INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
17–0540/2018/20–1–77–84
DOI: 10.17957/IJAB/15.0401
http://www.fspublishers.org

Full Length Article

Seed and Oil Yield Potential and Oil Quality of Vegetatively Propagated Moringa Landraces of Punjab, Pakistan

Muhammad Idrees Faisal[1*], Shahzad Maqsood Ahmed Basra[1], Irfan Afzal[1] and Abdul Wahid[2]
[1]*Department of Agronomy, University of Agriculture, Faisalabad, 38040, Pakistan*
[2]*Department of Botany, University of Agriculture, Faisalabad, 38040, Pakistan*
[*]For correspondence: idrees.1875@gmail.com

Abstract

Moringa (*Moringa oleifera* L.) seed oil has been recognized as premium quality oil. A field trial was conducted to explore seed and oil yield potential and oil quality of vegetatively propagated selected moringa landraces. Six locations in Punjab [Faisalabad (FSD), Rahimyar Khan (RYK), Bahawalpur (BWP), Layyah (LAY), Mulan (MUL) and Khanewal (KWL)] were selected and five mature stem cuttings from each location were planted at a farmer's field in district Khanewal (30° 17' 20.0724" N and 72° 0' 17.4852" E) for study. Data regarding seed and oil yield and oil quality were taken for two growing seasons (2014 and 2015). Seed yield and yield attributes of landraces were significantly ($P<0.05$) different during both years of study. Maximum pod length, pod weight, number of seeds per pod, seeds weight per pod, number of pods per tree, seeds weight per tree, oil yield per tree and oil yield per hectare were achieved by RYK landrace during both growing seasons (year 2014 and 2015). Oil physio-chemical characteristics, oxidative stability, fatty acid and sterol composition had little variability among all landraces. Non-significant difference among fatty acid and sterol composition were observed in oil of all landraces. Monounsaturated fatty acid, oleic acid was main fatty acid in seed oil of all landraces (>71% of total fatty acid) and β-sitosterol was the predominant plant sterol (more than 55% of total sterol composition) in oil of all selected landraces. On basis of above results it is concluded that RYK landrace produced highest seed and oil yield during both growing seasons while oil quality (Fatty acid and sterol composition) of selected landraces were similar. © 2018 Friends Science Publishers

Keywords: *Moringa oleifera*; Seed yield components; Oleic acid; Sterol composition

Introduction

Despite an agricultural country, Pakistan is chronically deficient in edible oil. During year 2015–2016 local oil production in Pakistan was 0.462 million tones which contributed only 14% of total demand. Pakistan is spending huge foreign exchange (US$ 2.710 billion during year 2015–2016) to import edible oil for meeting the deficit (Govt. of Pakistan, 2015–2016). It is the dire need of time to explore new oil seed crops with potential to fill the huge gap between production and consumption of edible oil within the country. Use of an oil yielding tree can be a potential option to increase local oil production in Pakistan. The scientists have focused on olive oil production where a limited success was achieved in olive tree. It increases the demand to explore new oil yielding trees which can be an alternate of oil production. *Moringa oleifera* is a good option which is native to Pakistan and its seeds have 30–40% premium quality oil (Anwar *et al.,* 2006; Rahman *et al.,* 2009; Leone *et al.,* 2015).

Moringa oleifera locally known as "Sohanjna" belongs to family Moringaceae with 13 known species. Plant is native to Sub-Himalayan tracks of India, Pakistan, Afghanistan and Bangladesh and now more widely distributed through Philippines, Combodia, African and Central and North America (Foidl *et al.,* 2001; Nouman *et al.,* 2013; Choudhary *et al.,* 2016). Moringa grows naturally at elevation up to 1000 m and ranges in height from 5–12 m (Parrotta, 2001; Choudhary *et al.,* 2016). Two species of family Moringaceae (*M. concanensis* and *M. oleifera*) have been reported in Pakistan (Verdcourt, 1985). Due to less attention given to *M. concanensis*, it is found rarely in Pakistan and limited only to distant desert areas of Tharparkar Desert in Sindh province while *M. oleifera* is very common, grown and cultivated in Punjab, Sindh and Khyber Pakhtunkhwa province (Anwar *et al.,* 2006).

Moringa is versatile tree which can be grown in subtropical to tropical regions of world with temperature around 25–40°C and can tolerate temperature up to 48°C (HDRA, 2002). It is a fast growing and drought tolerant tree that grows well in poor soils under the wide range of pH (5.0–9.0) and can tolerate salinity up to 12 dS m^{-1} (Palada and Chang, 2003; Thurber and Fahey, 2009; Nouman *et al.,* 2012, 2014a, b). Moringa is propagated through sexual (seed) or asexual (stem cutting) methods. Stem cutting is a very common method of moringa propagation in semi-arid

To cite this paper: Faisal, M.I., S.M.A. Basra, I. Afzal and A. Wahid, 2018. Seed and oil yield potential and oil quality of vegetatively propagated moringa landraces of Punjab, Pakistan. *Int. J. Agric. Biol.*, 20: 77–84

region of Pakistan and dry tropical region of India. During the rainy season, stem cuttings (1–2 m long and 4–15 cm diameter) are planted by burying 1/3 of length in soil (Palada, 1996; Animashaun, 2013). Trees propagated through stem cuttings may produce flowers and pods within one year of planting (Leone *et al.,* 2015; Hassanein and Al-Soqeer, 2017). Depending upon genotype and climate, tree bears flowers and fruits once or twice in a year. Rajangam *et al.* (2001) reported that seed production of moringa varies with soil, climate and landrace. Ndubuaku *et al.* (2014) reported that seed yield varies 4–24 t ha^{-1} depending upon environmental condition and soil type. Seed and oil yield per tree of moringa differed depending upon one or more factors such as temperature, soil type, light and nutrient availability and variety (Ayerza, 2011, 2012).

