http://dx.doi.org/10.1590/0100-2945-447/13

Cereus Peruvianus (KOUBO) NEW CACTUS FRUIT FOR THE WORLD[1]

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ABSTRACT-Several different species of the columnar cacti of the genera *Stenocereus* and *Pachycereus*, were introduced into different semi-arid ecozones in Israel and most of these efforts were of disappointing outcomes, the only exception being the *Cereus peruvianus* (L.) Miller,which bore plenty of fruits, some of them of good taste. The original seeds of this plant were obtained from the late Mr. Amram (Ron) Kodish, who collected seeds from various private gardens in Southern California which bore fruits of reasonable qualities. The initial success of this species led us to initiate an intensive research study, and today it is already fruit-crop, marketed mainly in Israel under the name "Koubo".
This paper will describe our work of domestication of this new cactus fruit crop in Israel.
Index terms: *Cereus peruvianus*, koubo, cacti.

KOUBO-UMA NOVA FRUTA PARA O MUNDO

RESUMO - Várias espécies diferentes de cactos colunares dos gêneros Stenocereus e Pachycereus, foram introduzidos em diferentes ecozones semiáridas em Israel, a maioria desses esforços foram de resultados decepcionantes, a única exceção sendo apenas o *Cereus peruvianus* (L.) Miller, que tinha muitas frutas, algumas delas com gosto bom. As sementes originais desta planta foram obtidas a partir do falecido Sr. Amram (Ron) Kodish, que coletou sementes de vários jardins privados do sul da Califórnia e que haviam dado frutos de qualidades razoáveis. O sucesso inicial desta espécie nos levou a iniciar um estudo intensivo, e atualmente existe uma produção dessas frutas, comercializados principalmente em Israel sob o nome "Koubo".
Termos para indexação: *Cereus peruvianus*, koubo, cacto.

INTRODUCTION

For centuries, the indigenous people of Latin America have been using the fruits of various species of columnar cacti, known to them as pitayas, for food and other applications (Pimienta-Barrios & Nobel 1994; Pimienta-Barrios, et al 1997, Ortiz-Hernández, 1999). During the past decades, we and others have been making attempts to convert these wild or semi-wild cacti, known only in their country of origin, into new cacti-fruit crops and introduce them into Western and other world markets (Pimienta-Barrios & Noble 1998; Pimienta-Barrios & Noble 1995; Pimienta-Barrios & Noble 1998; Pimienta-Barrios et al 1997; Pimienta-Barrios et al 1998; Nerd et al 1990; Nerd et al 1993; Nerd & Mizrahi 2002). Several different species of the columnar cacti of the genera *Stenocereus* and *Pachycereus*, were introduced into different semi-arid ecozones in Israel, most of these efforts were of disappointing outcomes, the only exception being the *Cereus peruvianus* (L.) Miller,which bore plenty of fruits, some of them of good taste (Nerd et al 1990; Nerd et al 1993; Nerd & Mizrahi 2002; Weiss et al 1993). The original seeds of this plant were obtained from the late Mr. Amram (Ron) Kodish, who collected seeds from various private gardens in Southern California which bore fruits of reasonable qualities. The initial success of this species led us to initiate an intensive research study, and today it is already fruit-crop, marketed mainly in Israel under the name "Koubo".

It is noteworthy that in South Africa this introduced species is known as an invader pest (Moran & Zimmermann 1991; Olckers 2004). This paper will describe our work of domestication of this new cactus fruit crop.

Taxonomy

We originally obtained the seeds of this new crop under the scientific name of *Cereus peruvianus* (L.) Miller. After publishing several papers which used this name, we received responses from Brazilian researchers asserting that the plant mentioned in our papers is in fact *Cereus jamacaru* from the semi-arid zones of Brazil, known there by the local name of

