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TWENTY YEARS OF JUJUBE (*ZIZIPHUS JUJUBA* MILL.) RESEARCH IN ROMANIA

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Abstract

Chinese jujube is a new fruit specie for Europe with a high potential to be planted on arid and semiarid areas on marginal, poor and even salty soils. Being a multimillennial fruit crop in China, jujube has a high importance in the Chinese diet due to its complex nutraceutical properties. Nearly 1000 varieties and local genotypes are cultivated in China on over 2 million hectares on low input production systems. Even if it was introduced in Dobrogea region, some 2,000 years ago by the Greek and Roman colonists, jujube plants and fruits are nearly unknown, as it happens in other countries from the Mediterranean basin. The first cultivated varieties were introduced at the Faculty of Horticulture in Bucharest from Shanxi Province, China within a common research project in 1997. During more than twenty years, a complex research program has been carried out. The first task was to determine the adaptation of the most important jujube genotypes at the local growing conditions. A second topic was to establish the best propagation method by using seedling production for rootstocks, grafting techniques and in vitro propagation. Other researches on jujube phenology, fruit biochemical composition, fruit storage and consumer preferences have been realized. The local jujube genotypes from Ostrov and Jurilovca have been studied and described. The paper presents a synthesis of the most important results achieved during two decades of jujube research and the opportunity to extend the cultivation of jujube in Romania as a new "super fruit".

Key words: *genotypes, seeds, seedlings, in vitro propagation, fruits, storage, sensorial analysis.*

Foreword:

I dedicate this work to the memory of my Chinese friend, Mr. Bi Ping from Shanxi Province that had an outstanding contribution to the first studies of *Ziziphus jujuba* Mill. in Romania.

INTRODUCTION

Gaius Plinius Secundus (AD 23 - 79), better known as Pliny the Elder, mentioned in his Historia Naturalis that a Counsellor of the Roman Emperor Octavian Augustus, introduced the Chinese jujube from Syria to Italy and from there it was distributed to other Mediterranean countries.

Most of ancient Chinese date populations are located in spontaneous status in Dobrogea region, between the Danube and the Black Sea (Ciocârlan, 2000) close to the antique sites (Greek, Roman and Byzantine ruins). It is possible that they have been introduced in those times by the colonists (Stănică, 1997).

Locals named jujube, "Dobrogea olive", for its bushy canopy and small olives live fruits. The most known jujube population can be found in Jurilovca, near Doloşman Cape on the Razelm lake shore, close to the Ancient Greek Colony Argamum. Recently, another jujube local genotype was identified close to Sasovia Fortress at Mahmudia, Tulcea, on Sfântu Gheorghe Danube Arm (Stănică, 2019).

In Ostrov, Constanța county, there exists another genotype with tree habitus and bearing bigger fruits near the Byzantine Fortress Vicina - Păcuiu lui Soare and the Roman City of Durostorum on the right shore of Danube.

Beginning with 1996, following a cooperation project between the University of Agronomic Sciences and Veterinary Medicine of Bucharest and Shanxi Academy of Agricultural and Forestry Sciences and Taigu Fruit Research Institute, few genotypes of jujube have been introduced in Romania, at the Faculty of Horticulture.

The collection was completed in May-June 1998, during the visit of Mr. Bi Ping from Taigu Fruit Research Institute, when few other varieties were grafted on a Romanian rootstock

(Figure 1). The Korean variety Hongan was introduced by grafting from Italy, soon after.
Other new varieties were introduced after 2016, within the scientific cooperation between Hebei Agricultural University from Baoding and USAMV of Bucharest. In January 2018, it was inaugurated the China-Romania Joint Jujube Key Research Laboratory with the same partner.