Fully matured dried seeds of moringa are round in shape, and kernels are covered with whitish or brownish semipermeable hull (Fig. 1). Seeds contain significant amount of oil (30–40%) commercially known as "Ben oil" which is rich in oleic acid, sterols and tocopherols (Lakshmipriya *et al.,* 2016). Among monounsaturated fatty acid, oleic acid (18:1) is the predominant fatty acid (contributes more than 70% in total fatty acid composition) while polyunsaturated fatty acids are less than 1%, which make oil less prone to oxidative damages as compared to other high oleic acid edible oils (sunflower, safflower and almond oil) (Anwar and Bhanger, 2003). Anwar *et al.* (2007) reported that, proper blending of moringa oil with traditional edible oils (palm, soybean and sunflower oil) improves physio-chemical characteristics and oxidative stability of oils. Seed oil contents varied depending upon variety, climate and extraction method (Lalas and Tsaknis, 2002; Anwar and Bhanger, 2003; Ogunsina *et al.,* 2014). Fatty acid composition of moringa oil has both edible and non-edible uses. It can be used in cooking as a substitute of olive oil, as lubricant and also in perfumes (Lalas and Tsaknis, 2002; Fahey, 2005). Moringa oil also used in cosmetic and source of biodiesel while the seedcake is excellent source green manure or fertilizer (Lakshmipriya *et al.,* 2016).

Previously, moringa seed oil yield and quality have been reported for drought and salinity prone areas with variation in oil quality while no comparison has been studied to find out a promising landrace which can contribute maximum in providing good quality moringa seed oil. Moreover, it has been previously reported for moringa to provide variation in its bioactive compounds nutritional quality among different moringa landraces and genotypes which can give reason to investigate oil yield and potential of moringa seeds among difference moringa landraces to find out promising one (Nouman *et al.,* 2016; Olson *et al.,* 2016). Anwar *et al.* (2016) reported variation in oil yield and quality of moringa seeds which invites the idea to propagate different moringa landraces on same piece of land for comparing oil yield and quality. By this, variation among moringa seed oil yield and quality among moringa landraces of Punjab, Pakistan can be explored keeping soil and environmental factors same for cultivated landraces. Considering the above, present study was designed to explore seed and oil yield potential and oil quality of vegetatively propagated moringa landraces of Punjab, Pakistan.

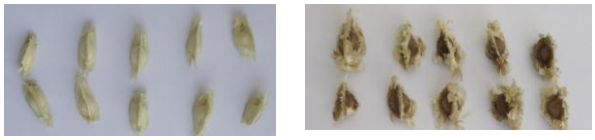

Fig. 1: White and brown seeds of Moringa

Materials and Methods

Trees Selection and Propagation

On the basis of morphological similarities confirmed by plant taxonomist, four moringa trees (at least five years old on the basis of farmer's knowledge) were tagged at six locations of Punjab, Pakistan [(Faisalabad (FSD), Rahimyar Khan (RYK), Bahawalpur (BWP), Layyah (LAY), Multan (MUL) and Khanewal (KWL)]. Five mature stem cuttings (180–185 cm length and 12–15 cm diameter), from tagged trees of each selected location were obtained and propagated on March 30, 2013 at a farmer's field at Chak 76/10-R (30° 17' 20.0724" N and 72° 0' 17.4852" E) district Khanewal in clay loam soil. Stem cuttings were planted in rows in square at 4.5 m × 4.5 m (494 plants ha^{-1}). Stem cuttings were planted by burying 1/3 length of stem cuttings into compost filled pits, after that pits were irrigated with canal water immediately. Subsequent irrigations were done fortnightly with canal and tube well water rotations until second harvest. Pits were supplemented with nitrogen and phosphorous @150:100 g/pit in two splits in a year followed by irrigation. Data regarding seed and oil yield and oil quality were taken for two growing seasons (May 2014 and 2015).

Soil Analysis

Soil samples of the experimental site were taken from three different depths (10, 20 and 30 cm), and composite sample was prepared and sent to soil and water testing laboratory of Fauji Fertilizer Company Multan to analyze following physio-chemical characteristics of soil.

Characteristic	Unit	Value
Textural class	-	clay loam
Saturation percentage	%	31.5
pH	-	7.9
EC	dS m^{-1}	1.32
Available phosphorus	mg kg^{-1}	3.27
Extractable potassium	mg kg^{-1}	78
Organic matter	%	0.74
Total nitrogen	%	0.7

Pod Harvesting and Seed Yield Data Collection

Mature dry pods (when turned brown and dry) were harvested from each tree manually during month of May 2014 and 2015. Pod length (cm), pod weight (g), number of seeds pod^{-1}, seed weight pod^{-1} (g), number of pods $tree^{-1}$, 1000 seeds weight (g), seed weight $tree^{-1}$ (kg) oil yield $tree^{-1}$ and oil yield ha^{-1} were determined. Data were taken for two growing seasons (2014 and 2015).

Oil Extraction and Degumming

After removing of the seed coat, 200 g seeds of each replication were grind and then fed into Soxhlet extractor filled with a 1 L round bottom flask and a condenser. The extraction was executed on a water bath for 6 h with 0.6 L of *n*-hexane. After extraction, the solvent was distilled off under vacuum in a rotary evaporator (EYELA, N.N. Series, Rikakikai Co. Ltd. Tokyo, Japan). The recovered oil samples were degummed by heating at 70°C on water bath. Water was added 18% to the final volume and mixed well with a glass rod. After cooling, the mixture was centrifuged @3000 rpm for 12 min in an automatic refrigerated centrifuge (Sorval RC-3). The degummed oil was dried over anhydrous sodium sulfate, filtered, and kept in separate sealed bottles under refrigeration (0–4°C) before use for analysis (Tsaknis *et al.,* 1998).