[1](Trabalho 447-13). Recebido em: 20-09-2013. Aceito para publicação em: 15-12-2013. Palestra II Simpósio Internacional de Fruticultura- Frutas Exóticas, 21 a 25 de outubro de 2013. Jaboticabal-SP.
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Mandacaru. Mandacaru is consumed by the local people as a wild-cactus-fruit. My colleague Dr. A. Nerd visited Brazil and brought with him seeds of *C. jamacru* collected from the wild near Independência, Ceará. Indeed, there is a close similarity between the two accessions but also some differences (Mizrahi & Nerd 1999; Gutman et al 2001). All *C. jamacaru* seedlings which we tested (several dozens), produced un-tasty almost inedible fruits. Today we believe that the *C. jamacaru* might be a subspecies of *C. peruvianus* (Gutman et al 2001). This notion was strengthened by performing crosses between the two species, which showed that the two can pollinate each other and produce viable seedlings (Gutman 2001). This compatibility is not necessarily an indication that the two are the same species, as we found that in vine-cacti inter-specific and even inter-generic crosses can produce viable offspring (Tel-Zur et al 2004; Tel-Zur et al 2011). However, it is important to note that *C. jamacaru* is another important genetic source for breeding. After successfully domesticating *C. peruvianus* in Israel, we nicknamed it "Koubo" - a name which is easy to pronounce (Fig.1).

Species characteristics, genetics and breeding.

Among several hundreds of seedlings germinated from the *C. peruvianus* seed batch we obtained from Mr. Amram Kodish, we found around 8 genotypes which were of good taste and selected them for further breeding. Those chosed for further breeding were also aromatic which adds a lot to the taste as we found later in our research into the fruit quality which will be discussed later (Ninio et al 2003,a & b). We found significant differences in spine length on the shoots. In the spiny genotypes the size of the spines declines with the seedling age, from 25 cm at the base of the young seedlings (old shoots) to 2-3 cm in the new emerging youngest shoots. The non-spiny genotypes have very small spines, of around 5 mm in length even on old shoots and are almost spineless at the top. The fruit itself is in all cases smooth with no spines (Figs 1, 2 & 3). Fruit flesh color is usually white (Fig. 2), while a very pale yellowish color may occur in the flesh of some genotypes. The peel color varies from shiny deep-red through pinkish-yellow to yellow (Figs 1, 2 & 3). The most important characteristic after taste was fruit cracking (Fig 4). Over 95% of the seedlings had fruits which cracked before full ripening took place. For further selection we chose genotypes with either almost no fruit cracking, or minor cracking of no more than 15%. It is interesting to note that almost all of the *C. jamacaru* seedlings we checked did not crack and thus these clones should be considered as future parents for further breeding of new clones, to avoid the fruit cracking problem. Four of the 8 selected clones showed peel blackening sometime after harvest even though their taste was still good, reminiscent of bananas (Fig 5). These genotypes serve today mainly as pollen sources for the commercial varieties. It is very interesting to note that these sensitive clones do not show this peel blackening in the Arava valley where average summer temperatures may rise to 40°C concomitant with 10% air humidity (unpublished data). There was no difference in fruit cracking between the Arava hot valley and other regions in Israel (Leshem et al 2003). This species can tolerate these high temperatures with no problem. However, subfreezing temperatures (-6°C for several hours) can eradicate a whole plantation. At -2°C some clones were heavily damaged while others survived and produced the same yield in the following summer as in normal years (unpublished data). Hence, it is possible to breed for sub-freezing tolerant varieties. Molecular typing of various genotypes analyzed by random amplified polymorphic DNA (RAPD) technique demonstrated that this species has a limited genetic base and additional germplasm is required for further efficient clone improvement (Gutman et al 2001).

The *C. peruvianus* species is basically self-incompatible (Weiss et al 1994). Unlike the vine-cacti, not a single genotype was found to be able to pollinate itself. Another important finding is that not every genotype can pollinate others. It is reminiscent of the S_1 S_2 mechanism of in-compatibility which is also found in other fruit trees (Newbigin et al 1993). To guarantee fruit production throughout the year, we have to match compatible clones for early, medium and late season of flowering and fruiting clones. It is of major interest to try producing self-compatible clones by doubling the chromosomes as was done with the vine-cacti and some other plants (Cohen & Tel-Zur 2012).