Figure 1. Mr. Bi Ping, doing Jujube grafting (May 1998)

MATERIALS AND METHODS

After the introduction of the first jujube genotypes from China, researches were conducted at the Faculty of Horticulture within the University of Agronomic Sciences and Veterinary Medicine of Bucharest (Stănică, 2016).
The trees were planted in the jujube collection, at 4.0 m between rows and 2.5 m between plants on the row. The soil was maintained covered with grass by regular mowing. No irrigation, chemical fertilizers or phytosanitary treatments were applied.
Regular studies regarding the behaviour of jujube genotypes at Romanian conditions, by registering the plant phenology, tree growth, characteristics of leaves, flowers, fruits and seeds, resistance to pest and pathogens, etc. were applied. Several studies on generative and vegetative propagation, including *in vitro* micro-propagation of jujube genotypes were also finalized. A study regarding the microbial charge on the fruit in the preharvest phase, prior to picking was done, underlining the high density of different pathogens existing on an untreated orchard (Mardare et al., 2016). In the same time, the presence of several emerging pest was monitored in the jujube orchard (Ciceoi et al., 2017).
Lately, for several genotypes, studies on fruit behaviour in controlled atmosphere storage conditions (temperature: -2-0°C, humidity: over 95%, O_2 between 3% and 5% and CO_2 lower than 2%) were conducted.
Recently, the consumer preferences were analysed by using a test group of 116 persons with an age range from under 14 to over 66 years
Some fruits physical characteristics related to fruit size as weight (g), length and diameter (mm) and fruit shape index were analysed prior to the sensorial test in order to notice any correlation between them and the fruit sensorial appreciation. A constant research work along the years was done in Dobrogea region for the identification and characterization of the local genotypes of jujube (Ciocârlan, 2000; Stănică F., 2009)

RESULTS AND DISCUSSIONS

Phenology studies

The earliest bud breaking was recorded in the beginning of April, having even 7 days difference between genotypes, according to the temperature in the spring. When the bud breaking occurred later, the difference between genotypes was of only 2-3 days.
It was noticed that the flowering starts at the beginning of June, with no major differences between the genotypes and continues till end of July-beginning of August.
Instead, the fruit ripening varied with the genotype, from the third decade of September till the third decade of October. From the genotypes analysed, three had a compact ripening period of only 10 days (selection R2P4, Da Bai Ling and Feng Mi Guan), while selections R1P8 and R1P9 maturated their fruits during 30 days. Selections R2P2, R2P3, R1P8 and R1P9 were early ripening, while Xuan Cheng Jian and Cheng Tuo, were late ripening genotypes (Stănică & Vasile, 2008).

Propagation by seeds

For rootstock propagation, the use of sour jujube (*Ziziphus acido-jujuba* Mill.) seeds is most common practice. One of the most difficult phase of seed propagation was the extraction of the seeds from the hard-woody kernels. The raw kernels have a long duration and a very low germination rate. The mechanical seed extraction is difficult without the use of an appropriate equipment an having a small quantity of kernels. Instead, the kernel endocarp digestion using concentrated acids can be a simple method. Studies regarding the influence of different time treatments from 9 to 24 hours with concentrated H_2SO_4 on seed germination were done on Ostrov and sour jujube genotypes. The optimum time for the concentrated acid treatment was 12 hours, with 77% germination rate for Chinese sour jujube (Stănică et al., 1997).

***In vitro* propagation**

In order to find a rapid clonal propagation method, some studies regarding the *in vitro* multiplication were done. Two culture media: Murashige and Skoog (1962) and Quoirin and Lepoivre (1977) modified by Standardi and Catalano (Standardi & Catalano, 1985) and four hormonal balances with 0, 1.0, 2.0 and 3.0 mg/l BAP were tested. Best results were obtained on MS medium with the macro elements reduced in half (Stănică, 1997). The highest shoots multiplication rate was recorded at the concentration of 1.0 mg/l BAP. The highest shoots rooting percentage was obtained on MS culture media without cytokinin and with low auxins concentration (IBA 0.1 mg/l) (Stănică, 2002).

