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Colocynth: Potential Arid Land Oilseed from an Ancient Cucurbit

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Citrullus colocynthis (L.) Schrad., Cucurbitaceae (colocynth or wild-gourd or bitter-apple), is a non hardy, herbaceous perennial vine, branched from the base. Originally from Tropical Asia and Africa, it is now widely distributed in the Saharo-Arabian phytogeographic region in Africa and the Mediteranean region. The stems are angular and rough; the leaves are rough, 5–10 cm in length, deeply 3–7 lobed; solitary pale yellow blooms. Each plant produces 15–30 round fruits, about 7–10 cm in diameter, green with undulate yellow stripes, becoming yellow all over when dry. Seeds are small (~6 mm in length), smooth and brownish when ripe. *C. colocynthis* occurs in many places in Israel, from the north to the hot desert, in sandy soils and wadis. It flowers between May and August (Feinbrun–Dothan 1978).

During biblical times, fruits were gathered and considered as a deadly poison (II Kings 4:39–40). The fruits are widely used medicinally, especially for stomach pains. The pulp, because of its content of glucosides, such as colocynthin, is a drastic hydragogue, cathartic, and laxative (Dafni et al. 1984; Burkill 1985). The fruits were exported as a laxative from the Gaza Strip to Europe in the early 20th century (Palevich and Yaniv 1991). The seeds are edible and when ground provide a rude bread for the desert Bedouins (Zohary 1982). The seeds have a high oil content (17–19%); in ancient times it was among the oils permitted to be used for candle light (Palevitch and Yaniv 1991)

In recent years there has been much interest in developing new oilseed crops which could be used in food, and for medicinal and industrial purposes (Yaniv et al. 1994). Many melon seeds (*Cucurbita* spp., *Citrullus* spp.) are rich in oil and protein (Al-Khalifa 1996) and although none of these oils has been utilized on an industrial scale, many are used as cooking oils in some African and Middle Eastern countries (El-Magoli et al. 1979). Melon seeds are utilized for oil production, especially in Nigeria (Girgis and Said 1968). Melon seed oil contains a large amount of linoleic acid (C18:2) which is important for human nutrition and an essential fatty acid and very little linolenic acid (C18:3) (Akoh and Nwosu 1992; Huang et al. 1994; Udayasekhara Rao 1994). Such oil composition resembles safflower oil (Yaniv et al. 1996) and is very beneficial for human diets.

Accessions of wild *Citrullus colocynthis* have been collected in arid zones in Israel and kept in the Israel Gene Bank. This gene pool was evaluated for chemical and agronomical characters, in order to test the seeds as candidates for a potential new oilseed crop in Israel—a crop adapted to arid zones.

METHODOLOGY

Plant Material

Seeds of wild *Citrullus colocynthis* were collected during 1983/84 throughout the Negev, Arava, and Sinai Deserts. The seeds were generally collected after fruit ripening, between October and December. Seeds of each accession are stored at the seed storage facilities of the Israel Gene Bank, in sealed aluminum cans, until needed for maintenance and evaluation.

Field Observation Trial of 28 Accessions of *C. colocynthis*

In 1995, 28 accessions of *Citrullus colocynthis* were studied at the Bet Dagan Experimental Farm, The Volcani Center, located in the coastal plain at latitude 32°01'N and an elevation of 50 m. An adequately

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fertilized and irrigated field, kept free of weeds and diseases, was used. The soil type at the farm is a deep fertile vertisol.

Seeds were sown in germination trays on 2 May 1995. The soil used was a mix of equal parts of tuff, peat, and vermiculite. Full germination (100%) was observed within one week. 23-day old seedlings were transplanted into the field on 25 May 1995 on raised beds, spaced at 2 m from center to center. There was one row per bed with five plants of each accession at a spacing of 50 cm between plants and 3 m between accessions. Each accession occupied an area of 10 m^2. Basic fertilization was done at the time of soil preparation at rates of 2N–2P–1K. "Trifluralin" (2.5 kg/ha) was applied as a herbicide. At the time of planting, the seedlings received 300 m^3/ha water. A drip irrigation system was used throughout the growing period (June to August). The plants were irrigated weekly with 150 m^3/ha water. Irrigation was terminated at the onset of fruit ripening. The total amount of water given to the plants was 2,100 m^3/ha. Fruits were harvested according to maturation of each accession, and the seeds were analyzed for their fatty acid composition.