This plant is also used as gardening plant known under the name "Princess of the Night". This is due to its nocturnal flowers which will open for only one night. In June we found that there are 2 groups of clones. In some of the genotypes the anthers will dehisce at around 18.00 and the flowers will start to open 30 minutes later at twilight, when honey bees are still active. They come to drink the nectar which is secreted at the base of the flower. If compatible clones are planted next to each other, pollination is possible at that time. The anther dehiscence of other clones takes place at 19.00, in which flowers will open a few minutes before sunset so the time

remaining for bee grazing is very limited and almost no pollination takes place (Weiss et al 1994). After sunrise bees and other day time insects will visit the flowers, however the flower is too big for efficient pollination by these insects (Fig.6). In nature pollination is performed by big insects and even by fruit bats (Silva & Sazima 1995). Today in commercial orchards all flowers are hand pollinated by the growers. The pollen is collected by car vacuum cleaners, mixed with various existing clones and applied by hand with a small brush. This action guarantees over 95% fruit set. Fruit sizes of open pollination from compatible clones grown in close proximity will be 60% of the size of hand pollinated clones (Weiss et al 1994). The flowers are produced throughout the warm season from June to October. One can find flower buds, flowers and fruits of various degrees of development on the shoots at the same time, (Fig.7). We expected columnar cacti to be tolerant to salinity but found them to be severely stressed by 3.5 dS/m of saline water. The picture presented in Fig. 8 was taken in the Arava valley where the main salinity source is Ca, Mg, and sulphates which are less toxic than Na, and Chloride ions. Salinity of 2 dS/m hardly stressed the Koubo and commercial yields were obtained by irrigation at this degree of salinity (Fig.9).

To the best of our knowledge no irrigation and/or fertilization experiments have been performed with this plant. However, since it is a CAM plant the applied irrigation is around 10% of what is used to irrigate C_3 fruit trees, around 100 mm per year (see table 4 in Mizrahi et al 2007). All orchards are drip irrigated and we use fertigation methods, namely with each irrigation we deliver fertilizers of 23/7/23 N/P/K with minor elements. When growers added in each irrigation 70 ppm N of this fertilizer they obtained yields of 20-25 ton/hectare/year. Since so far in Israel we have not found any pests, this crop is commonly grown using organic protocols, with animal manure as fertilizer and covering soil with plastic sheets to avoid the use of weed-killers (Fig. 9).

Propagation.

Since this species is self-incompatible, propagation from seeds is used for breeding, to obtain new genotypes. A lot of work was done by Machado, Mangolin and other coworkers on various techniques for vegetative propagation including mini-propagation ones. (Machado & Prioli 1996, Mangolin et al 2002). However, we found that propagation from cuttings is the easiest and cheapest way. Shoots are cut to 40-50 cm long sections, dried for a week under shade to heal the open wound and then put into any nursery mix to root. Within a few weeks roots are produced and plants are ready to be transferred into the orchard. This will guarantee the establishment of over 97% of plants. Direct planting of cuttings into the orchard is also feasible but the rate of plant establishment is lower ~ 80-85%. We also were able to regenerate whole plants from shoot slices containing a single areole (Fig 10); however, it took 4 more years to achieve full plant production. Today we recommend establishing the 40-50cm long cuttings in pots, and then transfer them into the orchard.

If planting an orchard for hand pollination, one clone can be grown in one separate section of the orchard. Pollen will be collected from the pollinizers section and applied daily from early morning to about 9.00 am. This is to allow efficient pollination when flower is open and the stigma is exposed (Figs 6&7). One thousand flowers can be pollinated per hour if a big wave of flowers exists. If pollination by bees and/or other pollinizers is considered, then near each plant, a compatible clone should be planted expecting reduction in fruit sizes of ~ 40% (Weiss et al 1994). We recommend planting 5X2 m distances in the Koubo orchard. Roots are much deeper than the vine-cacti, reaching a depth of 1.5 m. In areas where summer rains prevail, irrigation might be unnecessary.

Tree pruning is performed in winter, to reduce the plant's height to allow easy access to both flowers and fruits, and to open the tree canopy to allow easy access to fruits and flowers and allow light penetration (Fig. 11).

Fruit development, ripening and postharvest behavior.