Propagation by cuttings

Several tests for the jujube clonal propagation by cuttings were made during the vegetation period and during dormancy, using rooting hormones: NAA, IBA, Radistim, Rhizopon, mist atmosphere and basal heating. Until now, no satisfactory results obtained.

Propagation by grafting

Grafting is the most used and the most efficient jujube propagation method that we applied. Since 1998, the new introduced varieties were over grafted on mature rootstocks using the top under bark method. Scions were taken from mother plants during dormancy and preserved at 3-4°C, wrapped in plastic film till the grafting moment (May). The individual one node scion waxing with white paraffin at 80-100°C is another possibility for long term preservation of scions (Stănică, 2002). Top grafting was use also for the propagation of the new varieties on Romanian rootstock as Ostrov and Jurilovca selections. The varieties 'Da Gua Zao', 'Shengli', 'Ji Dan Zao', 'Da Bai Zao', 'Da Bai Ling Zao', 'Da Ma Ya', 'Feng Mi Guan Zao', 'Long Zao', 'Tai Li Hong Zao', 'Li Zao' and 'Ban Zao' were grafted with a success rate between 92.5 and 100%. After grafting, scions grew fast and 7 from 9 varieties produced fruits in the grafting year (Figure 2). Fruit average weight varied between 4.63 and 20.89 g.

Figure 2. Jujube fruit formation in the grafting year

Jujube fruit characteristics

A special attention was applied on fruit analyses, both morphological and biochemical. There were conducted comparative researches between Chinese, Korean genotypes and the local biotypes.

Sour jujube including the Jurilovca population was compared with Chinese sour jujube and

Ostrov biotype (Stănică, 2009). The weight of fruits varied from 0.96 g (Chinese sour jujube) to 1.35 g (Jurilovca 2 population). The Ostrov biotype weight was 6.29 g.
There were not registered significant differences between kernel shape and size at the sour jujube biotypes from China and Jurilovca (Table 1).

Table 1. Characteristics of Romanian jujube local populations fruit and kernel

Selection	Fruit weight (g)	Kernel weight (g)	Kernel length (mm)	Kernel diam. (mm)
Sour jujube	0.96	0.21	8.2	6.5
Jurilovca 1	1.30	0.22	7.8	6.7
Jurilovca 2	1.35	0.25	9.7	6.8
Ostrov	6.29	0.35	13.9	7.6

In the Faculty of Horticulture collection, the genotypes recorded a fruit weight between 5.89 g (R3P10 selection) to 28.57 g (Cheng Tuo Zao). The fruit length varied between 27.0 mm (Feng Mi Guan Zao) to 53.0 mm (R3P4 selection) and the fruit diameters varied between 10/20 mm (R3P10 selection) to 39/40 mm (R3P7 selection).
Chinese date is very well known for the highest content in soluble solids and ascorbic acid.
Most of the analysed genotypes had the soluble solid content higher than 30% Brix. R2P4 selection recorded 39.60 % Brix. Fruit content in minerals varied between 0.16% and 3.38% with an average of 1.78%. The ascorbic acid content varied between 110.0 mg/100 g fw and 1020.0 mg/100 g fw (R1P11 selection) with an average of 306.1 mg/100 g fw. Fruit acidity, expressed in content of malic acid, varied from 0.16% to 0.82% with an average of 0.36%.

Phytosanitary studies on jujube fruits
Several analyses on jujube fruits immediately after harvest and during storage period were conducted in the last years, comparing the differences between genotypes from Bucharest collection. It is important to mention that, no chemical treatments were applied after the establishment of the jujube orchard.
The Chinese jujube is highly tolerant to pests and diseases and only few phytopathological agents have been recorded (Ciceoi et al., 2017).
On the fruit samples, the following pathogens, *Alternaria* spp., *Rhizopus* spp., *Fusarium* spp. and *Monilinia* spp., were identified after harvest. The infection rate ranged from 2% to 30% on *Alternaria* spp., 20% to 75% on *Rhizopus* spp., 0 to 68% on *Fusarium* spp. and 0 to 70% on *Monilinia* spp. (Mardare et al., 2016).
The stored fruits are susceptible also to fungal diseases and quality losses. Several studies showed that in the ripening period several pathogens as *Alternaria* spp., *Stemphyllium* spp., *Rhizopus* spp., *Penicillium* spp., *Fusarium* spp. and *Monilinia* spp. can be present on the fruits and can affect the fruits quality during storage (Dicianu et al., 2017). Some basic antifungal treatments has to be applied during the preharvest phase in order to ensure a "clean" fruit production at the ripening moment and during postharvest period.