Analysis of Extracted Oils

Determination of Physical and Chemical Characteristics of Oil

Recommended AOCS methods were used to determine refractive index (method Cc 7-25), saponification value (method Cd 3-25), iodine value (method Cd 1-25), free fatty acid (method Ab 5-49), peroxide value (method Cd 8b-90), *p*-anisidine value (method Cd 18-90) and specific extension (method Ch 5-91) (AOCS, 2009).

Fatty Acid Composition

The IOC official method (COI/T.20/Doc. no. 33-2015) was carried out for fatty acid composition (IOC, 2015). In a 12 mL screw-top test tube 0.01 g oil sample was weighed and then 0.4 mL toluene was added and vortex. Then 3 mL of methanol and 0.6 mL of methanol:HCl mixture (80:20, v/v) was added and sample was kept at 40ºC overnight in a heat stock. Then 1.5 mL of hexane and 1 mL of naonpure water was added and vortexed the sample. The upper layer of methyl esters was decanted and anhydrous sodium sulfate was added to dry out water residue. The clear solution was transferred into GC vials for injection.

GC-FID analysis was conducted on a Varian 450-GC (Varian, Palo Alto, CA, U.S.A.). The injection volume was 0.2 µL. Helium at a flow rate of 1.5 mL per min was used as carrier gas. Separation of individual fatty acid compositions was achieved by using a 30 m × 0.25 mm × 0.1 µm DB-5 capillary column (Agilent Technologies, Santa Clara, CA, USA). The injector temperature was set at 240°C. The GC oven program was initially held isothermally at 80°C for 5 min; then ramped at 10°C per min to 230°C and held for 5 min; finally ramped at 20°C per min and held for 10 min. The detector temperature of FID was 260°C. The detector gas consisted of air (flow rate: 300 mL per min), hydrogen (flow rate: 30 mL per min), and helium make up gas (flow rate: 25 mL per min). Peak was identified by retention times using a FAME mix as standards.

Sterol Composition

Sterols were determined following IOC Official Method (COI/T.20/Doc. No. 30/Rev.1 2013) (IOC, 2013). Analysis was carried out on a Varian 450-GC (Varian, Palo Alto, CA, U.S.A.) equipped with a methyl phenyl polysiloxanes coated capillary column OV-17 (30 m 0.25 mm, 0.20-ím film thickness) and a flame ionization detector (FID). The column was operated at a 260°C while injector and FID temperatures were set at 270 and 280°C, respectively. Extra pure Helium flow at the rate of 20-35 cm per second was used as carrier gas. The internal standard used was α-cholestano was used as internal standard, while pure sterol standard mixture was used for identification and quantification of unknown sterol components.

Statistical Analysis

The experiment was laid out in randomized complete block design (RCBD) with four replications. Recorded data were analyzed using Fisher's analysis of variance technique and comparison of treatment means were done by Tukey's test at 5% probability level (Steel *et al.,* 1997). In case of no difference ($P>0.05$) between the values of different locations, the latter were excluded from the analysis of data.

Results

Seed Yield and Yield Components

All selected landraces cultivated through stem cuttings at Khanewal produced flowers and pods within a year except Faisalabad. Seed yield and yield attributes were significantly ($P>0.05$) affected by landraces during both years of study (Table 1; Table 2). Rahimyar Khan landrace trees attained highest mean pod length (46.05 cm), pod weight (11.44 g), number of seeds per pod (18.35) and seeds weight per pod (4.84 g) during both years of study (Table 1). Maximum number of pods per tree were harvested from Khanewal, which was at par with Rahimyar Khan, Bahawalpur and Multan while, the minimum in Layyah during year 2014. Similar trend was recorded during year 2015 (Table 1). Thousand seeds weight, seeds weight per tree was highest in

Table 1: Differences in seed yield and yield components of moringa trees of different landraces during year 2014 and 2015

Locations	Pod length (cm)		Pod weight (g)		Number of seeds per pod		Seeds weight pod^{-1} (g)		Number of pods tree^{-1}	
	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
FSD	n/a*	40.67 d	n/a	5.57 c	n/a	17.25 ab	n/a	2.11 d	n/a	292 a
RYK	46.16 a	45.95 a	11.32 a	11.55 a	18.75 a	17.96 a	4.90 a	4.77 a	235 ab	293 a
BWP	41.26 b	42.71c	8.27 b	8.22 b	16.96 ab	16.64 b	3.17 b	3.28 bc	239 ab	268 ab
LAY	42.89 ab	43.39bc	8.22 b	7.56 b	14.42 c	16.75 b	3.10 b	3.07 c	223 b	256 b
MUL	41.17 b	43.45 bc	7.56 b	8.23 b	16.00 bc	16.79 b	3.21 b	3.36 bc	250 a	283 ab
KWL	41.94 b	44.63 ab	7.99 b	7.98 b	17.20 ab	17.29 ab	3.34 b	3.45 b	253 a	295 a
CVC	4.17	1.54	0.70	0.75	2.53	0.69	0.28	0.29	19.59	28.82

Table 2: Differences in seed yield and yield components of moringa trees of different landraces during year 2014 and 2015

Locations	Seed weight tree^{-1} (kg)		1000 seeds weight (g)		Seed oil content (%)		Oil yield tree^{-1} (kg)		Oil yield ha^{-1} (kg)	
	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
FSD	n/a*	0.63 f	n/a	130.65 e	n/a	36.80	n/a	0.23 f	n/a	107.84 f
RYK	1.15 a	1.39 a	258.19 a	254.10 a	38.36	37.96	0.44 a	0.53 a	210.98 a	253.01 a
BWP	0.76 cd	0.87 d	222.93 bc	224.47 c	37.47	36.91	0.29 cd	0.32 d	135.22 c	154.60 d
LAY	0.69 d	0.79 e	213.27 c	210.03 d	37.44	37.11	0.26 d	0.28 e	120.77 d	135.69 e
MUL	0.80 bc	0.95 c	221.27 bc	216.55 cd	37.51	37.13	0.30 bc	0.36 c	143.77 bc	168.54 c
KWL	0.85 b	1.00 b	229.98 b	236.67 b	38.43	38.14	0.33 b	0.39 b	155.30 b	182.76 b
CVC	0.08	0.04	16.32	9.90	1.39	1.41	0.03	0.02	13.64	10.38