Field Experiment on Four Selected Lines

Based on the field-observation trial conducted in the 1995 season, four accessions representing various oleic and linoleic acid values were selected and tested at the Bet Dagan Experimental Farm during the 1996 season. Each accession was replicated four times in a random block design. Soil preparation and the size of the plots (10 m^2) were the same as during the 1995 trial. Seeds were sown in germination trays on 6 May 1996 and transplanted into the field on 6 June 1996. All the fruits were harvested from each plot at maturation (when the fruit changed its color from green to yellow and the seed coat was dark brown), from 1 Sept. until 14 Nov., and divided according to five maturation periods: Sept. 1 (1–15 Sept.), Sept. 15 (15–30 Sept.), Oct. 1 (1–15 Oct.), Oct. 15 (15–31 Oct.), and Nov. 1 (1–15 Nov.). In addition, fruits from each maturation period were divided according to size into five sub-groups.

Data regarding the following parameters were collected during the growing season: earliness in flowering and ripening; fruit yield, fruit diameter, number of seeds per fruit, 1000-seed weight, seed yield, oil content (% of dry weight), and fatty acid composition. Oil content was determined on samples taken from each plot. The data were subjected to a two-factorial variance analysis and arranged in a split-plot design (the main plots were accessions and the sub-plots were the various periods) having four replications.

Lipid Extraction

Seeds were dried overnight at 50°C and ground into powder in a Moulinex coffee grinder. Five grams of powder were mixed with 100 ml petroleum ether (40–60°C), and the lipid fraction was extracted in a Soxhlet apparatus for 16 h at 60°C. The solvent was evaporated, and the lipid fraction residues were weighed (Yaniv et al. 1991).

Direct Transesterification from Seeds

Seeds (200 mg) were dried overnight at 50°C and ground into powder with a mortar and pestle, after which 0.3 ml of dichloromethane and 2.0 ml of 0.5N sodium methoxide (MeONa) were added. The tube was shaken and heated for 30 min at 50°C. The reaction was stopped by adding 5.0 ml of water containing 0.1 ml of glacial acetic acid. The esterified fatty acids were extracted with 2.0 ml petroleum ether (40–60°C). The clear fraction was kept at –20°C until further analysis. Samples of 2.0 ml were injected into the gas-chromatograph for fatty acid analysis (Yaniv et al. 1991).

Gas Chromatography of Methylated Fatty Acids

A Megabore column (DB-23, 0.5 mm film thickness, 30 m × 0.54 mm, J&W Scientific) was used in a gas-chromatograph equipped with a flame ionization detector (Varian 3700 GC) and an automatic area integrator (3390A-HP). The flow rate of N_2 was 30 ml/min and the oven temperature range was 135–200°C, programmed at a rate of 4°C/min.

The following fatty acids were identified by comparison with known standards (Supelco): C16:0, palmitic; C18:0, stearic; C18:1, oleic; and C18:2, linoleic acid (Yaniv et al. 1991).

Statistical Analysis

All data were analyzed by means of procedures of the SAS package (SAS Institute 1985). Statistical differences were calculated as Least Significance Difference (LSD) and evaluated with Duncan's Multiple Range Test (DMRT) at P=0.05.