Jujube pests
During the last 20 years, since we first introduced the jujube trees in the experimental field of the University of Agronomic Sciences and Veterinary Medicine of Bucharest, no pests were observe with the exception of the Mediterranean fruit fly (*Ceratitis capitata* Wied.), identified in 2013 (Chireceanu et al., 2013). Since only few early matured fruit have been affected, no chemical applications were needed. Instead, the year 2016 was totally exceptional from the point of view of climatic conditions and pests evolution, especially for the new invasive species. Four species were damaging the jujube crop, namely *Metcalfa pruinosa* Say, *Ceratitis capitata* Wiedemann, *Nezara viridula* (L.) and *Halyomorpha halys* Stal, while another 2 species producing damages were not yet identified (Ciceoi et al., 2017). Using online databases, we estimate the possible risk raised by other recorded pests of jujube crop in Romania.

Jujube fruit storage
After one month of storage, the jujube fruit weight losses varied between 1.05% at Taigu Ban Zao and Xuan Cheng Jian Zao and 4.65% at Hongan. After 60 days of storage the highest weight losses was registered at Xuan Cheng Jian Zao (5.70), while the lowest at Taigu Ban Zao (0.07%).

The qualitative losses were determined by several fungi infections as: *Alternaria* spp., *Rhizopus* spp., *Fusarium* spp. and *Monilinia* spp. (Figure 3). Even the fruits are carrying the pathogen agents from the field, the infection occurring in the preharvest phase, the incidence of qualitative losses was influenced by genotype. After one month of storage, few genotypes lost most of the fruits: R1P4 (81.64%), R1P7 (83.11%) and Taigu Ban Zao (89.65%). No qualitative losses were registered at: Sour jujube, Xuan Cheng Jian Zao, Shanxi Li Zao and Hongan (Dicianu et al., 2017).

Figure 3. Qualitative losses of Hu Ping Zao fruit after two months of storage, determined by preharvest fungi infections

The highest percentage of healthy fruits left after two months of CA storage were registered at Da Bai Ling Zao (75.00%), Sour jujube (73.45%) and Hongan (63.46%) (Stănică et al., 2018a).

Fruit Sensorial Analysis

Sensorial analysis was made beginning with the first fruits obtained in the Bucharest collection. Several external parameters like fruit size, typical shape, skin colour and skin status were studied. In the same time, some internal parameters like flesh colour, firmness and juiciness, taste and aroma were analysed.

The target public were very diverse, from experts in fruit growing, students to general public in different open day's events.

The results showed no significant correlations between the fruit weight and the consumer appreciation of the fruit size, the correlation coefficient being 0.45. In the same time, there were no significant correlations between the shape index and the consumer appreciation (correlation coefficient - 0.30).

By analysing the rates of different aspects that the panellists had to appreciate, one can see that the fruit size was noted with only an average of 3.22 while the colour with 3.11 out of 5 points possible. We think, both characters were subjective judged in comparison with similar know fruits and without having a clear base reference. Instead, the flavour and the general taste received both higher scores 4.14 and 4.15 respectively, showing that jujube fruits are appreciated by the consumers, even the analysed genotypes are mainly used for dried products and not for fresh consumption (Stănică et al., 2018b).