Table 3: Differences in physical and chemical characteristics of seed oil of moringa trees of different landraces during year 2014 and 2015

Locations	Refractive index (40°C)		Saponification value (mg of KOH/g of oil)		Iodine value (g of I/100 g of oil)		Free fatty acid (% oleic acid)	
	2014	2015	2014	2015	2014	2015	2014	2015
FSD	n/a*	1.4627	n/a	179.66 a	n/a	69.14	n/a	0.45
RYK	1.4627	1.4632	183.33 a	179.24 ab	68.65 ab	68.80	0.52	0.48
BWP	1.4621	1.4623	177.59 b	176.30 abc	68.2 ab	69.65	0.45	0.45
LAY	1.4623	1.4625	180.83 ab	176.86 abc	68.03 b	71.20	0.44	0.42
MUL	1.4626	1.4626	178.02 b	175.45 c	69.14 a	69.71	0.49	0.43
KWL	1.4621	1.4623	178.02 b	175.88 bc	68.06 b	71.81	0.45	0.41
CVC	n/a	1.4627	5.26	3.52	0.99	8.66	0.21	0.16

FSD (Faisalabad); RYK (Rahimyar Khan); BWP (Bahawalpur); LAY (Layyah); MUL (Multan); KWL (Khanewal)

* n/a (not availed), during year 2014, trees of FSD locations didn't produce seed so value is missing,

CVC (critical value of comparison)

In a column, means with the same letter or means with no lettering are not statistically different (P <0.05)

Rahimyar Khan landrace during year I while, similar trend was observed during year II (Table 2). Moreover, maximum oil yield per tree (0.49 kg) and per hectare (231.99 kg) were attained by Rahimyar Khan landrace.

Physio-chemical Characteristics of Oil

Results of various physio-chemical characteristics of seed oil are given in Table 3. Refractive index and free fatty acids of oil were not affected by landraces (P>0.05) during both years of study (Table 3). However, saponification value of oil was significantly (P<0.05) different among landraces during both years of study. Maximum saponification value (183.33 mg of KOH/g of oil) was observed in Rahimyar Khan landrace, which was at par with Layyah (180.83 mg of KOH/g of oil), while the minimum in Bahawalpur (177.59 mg of KOH/g of oil) during year 2014. During year 2015, highest saponification value was noticed in Faisalabad (179.66 mg of KOH/g of oil), which was at par with Rahimyar Khan, Bahawalpur and Layyah while minimum in Multan (175.45 mg of KOH/g of oil). Iodine value of oil from landraces was significantly (P<0.05) different during year I while non-significant during year II (Table 3). During year I, maximum iodine value of oil was noted in Multan tree, which was at par with Rahimyar Khan and Bahawalpur while minimum in Layyah.

Oxidative State of Oil

Peroxide value of oil was significantly (P<0.05) different among landraces during year 2014 while non-significant during year 2015 (Table 4). Maximum peroxide value was recorded in Layyah (1.88 meq of O_2 kg^{-1} of oil) landrace oil which were at par with Bahawalpur while the minimum in Khanewal (1.20 meq of O_2 kg^{-1} of oil), which was at par with Multan and Rahimyar Khan during year 2014. Maximum peroxide value was noticed in Rahimyar Khan trees during year 2015. The *p*-anisidine value and specific extensions of oil were at par in all landraces during both growing seasons.

Fatty Acids Composition

Non-significant (P>0.05) differences among fatty acid composition of selected landraces were observed during both years of study (Table 5). The content of saturated fatty acids, palmitic (16:0), stearic (18:0), arachidic (20:0) and behenic (22:0) acids were ranged from 9.22–11.01, 3.63–5.37, 2.51–3.34 and 3.66–4.85%, respectively during both years of study (Table 5). Oil of all landraces was found to contain high level of monounsaturated ω-9 fatty acid (oleic acid 18:1). The content of oleic acid was ranged from 71.73–72.85% during both growing seasons, while small amount of palmitoleic (16:1), linoleic (18:2), linolenic (18:3) and gondoic (20:1) acid were also observed and ranged from 1.70–2.03, 0.62–0.76, 0.09–0.11 and 2.20–2.45%, respectively.

Sterols Composition

Sterols compositions of oil of selected landraces were statically similar during both years of study (Table 6). The β-sitosterol was the main plant sterol in oil of all landraces which accounted for 55.14–57.23% of total sterols composition. The content of other major sterols compounds, campesterol, stigmasterol and Δ^5-avenasterol were ranged from 16.42–17.80, 15.94–18.74 and 4.97–6.09%, respectively during both years of study. A small amount of cholesterol, 24-methylenecholesterol, campestanol,

Table 4: Oxidative state of seed oil of moringa trees of different landraces during year 2014 and 2015

Locations	Peroxide value (meq of O_2 kg^{-1} of oil)		K232 ($K^{1\%}_{1cm}$)		K268 ($K^{1\%}_{1cm}$)		*p* anisdine-value	
	2014	2015	2014	2015	2014	2015	2014	2015
FSD	n/a*	1.25	n/a	1.37	n/a	1.65	n/a	0.40
RYK	1.43 bc	1.45	1.30 c	1.49	1.67	1.65	0.42	0.40
BWP	1.60 ab	1.45	1.75 ab	1.38	1.75	1.78	0.44	0.43
LAY	1.88 a	1.05	1.94 a	1.48	1.67	1.69	0.42	0.42
MUL	1.48 bc	1.05	1.71 b	1.67	1.72	1.65	0.43	0.41
KWL	1.20 c	1.40	1.38 c	1.37	1.66	1.65	0.42	0.39
CVC	0.37	0.80	0.22	0.38	0.11	0.03	0.17	0.03