EXPERIMENTAL RESULTS

Evaluation of *C. colocynthis* Wild Accessions

The results of the chemical analyses of the 28 accessions are summarized in Table 1. The predominant fatty acid of the seed oil was linoleic acid, C18:2, ranging from 67.0% to 73.0% of total fatty acids. The oleic acid (C18:1) content ranged from 10.1 to 16.0%. A similar pattern has been observed previously (Akoh and Nwosu 1992; Huang et al. 1994; Udayasekhara Rao 1994; Al-Khalifa 1996) but, the contents of both linoleic acid and oleic acid were generally higher in our study than those reported in these studies (i.e., 77.1% to 89% in our studies as compared with 75.8% to 82.3% in the literature). The fatty acid composition of the seed oil of *C. colocynthis* is very similar to that of safflower oil (Yaniv et al. 1996).

Field Evaluation of Four Selected Lines

Tables 2 and 3 present agronomical and chemical data collected during the cultivation of four selected lines of *C. colocynthis* in the 1996 season. The number of fruits harvested per plot (10 m^2) varied within lines from 112 to 129, with Line 4 being the highest. Fruit size was determined by its diameter and varied from 5.7 cm (Line 1) to 6.3 cm (Line 2). Fruits of *C. colocynthis* contain a large number of seeds. Numbers varied, in lines, between 291 and 404 seeds per fruit; 1000-seed weight was the same in Lines 2, 3, and 4 (about 44 g) while Line 1 had significantly smaller seeds (40 g). Lines 2 and 4 excelled in seed yield (2.1 kg/10 m^2) while Line 1 yielded only 1.5 kg/10 m^2.

Table 1. Chemical variability in seed oil of 28 accessions of *Citrullus colocynthis* collected in Israel and grown at Bet Dagan experimental farm during 1995.

Fatty acids		% of total oil	
		Mean	Range
Palmitic	(C16:0)	10.1	8.6–12.0
Stearic	(C18:0)	6.7	5.2–8.2
Oleic	(C18:1)	13.1	10.1–16.0
Linoleic	(C18:2)	70.1	67.0–73.0

Table 2. Agronomic data of *Citrullus colocynthis* lines evaluated in Bet Dagan during the 1996 season. (The observations are averages of four replications.)

Line	No. fruit/ 10 m^2	Fruit diam. (cm)	No. seed/ fruit	1000-seed weight (g)	Seed yield (g/10 m^2)
IN 34250	127.3a[z]	5.70b	291b	40.0b	1,479c
IN 34256	120.6b	6.32a	396a	43.4a	2,107a
IN 34262	112.5c	6.18a	404a	44.0a	1,893b
IN 34267	128.8a	6.12a	370a	43.6a	2,143a

[z]Mean separation by Duncan's multiple range test, 5% level.

Table 3. Chemical data of *Citrullus colocynthis* lines evaluated in Bet Dagan during the 1996 season. (The observations are averages of four replications.)

Line	Oil content[z] (%)	Calculated oil yield (L/ha)	Oleic FA content (%)	Linoleic FA content (%)	Oleic+linoleic FA yield (L/ha)
IN 34250	17.1c[y]	253b	11.7c	70.9a	209b
IN 34256	19.0ab	400a	15.0a	66.7c	327a
IN 34262	19.5a	369a	14.2b	68.2b	304a
IN 34267	18.5b	396a	14.3b	67.9b	325a

[z]Oil content based on seed dry weight.
[y]Mean separation by Duncan's multiple range test, 5% level.

Evaluation of Chemical Characteristics

Oil content in seeds varied from 17 to 19.5%. Based on this data and seed yield, oil yield of 250 to 400 L/ha was calculated. The two major fatty acids in seed oil of *C. colocynthis* are C18:1 oleic (11.7–15%) and C18:2 linoleic (66–70%). The total yield of unsaturated fatty acids varied from 209 to 327 L/ha. This oil contains only traces of C18:3 linolenic acid and thus could provide a good source of edible oil. Comparison between the lines, which were collected from different locations in Israel, points to the fact that Line 1 was significantly lower in almost all parameters evaluated, including seed and oil yield.

Yield Parameters

Seed yield (Fig. 1) was maximal in the first month of ripening and declined drastically until the end of the ripening period. The differences among lines indicated biodiversity. Early-ripening fruits (first month) contained heavier seeds than later-ripening fruits (Fig. 2), as measured by 1000-seed weight, perhaps late ripening seeds contain less water then early ripening seeds.