Identification of local Romanian jujube genotypes

After 1997, several expeditions have been organized in Dobrogea Region in order to identify Romanian jujube genotypes (Stănică, 1997; Ciocârlan, 2000). The Ostrov population located in the Southern part of Dobrogea is represented by highly productive trees (Figure 4) producing small fruits of over 6 g, sweet and sour, resistant to cracking (Figure 5).

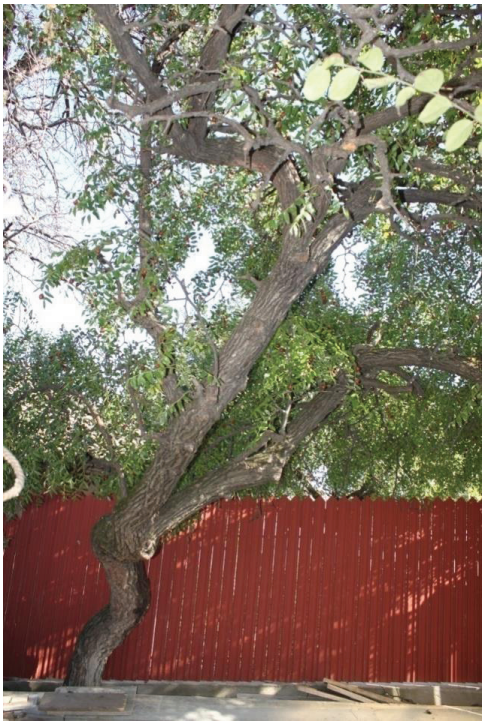

Figure 4. Tree of Ostrov Jujube population

Figure 5. Fruits of Ostrov Jujube population

In Jurilovca area at Doloşman Cape, jujube plants grow as high vigour bushes (Figure 6), while the fruits are similar with the sour jujube ones with 1.3 g, average size.

Figure 6. Jujube high bushes at Doloşman Cape, Jurilovca

Three Jujube genotypes were identified at Jurilovca, maybe being natural hybrids produced by free pollination followed by seed propagation (Figure 7).

Figure 7. Fruits of Jurilovca 1, 2 and 3 Jujube genotypes (from left)

In October 2009, a new local Jujube genotype was identified at Mahmudia, on the Sf. Gheorghe Danube Arm (Figure 8), close to the Sasovia Fortress (Stănică, 2019). The plants are similar with the Jurilovca genotypes, even they are located some 60 km farer. Two fruit types were found and described (Figure 9).

Figure 8. Jujube plants at Mahmudia, close to Sasovia Fortress on Sf. Gheorghe, Danube Arm

Figure 9. Fruits of Mahmudia 1 Jujube genotype

Rumours that some other Jujube genotypes are growing in Dobrogea has to be verified.
In the same time, genetic finger prints of the Romanian Jujube genotypes have to be made in order to see their parentage with some Chinese genotypes, and to establish from which Province of China they are originated.

CONCLUSIONS

Chinese Jujube is a new fruit specie for Romania being studied during over 20 years at the Faculty of Horticulture in Bucharest. The plant can be successfully propagated both by seeds or by vegetative ways. Even the grafting can be applied with good results, for the fast propagation of the new varieties, *in vitro* multiplication is recommended. The planting material starts to produce fruits in the first year after planting due to the Jujube high precocity.

Considering the complex composition of sugars, vitamins, amino acids, etc, Jujube fruits have an important nutraceutical effect, being extremely useful for the human healthy diet. Jujube dried fruits can be used as a source of raw material for other products. Having in mind that, in Romania, Jujube can be cultivated with organic technologies gives a highly value to this crop. The jujube trees are resistant to drought, tolerant to salt and can be cultivated on sandy soils. Over 700,000 ha are affected by these two problems especially in the Southern and North-Western part of Romania. With a high resilience to extreme temperatures: very low and very high, Jujube is the fruit tree recommended to cope with the climate change effects and to valorise the marginal soils. The interesting ornamental look of some varieties, recommends Jujube trees for landscaping too (Stănică, 2000).

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