Fruit start to develop after pollination with compatible pollen. The first stage is a fast growth period (~ 15 days) followed by a slower-rate period (about 10 days) which is then followed by another fast growth period, until full ripening. Fruit reach maximum mass about 45-50 days after anthesis. The first color appears after ~ 35 days while full color is reached at 45-50 days after anthesis. Seeds develop mainly during the second period when growth rate is slowed down. After this stage seeds have a hard black coat and can germinate. At the end of the fruit-development-stage (~ 45-50 days) over 95% of the fruits start to dehisce and crack (Fig. 4). This is a major obstacle for growing various clones for the market. Lack of fruit cracking is an essential characteristic for development of this species. Unfortunately harvesting the fruits in early stages of fruit

development leads to un-tasty inedible fruit (Wang 1997). To reach reasonable taste one has to harvest the fruits around full color stage where most clones will crack. The clones we selected can be harvested almost at full color stage and then stored for a few weeks. They should be stored at 10ºC since lower temperatures will induce chilling injury, not only in blackening of the peel but also development of off-flavor. We analyzed fruit composition both immediately after harvest and 2 weeks later after storage at 10ºC. Results are given in Table 1. One should emphasize the following; water content is around 85%, the fruit is rich in fibers (mucilage), and low in sugars. Before ripening the polysaccharide content declines concomitant with rise in soluble sugars. The main sugars are glucose and fructose with traces of sucrose. During ripening, the titratable acidity value decreased by 50% concomitant with the rise in pH. The main acid was malic (~80%), along with Citric, Oxalic and Succinic acids (Wang 1997; Ninio et al 2003a and Ninio et al 2003b). Due to its low sugar content this fruit can possibly be recommended for diabetics. The fruit emits a unique perfume-like odor. Volatiles analysis showed that the main component is linalool and its derivatives. When the fruit is allowed to reach full ripening in storage, the amount of linalool is 17 times higher than in tree-ripe cracked fruit (Ninio et al 2003b). This aroma adds a lot to the acceptance of the fruit by tasters (Wang 1997). We can use the fruit peel color as a reliable ripening index. Before ripening the fruit is green, then changes its color to violet where chlorophyll and betalains red pigments exist together. As the chlorophyll disappears the color is changed to full red in the red clones. The fruit peel stretches and becomes smooth. The best eating quality is reached when the fruit is fully red, however, since fruit cracking appears in most clones, it is recommended to harvest the fruit where some violet still exists and peel is fully stretched. When fruit is stored at 10ºC, shelf-life is over one month which allows the sea-freight to Europe from Israel which is around 10% of the air-freight cost. Reducing the storage temperature to 4ºC, will result in sever chilling injury symptoms such as; peel shriveling and blackening, watery pulp concomitant with off-flavor (Wang 1997).

The fruit is of un-climacteric type but when exposed to ethylene rapid ripening and deterioration will occur (Wang 1997). Hence, it is advisable to ship this fruit in a separate container, not mixed with climacteric fruits.

Marketing of new crop is a major obstacle to the success of establishing sustainable crop (Mizrahi et al 2002). In 2002 a survey was performed by Rut Ela'azar from the Israeli Ministry of Agriculture. She exposed around 100 persons to taste test of koubo fruits. These testers were first time tasters of kuobo and 84% of them claimed that they liked it and were ready to consume it on a regular basis provided the price will be reasonable. Today the fruit is sold for 8-10 Shekels/Kg as a gate-price (1 $US = 3.7 New Israeli Shekels). Fresh fruits are available in the markets from July to January.

Uses other than fresh fruit.

Stems of this species include many kinds of polysaccharides. The arabinogalactan was found to have anti-ulcer properties and can be used for phytotherapy (Tanaka et al 2010). In Israel polysaccharides (mucilage) from fruit and stems are used as a base for various cosmetic products (unpublished and confidential information). Shoot waxes were identified and may be used for various wax needs (Dembitsky & Rezanka 1996; Hughs, et al 1980). We developed several food products from the ripe fruit, among them jam, dry slices of the fruit and excellent aromatic liquor.