FSD (Faisalabad); RYK (Rahimyar Khan); BWP (Bahawalpur); LAY (Layyah); MUL (Multan); KWL (Khanewal)
* n/a (not availed), during year 2014, trees of FSD locations didn't produce seed so value is missing,
CVC (critical value of comparison)
In a column, means with the same letter or means with no lettering are not statistically different (P <0.05)

Table 5: Fatty acids composition (g per 100 g of fatty acids) of seed oil of different moringa landraces during year 2014 and 2015

Fatty acids	FSD		RYK		BWP		LAY		MUL		KWL		CVC	
	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
Palmitic acid (16:0)	n/a	9.87	9.27	10.85	10.1	10.01	9.22	10.23	9.48	10.58	9.48	11.01	2.61	2.51
Palmitoleic acid (16:1)	n/a	1.98	1.7	1.94	1.85	2.06	1.85	2.00	1.61	1.97	1.72	1.94	1.64	0.5
Stearic acid (18:0)	n/a	4.63	5.37	3.9	4.61	4.31	4.32	4.13	5.16	3.83	5.16	3.63	3.91	1.19
Oleic acid (18:1)	n/a	71.9	71.79	72.27	72.7	71.79	72.53	72.06	72.36	72.84	71.73	72.85	2.95	1.39
Linoleic acid (18:2)	n/a	0.65	0.62	0.74	0.73	0.66	0.71	0.68	0.67	0.73	0.64	0.76	0.58	0.2
Linolenic acid (18:3)	n/a	0.09	0.1	0.09	0.1	0.1	0.09	0.09	0.1	0.09	0.11	0.09	0.05	0.04
Arachidic acid (20:0)	n/a	3.12	3.34	2.68	2.89	3.04	2.87	2.91	3.2	2.65	3.28	2.51	3.29	0.77
Gandoic acid (20:1)	n/a	2.35	2.2	2.37	2.2	2.41	2.4	2.38	2.15	2.43	2.21	2.45	0.4	0.19
Behenic acid(22:0)	n/a	4.29	4.47	4	3.74	4.46	4.85	4.34	4.15	3.78	4.51	3.66	1.37	1.41
Lignoceric acid (24:0)	n/a	1.11	1.13	1.16	1.08	1.16	1.16	1.17	1.13	1.09	1.17	1.1	0.69	0.18

Table 6: Sterol composition (%) of seed oil of different moringa landraces during year 2014 and 2015

Sterol composition	FSD		RYK		BWP		LAY		MUL		KWL		CVC	
	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
Cholesterol	n/a	0.14	0.13	0.13	0.13	0.14	0.14	0.13	0.15	0.13	0.13	0.12	0.04	0.09
24-Methylenecholesterol	n/a	0.21	0.30	0.25	0.36	0.27	0.26	0.27	0.21	0.25	0.31	0.25	0.32	0.16
Campesterol	n/a	16.62	17.35	17.73	16.69	16.76	16.63	16.81	16.42	17.74	16.41	17.80	1.92	3.06
Campestanol	n/a	0.07	0.05	0.07	0.06	0.10	0.10	0.10	0.07	0.07	0.05	0.07	0.13	0.14
Stigmasterol	n/a	17.74	18.07	17.09	18.74	16.66	17.98	16.82	18.22	16.68	18.31	15.94	1.82	4.27
Clerosterol	n/a	0.56	0.46	0.58	0.46	0.53	0.50	0.53	0.50	0.56	0.45	0.57	0.15	0.19
β-Sitosterol	n/a	55.83	55.51	55.15	55.48	57.14	56.70	56.78	57.26	55.80	55.94	56.44	4.33	2.18
Δ^5- Avenasterol	n/a	6.00	6.09	5.70	4.97	5.53	5.35	5.65	5.76	5.57	5.62	5.65	3.03	1.27
$\Delta^{5\text{-}24}$-Stigmastadienol	n/a	0.47	0.36	0.52	0.37	0.47	0.45	0.48	0.34	0.50	0.35	0.51	0.26	0.12
Δ^7-Stigmastadienol	n/a	1.14	0.54	1.26	1.23	1.16	0.71	1.17	0.30	1.23	1.10	1.25	1.89	0.26
Δ^7. Avenasterol	n/a	1.21	1.13	1.50	1.46	1.26	1.18	1.28	0.76	1.47	1.33	1.49	0.57	0.66

FSD (Faisalabad); RYK (Rahimyar Khan); BWP (Bahawalpur); LAY (Layyah); MUL (Multan); KWL (Khanewal)
* n/a (not availed), during year 2014, trees of FSD locations didn't produce seed so value is missing,
CVC (critical value of comparison)

clerosterol, Δ^{5-24}-stigmastadienol, Δ^{7}-stigmastadienol and Δ^{7}-avenasterol were also observed in seed oil of all landraces (Table 6).

Discussion

Moringa is native to Pakistan but its seed and oil yield potential and oil quality of different regions yet to be explored. Selected moringa landraces propagated through stem cuttings produced seed within year except Faisalabad. However, during second year all landraces produced seeds. Rahimyar Khan landrace attained highest seed and oil yield during both growing seasons while oil quality of all landraces were similar.

