al., 2016), various plant parts used as medicines (Caceres et al., 1991; Holst, 2000), tree bark and gum used in tanning hides (Emmanuel et al., 2011), mature plants grown as ornamentals, for live fencing and for paper pulp (Babu and Rajasekaran, 1991).

The second most economically important species is *Moringa peregrina* (Forssk.) Fiori. (*M. peregrina*); an endangered plant endemic to the desert regions of the Arab peninsula and Northern Africa (Hegazy et al., 2008). It is one of the most economically important medicinal plant in Egyptian markets (Abd El-Wahab et al., 2004). The seeds of *M. peregrina* provided oil for food and cosmetics in antiquity, as far as the time of the pharaohs, and is currently in demand for folk medicines (Migahid, 1978 Wilkinson, 1998). The plant is highly drought and heat tolerant (Hegazy et al., 2008) and in contrast to *M. oleifera* it is essentially a wild species and its commercial value has rarely been considered (Somali

*Abbreviations:* GC, Gas chromatography; HSD, Honest significant difference; *M. oleifera*, *Moringa oleifera*; *M. peregrina*, *Moringa peregrina*; NIRS, Near infrared spectroscopy; NPK, Nitrogen phosphorus and potassium; SEC, Standard error of calibration; SECV, Standard error of cross validation; SEP, Standard error of prediction

* Corresponding author.
*E-mail address:* yiftachv@volcani.agri.gov.il (Y. Vaknin).

http://dx.doi.org/10.1016/j.scienta.2017.07.039
Received 12 December 2016; Received in revised form 23 July 2017; Accepted 24 July 2017
Available online 30 July 2017

et al., 1984).

Most breeding efforts on *M. oleifera* have been concentrated at the production of high yields of edible and fleshy green fruits/pods with few varieties reported in India, including 'Jaffna', 'Chauakacheri Murunga', 'Chem', 'Kadu', and 'Palmurungai', 'GKVK-1', 'Dhanraj', 'CO-1', 'KDM-1', 'Bhagya', 'Amar-32' and 'PAVM-1' (Ramachadran et al., 1980; Morton, 1991; Saini et al., 2013), and the commercially available varieties Periyakalum-1, 'PKM-1' and 'PKM-2' (Lalas and Tsaknis, 2002; Saini et al., 2013). Several breeding efforts have also been made at the development of high seed-oil yielding *M. oleifera* varieties as feedstock for edible oil, with most efforts concentrated on varieties that were previously selected for edible pods ('PKM-1'; Lalas and Tsaknis, 2002; Ayerza, 2011, 2012) or on indigenous genotypes to the Indian subcontinent (Anwar and Bhanger, 2003) and Africa ('Mbololo'; Tsaknis et al., 1999, and 'Congo-Brazzaville'; Nzikou et al., 2009; Ayerza, 2011). The development of *M. oleifera* seed-oil varieties as feedstock for biodiesel production was also considered (Rashid et al., 2008; Schil, 2008; Kafuku and Mbarawa, 2010; Wakil et al., 2014).

The potential of *M. oleifera* as a seed-oil crop under Mediterranean conditions was evaluated on rare and sporadic small projects such as "Mediterranean Moringa", a pioneer project to adapt *M. oleifera* trees using permaculture to the Mediterranean climate in Mallorca, Spain (PERMACULTURE WORLDWIDE NETWORK, 2015) with promising, yet, preliminary results. *Moringa peregrina*, to the best of our knowledge, remains unexplored under such conditions. The main aim of this research was to evaluate the potential of the tropical *M. oleifera* and the arid *M. peregrina* as seed-oil as well as seed-protein crops under Mediterranean conditions.

## 2. Materials and methods

### 2.1. Seed trait analyses

Measurements of seed, oil and protein traits are vital for the estimation of seed yield and seed quality, however, such measurements, are expensive, require handling of unpleasant chemicals, and are relatively time consuming. Therefore, concurrent with the establishment of an evaluation plot, we developed a quick and nondestructive NIRS (Near-infrared reflectance spectroscopy) technology to estimate seed traits such as seed weight, seed oil content, seed protein content and fatty acid composition, currently used in a number of crops such as wheat, maize, sorghum and soybeans (Shenk and Westerhaus, 1993; Tallada et al., 2009; Kandala et al., 2012).