Larger fruits were obtained mostly during the first month of ripening (Fig. 3). Later in the season, ripe fruits became smaller and smaller. The best harvest time, in terms of fruit yield, fruit size, seed yield, and total oil yield was during the first four weeks of ripening.

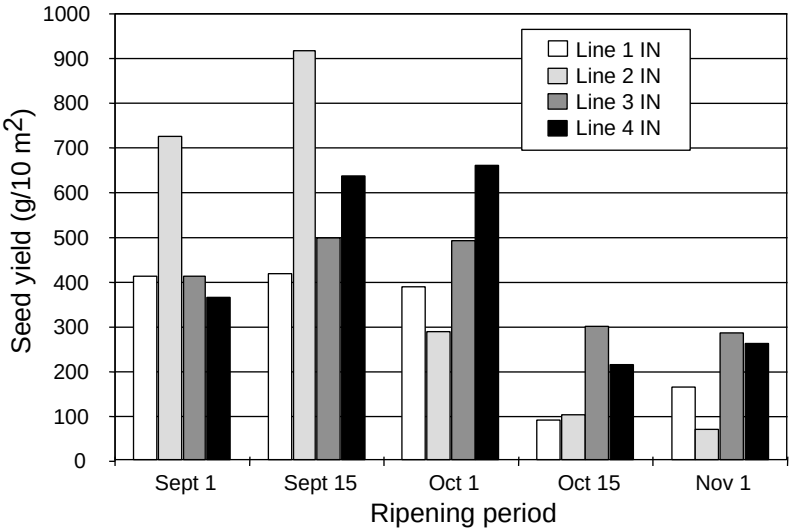

Fig. 1. The relation of seed yield to ripening.

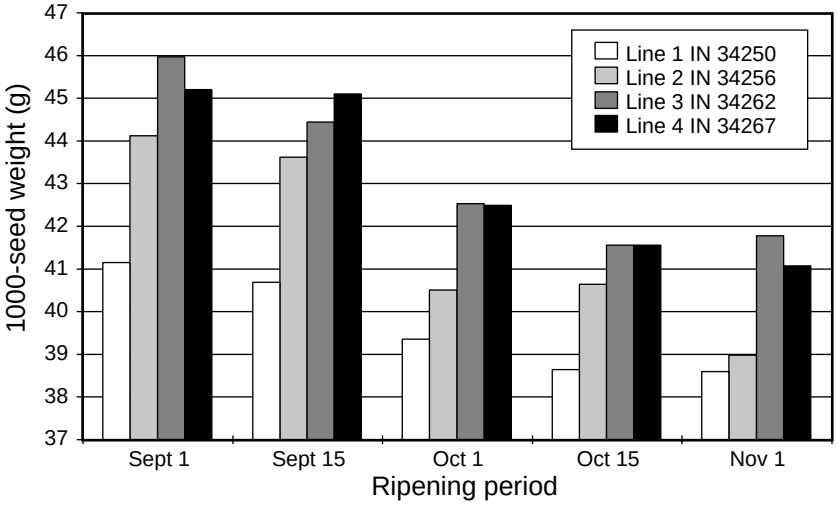

Fig. 2. The relation of 1000-seed weight to ripening period.