Ramachandran *et al.* (1980) reported that moringa tree propagated through stem cutting may produce seeds within one year. Moringa propagated through seeds produce poor fruits (pods) due to genetic diversity, as moringa is highly cross pollinated specie (Ramachandran *et al.,* 1980). Scientists reported that pod yield of moringa significantly affected by landraces (Rajangam *et al.,* 2001; Ndubuaku *et al.,* 2014). In present study, significant differences in seed yield and yield attributes of moringa landraces were found. Landrace selected from Rahimyar Khan excelled during both years of study and attained maximum pod length, pod weight, number of seeds per pod, seed weight per pod, number of pods per tree and ultimately seed weight per tree (Table 1; Table 2). Values of these seed yield and yield parameters were higher than the reported value of Ferrao and Ferrao (1970). Such variations among different moringa genotypes have been previously reported by Ayerza (2011). Moreover, seed oil contents of all landraces were similar during both years of study and their values were in range of 36.80–38.43% (Table 2). Seed oil contents were within the range of previously reported work in Pakistan and other countries (Anwar *et al.,* 2006; Anwar and Rashid, 2007; Orhevba *et al.,* 2013) and even higher than reported value of African moringa variety (Mbololo) (Tsaknis *et al.,* 1999) and conventional oilseed crops like cotton (15–24%), soybean (17–21%), safflower (25–40%) and mustard (24–40%) (Pritchard, 1991). Soil and environmental conditions may affect seed oil contents more than genotype (Ayerza, 2011). Muhl *et al.* (2014) also reported that inadequate water supply during reproductive and flowering stage ensures better fruit set and ultimately greater yield. Maximum oil yield per tree and oil yield per hectare were recorded in Rahimyar Khan trees during both years of experiment, which indicates that Rahimyar Khan landrace has more potential than other landraces. Rajangam *et al.* (2001) reported variation observed in seed and oil yield of moringa. Ndubuaku *et al.* (2014) also reported that, in Nigeria seed yield ranges between 4–24 tons per hectare depending upon climate, soil, available nutrients and precipitation.

On the other hand, physio-chemical characteristics of seed oil had little variability and comparable with the earlier reports of moringa seed oil (Table 3). Refractive index at 40°C (1.4622–1.4627), saponification value (176.74–181.28 KOH/g of oil), iodine value (68.80–71-81 g of iodine/100 g of oil) and free fatty acids (0.82–0.87% oleic acid) of selected landraces in this study are within same range as of earlier reported work in Pakistan, Kenya, Malawi and India (Tsaknis *et al.,* 1999; Lalas and Tsaknis, 2002; Anwar and Bhanger, 2003; Anwar *et al.,* 2006).

Peroxide value (PV), specific extinction and *p*-anisdine value (*p*-AV) are indicators of oxidative state of oil. These values in present study depict that moringa trees of different landraces can produce oil of good oxidative state. However, peroxide value of different landraces oil was higher than earlier reported value in Pakistan and wild trees of Malawi (Anwar and Bhanger, 2003; Anwar *et al.,* 2006) and less than Indian variety PKM-1 (Lalas and Tsaknis, 2002). Specific extinction indicates oxidative deterioration of oil. During both years of experiment, specific extinction values of oil were statically similar in all landraces. Values are within range of earlier reported work in Pakistan (Anwar and Bhanger, 2003; Anwar *et al.,* 2006) however, less than Indian variety PKM-1 and African variety Mbololo (Tsaknis *et al.,* 1998; Lalas and Tsaknis 2002).

Different landraces of moringa propagated through stem cuttings produced seed oil having similar in fatty acids composition (Table 5). The contents of saturated fatty acids ranged between 21.40–22.50%. High behenic acid content in oil is the reason why moringa oil commercially known as "Ben oil" or "Behen oil." Moringa oil contains high level of monounsaturated fatty acid. In seed oil of all landraces, oleic acid (ω-9) was major fatty acid (>71%), while polyunsaturated fatty acids (linoleic and linolenic acid) were less than 1% (Table 5). Amount of monounsaturated fatty acid (oleic acid) in moringa oil was higher than values of conventional oil seed crop like palm oil (45.6%), canola oil (57.4%) and soybean oil (24.8%) (Abdulkarim *et al.,* 2005). Current study revealed that moringa oil fall in the category of high oleic acid oil, and contains high monounsaturated to the saturated fatty acid ratio (MUFA/SFA). High MUFA/SFA is associated with health benefits and decreases chances of heart diseases (Schwingshackl and Hoffmann, 2014). Moringa seed oil also resembles with olive oil regarding fatty acid composition (Ramachandran *et al.,* 1980). Warner and Knowlton (1997) reported that moringa oil is more stable than other edible oils due to less oxidation both at ambient and high temperatures as it contains more monounsaturated and less polyunsaturated fatty acids. Anwar *et al.* (2007) reported that proper blending of moringa oil in high linoleic oils not only improves oil nutritional value but also improves oil stability to oxidation during cooking and deep frying. The content of PUFA (polyunsaturated fatty acid) in moringa oil was less than 1% in all landraces. Cosmetic industry use moringa oil over other high oleic acid source due to very low content of PUFA, oil is less prone to oxidation (Kleiman *et al.,* 2006).

There was no significant difference in sterol composition of oil from different landraces in the study. The

sterol fractions of the oil from cultivated landraces mainly comprised of campesterol, stigmasterol, β-sitosterol and delta 5-avenasterol. These four plant sterols comprised of 96% total sterols in all selected landraces. β-sitosterol was most important and predominant sterol (>55%) compound in oil of all landraces. β-sitosterol is involved in metabolism of cholesterol and reduces the LDL cholesterol level in blood and ultimately decreases risk of coronary heart diseases (Ras *et al.*, 2014). Gupta *et al.* (2011) also reported that β-sitosterol had an antidiabetic potential.

Sterol composition of moringa landraces of current study were more than 55%, which is higher than earlier reported work in Pakistan, India (PKM-1) and Kenya (Tsaknis *et al.*, 1999; Lalas and Tsaknis, 2002; Anwar and Bhanger, 2003; Anwar *et al.*, 2006).

Conclusion

Moringa landraces propagated through stem cuttings produced flowers and seeds within one year except Faisalabad (which produced seed yield during second year). Significant variability in seed production and oil yield was observed during both years of experiment. Maximum seed and oil yield were recorded in Rahimyar Khan landrace. However, physio-chemical characteristics, oxidative state and oil quality had little variability between landraces and their values are much comparable with reported work in Pakistan and other countries. It is concluded that Rahimyar Khan landrace has significantly higher seed and oil yield potential as compared to other selected landraces.