To ensure maximal variation, we collected seeds from twelve *M.* oleifera accessions ('MOHZ1' – 'MOHZ6' from Kibbutz Hazerim in the Negev desert (31°14′N, 34°43′E), 'MOSG' and 'MOSP' from Kibbutz Samar in the Arava Valley (29°49′N, 35°1′E), 'MOVP', 'MOPP2008', 'MOPP2009' and 'MOLH1' a progeny of an old genetic line brought to Israel > 50 years ago by immigrants from Kochi, southwestern India; 9°55′N, 76°11′E) and two *M. peregrina* accessions ('MP' from Kibbutz Samar in the Arava Valley (29°49′N/35°1′E) and 'MP2009' from Volcani Center; 31°59′N, 34°49′E) (~10 seeds from each accession to a total of 150 seeds). One hundred and twenty eight *M. oleifera* seeds were used, with 100 assigned to the calibration set, and the remaining 28 randomly selected seeds were reserved for the validation set. The seeds were weighed before and after their hulls were removed. Intact kernels were scanned at NIR wavelengths between 1104 and 2492 nm, in 2-nm increments, with a Foss NIRSystems 5000 NIR reflectance (R) monochromator spectrometer (Foss Tecator, Hoganas, Sweden) (For a detailed description see Vaknin et al., 2011). The coefficient of determination $R^2$ and the standard errors of calibration (SEC) and of cross validation (SECV), and standard errors of prediction (SEP) of external validations, served as indicators of prediction quality. We also attempted to use the calibration equations developed for the 128 *M. oleifera* samples in order to predict an additional external set of 22 *M. peregrina* kernels.

Following the NIRS scan of both *Moringa species*, each kernel was ground manually with a mortar and pestle and transferred into a 2-mL pre-weighed Eppendorf vial, which was then re-weighed. A 2-mL aliquot of hexane was added to each vial; the vials were placed in a 360° rotating mixer (Intelli-Mixer RM-2; ELMI, Ltd., Russia) for 60 min, and then centrifuged at 10,000 rpm for 20 min. The supernatant was decanted into a 2-mL pre-weighed Eppendorf vial and placed inside a fume hood with the cap open to enable the solvent to evaporate. An additional 2 mL of hexane was added to the precipitated ground seed in each vial and the whole process was repeated. Two successive solvent extractions were found to be sufficient to extract > 99% of the oil content. The extracted oils were analyzed for fatty acid composition using GC analysis after methyl-esterification (For a detailed description see Vaknin et al., 2011). The seed cake remaining after solvent extraction was further analyzed for nitrogen content by the micro-Kjeldahl method (Bremner, 1965), and protein content was estimated by multiplying the N content by a conversion constant of 5.75 (Mosse, 1990). The resulting calibration equations were further used for the analyses of seed oil and protein content in the evaluation plot, described hereinafter.

### 2.2. Study site and evaluation plot

In June (summer) 2011, we established an evaluation plot of *M. oleifera* and *M. peregrina* seedlings at Volcani Center, Rishon Lezion, Israel (31°59′N, 34°49′E). Local climatic conditions are classified as Mediterranean, with hot dry summers (maximal and minimal temperature and relative humidity; 32 and 22 °C and 95 and 24%, respectively) and mild to cool wet winters (maximal and minimal temperature and relative humidity; 18 and 8 °C and 100 and 21%, respectively) with an average of 524 mm rainfall per year (Israel Meteorological Service). The planting density was 3 × 3 m. Seedlings from *M. oleifera* (local progenies of 'PKM-2') and *M. peregrina* (a local genetic line collected in the wild in the harsh desert of the Arava Valley; 'MP'), were planted in a random block design, with two blocks, each containing both species, 5 plants per species, randomly distributed along two rows (A total of 10 plants per species). Irrigation and fertilization were applied via drip irrigation throughout the summer and early autumn during the morning hours at ~5–7 am (30 cubic meters a day per hectare), with NPK fertilizer 20-20-20 1 gr per 10 L.

### 2.3. Bloom phenology and seed-oil and seed-protein production under Mediterranean conditions

Bloom phenology of six randomly chosen trees, from each species, at the evaluation plot, was documented throughout the year starting in spring 2012 until spring 2016. In winter 2012, the young trees were pruned down to 1 m height to spur on branching and subsequently elevate yield. In each tree, the number of flowering inflorescences, during summer and early autumn, was noted from 2014 to 2015. To estimate the number of flowers per tree and fruit set, three random branches were marked and the number of inflorescences, the number of flowers per inflorescence and the number of maturing pods was noted. From each bloom period, 10 mature pods, per tree, were sampled and measurements of pod length, seed number per pod and seed weight were taken. Seed weight and oil and protein contents were estimated using the specially developed NIRS method described above. Oil and protein yield per hectare were calculated by multiplying average oil yield per tree with number of trees per hectare.

### 2.4. Statistical analyses

Statistical analyses were performed with the JMP 12.0.1 software (SAS Institute Inc., Cary, NC, USA), according to Sokal and Rohlf (1994). The effects of seed source (various *Moringa* accessions or *M. oleifera* vs. *M. peregrina*) on kernel weights, kernel-oil content, kernel-