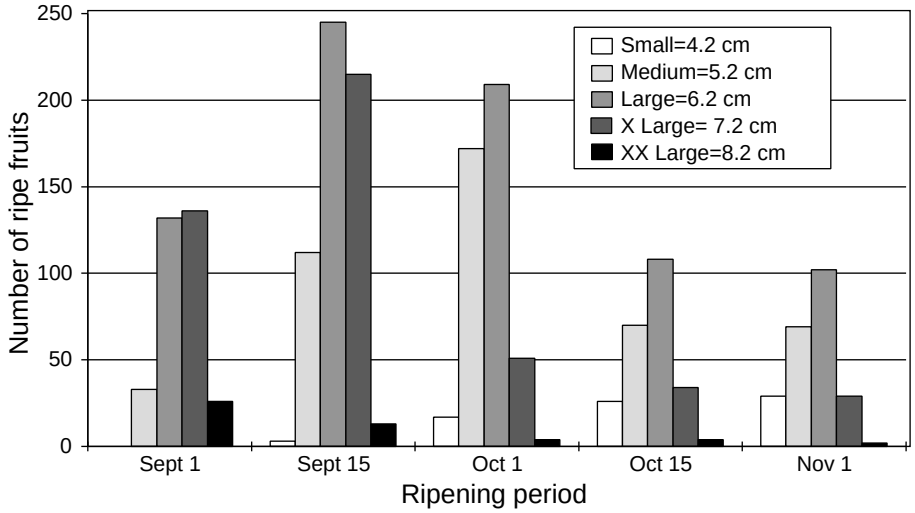

Fig. 3. Number of ripe fruits collected in each ripening period and arranged by fruit size.

CONCLUSIONS

C. colocynthis is a plant of dry climate and its cultivation should now be evaluated under arid conditions. To improve its agronomical properties it is desired to select lines with uniform harvest time and with higher oil content. A special attention should be given to evaluate its medicinal and diuretic potential as a source of high value by-products.

REFERENCES

Akoh, C.C. and C.V. Nwosu. 1992. Fatty acid composition of melon seed oil lipids and phospholipids. J. Am. Oil Chem. Soc. 69:314–316.

Al-Khalifa, A.S. 1996. Physicochemical characteristics, fatty acid composition, and lipoxygenase activity of crude pumpkin and melon seed oils. J. Agr. Food Chem. 44:964–966.

Burkill, H.M. 1985. The useful plants of west tropical Africa. Vol. 1, Families A-D. Royal Botanic Garden. Kew, UK.

Dafni, A., Z. Yaniv, and D. Palevitch. 1984. Ethnobotanical survey of medicinal plants in Northern Israel. J. Ethnopharmacol. 10:295–310.

El-Magoli, S.B., M.M. Morad, and A.A. El-Fara. 1979. Evaluation of some Egyptian melon seed oils. Fette Seifen Anstrichmittel 81(5):201.

Feinbrun-Dothan, N. 1978. Flora Palaestina—Part III. The Israeli Academy of Sciences and Humanities, Jerusalem.

Girgis, P. and F. Said. 1968. Characteristics of melon seed oil. J. Sci. Food Agr. 19:615–616.

Huang, K., C.C. Akoh, and M.C. Erickson. 1994. Enzymatic modification of melon seed oil: Incorporation of eicosapentaenoic acid. J. Agr. Food Chem. 42:2646–2648.

Palevitch, D. and Z. Yaniv. 1991. Medicinal plants of the Holyland. (in Hebrew) Tamus Modan Press, Tel Aviv. p. 56–58.

SAS Institute. 1985. SAS Users' Guide: Statistics, version 5. Cary NC.

Udayasekhara Rao, P. 1994. Nutrient composition of some less-familiar oil seeds. Food Chem. 50: 379–382.

Yaniv, Z., Y. Elber, M. Zur, and D. Schafferman. 1991. Differences in fatty acids composition of oils of wild Cruciferae seeds. Phytochemistry 30:841–843.

Yaniv, Z., D. Schafferman, Y. Elber, E. Ben-Moshe, and M. Zur. 1994. Evaluation of *Sinapis alba*, native to Israel, as a rich source of erucic acid in seed oil. Indust. Crops Prod. 2:137–142.

Yaniv, Z., D. Schafferman, M. Zur, and I. Shamir. 1996. *Matthiola incana*: Source of omega-3-linolenic acid. p. 368–372. In: J. Janick (ed.), Progress in new crops. ASHS Press, Alexandria, VA.

Zohary, M. 1982. Plants of the Bible. Cambridge Univ. Press. Cambridge.