Acknowledgements

Authors acknowledge with thanks Ch. Muhammad Akram Sahu for providing land to conduct experiment at Chak # 76/10-R, Khanewal.

References

Abdulkarim, S.M., K. Long, O.M. Lai, S.K.S. Muhammad and H.M. Ghazali, 2005. Some physico-chemical properties of *Moringa oleifera* seed oil extracted using solvent and aqueous enzymatic method. *Food Chem.*, 93: 253–263

Animashaun, J., 2013. Prospects of Agriculture Enterprise for Sustainable Economic Development: Success Story of University of Ilorin Moringa Value-Addition Activities. *In*: *Proceedings of the 4th International Conference of the African Association of Agricultural Economists*, Hammamet, Tunisia

Anwar, F. and M. Bhanger, 2003. Analytical characterization of *Moringa oleifera* seed oil grown in temperate regions of Pakistan. *J. Agric. Food Chem.*, 51: 6558–6563

Anwar, F. and U. Rashid, 2007. Physico-chemical characteristics of *Moringa oleifera* seeds and seed oil from a wild provenance of Pakistan. *Pak. J. Bot.*, 39: 1443–1453

Anwar, F., G.M. Kamal, F. Nadeem and G. Shabir, 2016. Variations of quality characteristics among oils of different soybean varieties. *J. King Saud Univ. Sci.*, 28: 332–338

Anwar, F., N.S. Zafar and U. Rashid, 2006. Characterization of *Moringa oleifera* seed oil from drought and irrigated regions of Punjab, Pakistan. *Grasas Y. Aceites.*, 7: 160–168

Anwar, F., S. Latif, M. Ashraf and A.H. Gilani, 2007. *Moringa oleifera*: a food plant with multiple bio-chemical and medicinal uses – A review. *Phytother. Res.*, 21: 17–25

AOCS, 2009. *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 7th edition. Champaign, Illinois, USA

Ayerza, R., 2011. Seed yield components, oil content, and fatty acid composition of two cultivars of moringa (*Moringa oleifera* Lam.) growing in the Arid Chaco of Argentina. *Ind. Crops Prod.*, 33: 389–394

Ayerza, R., 2012. Seed and oil yields of *Moringa oleifera* variety Periyakalum-1 introduced for oil production in four ecosystems of South America. *Ind. Crops Prod.*, 36: 70–73

Choudhary, S.K., S.K. Gupta, M.K. Singh and Sushant, 2016. Drumstick tree' (*Moringa oleifera* Lam.) is multipurpose potential crop in rural area of India. *Int. J. Agric. Sci.*, 12: 115–122

Fahey, J.W., 2005. *Moringa oleifera*: a review of the medical evidence for its nutritional, therapeutic, and prophylactic properties: part 1. *Tree. Life J.*, 1: 1–15

Ferrao, A.M.B.C. and J.E.M. Ferrao, 1970. Acidos gordos em oleo de Moringueiro (*Moringa oleifera* Lam.). *Agron. Angolana*, 8: 3–16

Foidl, N., H.P.S. Makkar and K. Becker, 2001. The potential of *Moringa oleifera* for agricultural and industrial Uses. *In*: *The miracle tree: The multiple uses of Moringa*, pp: 45–76. Lowell, J. and C.T.A. Fuglie (eds.). Wageningen, The Netherlands

Government of Pakistan, 2015–2016. *Agricultural Statistics of Pakistan*. Ministry of Food, Agriculture and Livestock, Islamabad, Pakistan

Gupta, R., A.K. Sharma, M.P. Dobhal, M.C. Sharma and R.S. Gupta, 2011. Antidiabetic and antioxidant potential of β-sitosterol in streptozotocin-induced experimental hyperglycemia. *J. Diabetes*, 3: 29–37

Hassanein, A.M.A. and A.A. Al-Soqeer, 2017. Evaluation of seed germination and growth characteristics of *Moringa oleifera* and *M. peregrina* under laboratory, greenhouse and field conditions. *Int. J. Agric. Biol.*, 19: 873–879

HDRA, 2002. *Moringa oleifera*-A Multipurpose Tree, Produced by the Tropical Advisory Service, HDRA - The Organic Organization. [Online] http://www.hdra.org.uk, Accessed on June 14, 2017

International Olive Council (IOC), 2013. Determination of the Composition and Content of Sterols and Triterpene Dialcohols by Capillary Column Gas Chromatography; COI/T.20/Doc. No. 30/Rev.1 2013; International Olive Council (IOC): Madrid, Spain

International Olive Council (IOC), 2015. Determination of Trans-Unsaturated Fatty Acids by Capillary Column Gas Chromatography; COI/T.20/Doc. no. 33-2015; International Olive Council (IOC): Madrid, Spain

Kleiman, R., J. Brown and J. Hill, 2006. Moringa oil: a "new yet old" unique cosmetic emollient. *Inform. Champaign*, 17: 739–743

Lakshmipriya, G., K. Doriya and D.S. Kumar, 2016. *Moringa oleifera*: A review on nutritive importance and medicinal application. *Food Sci. Hum. Wellness*, 5: 49–56

Lalas, S. and J. Tsaknis, 2002. Characterization of *Moringa oleifera* seed oil variety "Periyakulam L". *J. Food Compos. Anal.*, 15: 65–77

Leone, A., A. Spada, A. Battezzati, A. Schiraldi, J. Aristil and S. Bertoli, 2015. Cultivation, genetic, ethnopharmacology, phytochemistry and pharmacology of *Moringa oleifera* leaves: An overview. *Int. J. Mol. Sci.*, 16: 12791–12835

Muhl, Q.E., E.S. du Toit, J.M. Steyn and Z. Apostolides, 2014. Bud development, flowering and fruit set of *Moringa oleifera* Lam. (horseradish tree) as affected by various irrigation levels. *J. Agric. Rural Dev. Trop. Subtrop.*, 114: 79–87