TABLE 1 - Fruit composition of 2 clones of *Cereus peruvianus* at harvest and after 2 weeks of storage at 10ºC

Yellow pitaya clones	Hylocereus spp clones
Spiny fruit	No spines on fruit
Tiny fruit scales	Big fruit scales
Three years to production	One year to production
Weak vegetative growth	Vigorous vegetative growth
Small weak root system	Well-developed root system
Most sensitive to low temperature	Relative resistance to low temperature
Superior taste	Taste from bland to good
Long shelf-life	Short shelf-life
Long fruit development time	Short fruit development time
Flowering in autumn	Flowering in summer
Tetraploid, self-compatible	Diploid only 10% self-compatible
Fruit size is small-medium	Fruit size is medium-large
Seed number in fruits is low	Seed number in fruits is high
Stomata are big	Stomata are small
Many aborted seeds	All seeds are viable
Parenchyma & chlorenchyma are mixed, as one layer	Parenchyma & chlorencyma are separate layers.
All clones sensitive to nematodes	Many genotypes are resistant to nematodes
Seeds are big	Seeds are small

FIGURE 1- Export box of ripe *C. peruvianus* fruits, for European markets.

FIGURE 2- Appearance of ripe red clone of *C. peruviaus* fruit. The black seeds embedded in the white flesh are soft and edible, reminiscent of Kiwi fruit.

FIGURE 3- Spineless, smooth, fully ripe yellowish –pinky fruit of *Cereus peruvianus* attached to a spiny shoot.

FIGURE 4- Violet wrinkled and fully ripe red cracked fruits of *Cereus peruvianus*.

FIGURE 5- Black patches over the red peel of *C. peruvianus* fruit appeared after storage. The fruit is still aromatic, tasty and edible, similar to ripe bananas.

FIGURE 6- Open flower of *C. peruvianus* with bee and other insect at 8.00 am in Beer-Sheva experimental orchard (university campus). These insects can go in and out of the flower without touching the stigma. Hence, hand pollination is necessary to guarantee fruit set.

FIGURE7- *Cereus peruvianus* shoot with flower buds, flowers at full anthesis and closed old flowers, together with developing wrinkled fruits. The black dry corolla abscised from the top end of the set fruit.

FIGURE 8- Nine-year-old *Cereus peruvianus* orchard, suffering from irrigation-water-salinity of 4dS/m, with major ions of Ca, Mg and Sulphates. Note the white circles of salt around some plants. The picture was taken in Qetura (southern Arava Valley, Israel).

FIGURE 9- Productive *Cereus peruvianus* orchard in Ein-Yahav (Northen Arava valley), irrigated with 2 dS/m water where major ions are similar to Qetura (as seen in Fig. 8). This orchard is treated with an organic protocol. Note the plastic sheets on the soil to avoid weeds.

FIGURE 10- One year old *Cereus peruvianus* plant, propagated by a single areole of a shoot slice. These plants are lagging behind plants which were propagated in the recommended way, namely, 40-50cm long shoot cuttings.

FIGURE 11- Five-year-old pruned orchard of *C. peruvianus*. The plants were trained and shaped to ease pollination and fruit harvest, and maximize light passage into the plant canopy.

CONCLUSIONS

To the best of our knowledge, Israel is the only country which grows this plant as a fruit crop. It has many advantages: a very high water use efficiency- ~ 10 times higher than other C_3 fruit tree species, hence, an ideal fruit crop for semi-arid zones; it can tolerate very high temperatures accompanied with very low relative humilities; no pests are known in Israel, hence, an ideal crop to be grown under organic protocols; easy and cheap to propagate from cuttings; only three years to commercial production; can be used to produce various fruit products; long shelf-life suitable to reach the world markets; other products can be made from the stems. In spite of the fact that it is really new crop, with all difficulties involved in marketing, first time consumers were ready to buy it on a permanent basis; it has low sugar content, suitable for diabetic consumers. Special attention should be given to the fact that it can be introduced as a weed. This risk is minimal in semi-arid areas like in Israel. There is still a tremendous amount of knowledge to be accumulated about this crop, since research is still in its infancy.

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ACKNOWLEDGEMENT

The author would like to thank Dr. Tali Brunner for editing this manuscript.

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