Ndubuaku, U.M., T.C.N. Ndubuaku and N.E. Ndubuaku, 2014. Yield characteristics of *Moringa oleifera* across different ecologies in nigeria as an index of its adaptation to climate change. *Sustain. Agric. Res.*, 3: 95–100

Nouman, W., F. Anwar, T. Gull, A. Newton, E. Rosa and R. Domínguez-Perles, 2016. Profiling of polyphenolics, nutrients and antioxidant potential of germplasm's leaves from seven cultivars of *Moringa oleifera* Lam. *Ind. Crops Prod.*, 83: 166–176

Nouman, W., S.M.A. Basra, A. Yasmeen, T. Gull, S.B. Hussain, M. Zubair, R. Gul, 2014b. Seed priming improves the emergence potential, growth and antioxidant system of *Moringa oleifera* under saline conditions. *Plant Growth Regul.*, 73: 267–278

Nouman, W., S.M.A. Basra, M.T. Siddiqui, A. Yasmeen, T. Gull, and M.A.C. Alcayde, 2014a. Potential of *Moringa oleifera* L. as livestock fodder crop: A review. *Turk. J. Agric. For.*, 38*:* 1–14

Nouman, W., M.T. Siddiqui, S.M.A. Basra, H. Farooq, M. Zubair and T. Gull, 2013. Biomass production and nutritional quality of *Moringa oleifera* as field crop. *Turk. J. Agric. For*., 37: 410–419

Nouman, W., M.T. Siddiqui, S.M.A. Basra, I. Afzal and H. Rehman, 2012. Enhancement of emergence potential and stand establishment of *Moringa oleifera* Lam. by seed priming. *Turk. J. Agric. For*., 36: 227–235

Ogunsina, B.S., T.N. Indira, A.S. Bhatnagar, C. Radha, S. Debnath and A.G.G. Krishna, 2014. Quality characteristics and stability of *Moringa oleifera* seed oil of Indian origin. *J. Food Sci. Technol*., 51: 503–510

Olson, M.E., R.P. Sankaran, J.W. Fahey, M.A. Grusak, D. Odee and W. Nouman, 2016. Leaf protein and mineral concentrations across the "Miracle Tree" genus *Moringa*. *PloS ONE*, 11: 11: e0159782

Orhevba, B.A., M.O. Sunmonu and H.I. Iwunze, 2013. Extraction and characterization of *Moringa oleifera* seed oil. *J. Food Dairy Technol.*, 1: 22–27

Palada, M.C., 1996. Moringa (*Moringa oleifera* Lam.): A versatile tree crop with horticultural potential in the subtropical United States. *HortScience*, 31: 794–797

Palada, M.C. and L.C. Chang, 2003. *Suggested Cultivation Practice for Moringa*. AVRDC publication, Shanhua, Tainan, Taiwan

Parrotta, J.A., 2001. *Healing Plants of Peninsular India*. Cabl Publishing, Wallingford, New York, USA

Pritchard, J.L.R., 1991. Analysis and properties of oilseeds. *In*: *Analysis of Oilseeds, Fats and Fatty Foods*, pp: 80–98. Rossell, J.B. and J.L.R. Pritchard (eds.). Elsevier Applied Science: New York, USA

Rahman, I.M.M., S. Barua, M. Nazimuddin, Z.A. Begum, M.A. Rahman and H. Hasegawa, 2009. Physicochemical properties of *Moringa oleifera* Lam. Seed oil of the indigenous-cultivar of Bangladesh. *J. Food Lipids*, 16: 540–553

Rajangam, J., R.S.A. Manavalan, T. Thangaraj, A. Vijayakumar and N. Muthukrishan, 2001. Status of production and utilisation of moringa in southern India. *In*: *Proceedings of the 2001 Workshop Developmental Potential for Moringa Products*, pp: 33–42. Dar Es Salaam, Tanzania, October 20–November 2, 2001

Ramachandran, C., K.V. Peter and P.K. Gopalakrishnan, 1980. Drumstick (*Moringa oleifera*): a multipurpose Indian vegetable. *Econ. Bot.*, 34: 276–283

Ras, R.T., J.M. Geleijnse and E.A. Trautwein, 2014. LDL-cholesterol-lowering effect of plant sterols and stanols across different dose ranges: A meta-analysis of randomised controlled studies. *Brit. J. Nutr*., 112: 214–219

Schwingshackl, L. and G. Hoffmann, 2014. Monounsaturated fatty acids, olive oil and health status: A systematic review and meta-analysis of cohort studies. *Lipids Health Dis.*, 13: 154

Steel, R.G.D., J.H. Torrie and D.A. Dickey, 1997. *Principles and Procedures of Statistics: A Biometrical Approach*, 3rd edition, pp: 400–428. McGraw Hill Book Co. Inc. New York, USA

Thurber, M.D. and J.W. Fahey, 2009. Adoption of *Moringa oleifera* tocombat under-nutrition viewed through the lens of the "Diffusion of Innovations" theory. *Ecol. Food Nutr.*, 48: 212–225

Tsaknis, J., S. Lalas, V. Gergis and V. Spiliotis, 1998. A total characterisation of *Moringa oleifera* Malawi seed oil. *Riv. Ital. Sostanze Gr.*, 75: 21–27

Tsaknis, J., S. Lalas, V. Gergis, V. Dourtoglou and V. Spiliotis, 1999. Characterization of *Moringa oleifera* variety Mbololo seed oil of Kenya. *J. Agric. Food Chem.*, 47: 4494–4499

Verdcourt, B., 1985. A synopsis of the Moringaceae. *Kew Bull*., 40: 1–15

Warner, K. and S. Knowlton, 1997. Frying quality and oxidative stability of high-oleic corn oils. *J. Amer. oil Chem. Soc.*, 74: 1317–1322

(Received 19 May 2017; Accepted 15 June 2017)