

## REVIEWS

# **MORUS SPP. Material Conservation and Characterization and its Importance for Romanian Sericulture and GCEARS-PSP Development- A Review**

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## **Abstract**

*Morus spp.* is a perennial and woody plant, belonging to *Moraceae* family, genus *Morus* and is essential for sericulture. One of the main aims of Global Centre of Excellence for Advance Research in Sericulture and Promotion of Silk Production (GCEARS-PSP) is to create a reserve of *Morus spp.*, to multiply the vegetal material and to provide it to farmers and also to create an important biotechnological support for further scientifically works. As in Romania the intensive plantations are almost non-existent, one of the main objective of the centre is to create a real base for sericulture revival (the existence of nutritional base for silkworms) and to develop the biotechnological scientific component of the centre.

The aims of this review are to create a clear overview of moriculture for sericulture and to reveal the biotechnological potential and pharmacological uses of different mulberry parts. In this review the major findings regarding description of Kokuso 21 variety, mulberry micropropagation and bioactive compounds of different vegetal parts from *Mulberry spp.* are presented, and represent only the first stage of the GCEARS-PSP activity.

**Keywords:** *GCEARS-PSP, mulberry, conservation, bioactive*

## **INTRODUCTION**

**Global Centre of Excellence for Advance Research in Sericulture and Promotion of Silk Production (GCEARS-PSP)** ([www.usamvcluj.ro/crcas-ppm](http://www.usamvcluj.ro/crcas-ppm)) is located in UASVM CN and it is recognised by the International Sericulture Commission (<http://www.inserco.org/>) since 2014. The centre was created in order to achieve development of advanced research in sericulture and moriculture, to offer diagnostic services using modern techniques, to maintain and reproduce the gene pool of Romanian and foreign silkworm`s breeds and to promote the silk production.

Working in close collaboration with Laboratory of Bee's Products Control – APHIS, the GCEARS-PSP

researches regards the biotechnological aspects of *Morus spp.* The most popular species of mulberry are *Morus alba* and *Morus nigra*, and Yongkang (2000) states that the intensive selection and mutation breedings have resulted in thousands of cultivars, hybrids and polyploids. Mulberry trees are valuable species not only for silkworm rearing, but also for gardening and landscaping, street shade and reduction of pollution level in the environment and soil (Rafati et al., 2011).

The purpose of the present review is to create a realistic image of the main GCEARS-PSP activities in the past and in the future regarding the moriculture.

As it was stated by literature, the components of mulberry trees exhibiting their biological activity in the various pathological and health human ailments. Our research team want to draw the attention that mulberry is a source of potential indigenous nutraceuticals and functional food.

### Description of Kokuso 21 variety

One of the main achievements of GCEARS-PSP in 2017 was to set up a mulberry plantation in UASVM CN. This plantation was necessary for providing the vegetal material as food for the silkworms that the Department of Apiculture and Sericulture owns and material for the further biotechnological researches in moriculture (i.e., *in vitro* micropropagation, the extraction of biological active compounds, etc.). The mulberry from Kokuso 21 variety was purchased from Sericulture and Agriculture Experiment Station Vratza (Bulgaria) (<http://ses-vratza.bacsa-silk.org>), accompanied by phytosanitary certificate. The Sericulture Experiment Station (SES) in Vratza collects, characterizes and evaluates both indigenous and exotic mulberry varieties. Currently more than 140 mulberry accessions are maintained in the germplasm at SES-Vratza (Tzenov, 2002, cited by Weiguo, 2011). A number of 570 saplings were planted on 0.12 ha.

Kokuso 21 (*Morus latifolia*) has Japanese origins; it derives from the crossing between Naganua, Gariin and Shiso varieties and it is a bisexual variety.

In intensive type plantations it can produce from the first years a high quantity of leaves with high protein content. Kokuso 21 has a good development with long branches, large unlobed (23 cm x 17 cm), thick and juicy nutritive leaves with slow withering. Leaves yield under rain fed conditions is higher than 15 000 kg/ha (<http://ses-vratza.bacsa-silk.org/en/chernichevi-fidanki>).

FAO Electronic Conference on Mulberry for Animal Production (Morus1-L) states that "Ichinose" and "Kokuso 21" varieties have been used as parents for other varieties. One of the major reasons why "Ichinose" and "Kokuso 21" have been selected as parents is that "Ichinose" is female and "Kokuso 21" is male. They have desirable traits, and crossing is easy and simple.

Fertilization of *M. alba* Kokuso 21 with 120 kg N/ha applied in a large study described by

Papanastasis *et al.* (1995) was found very effective in increasing the production of this shrub.

Dolis *et al.* (2016) reported in leaves a general chemical composition, containing proteins 21.16%, fat 3.54%, crude fibre 17.88%, nitrogen free extract 43.42% and ash content 13.96%.

The biological and productive parameters of Kokuso 21, gived by Tănase (2007) are: number of branches/shrub – 24.6, branches length 115.2 cm, branches thickness 15 cm, internodes length 3.8 cm, leaf surface 292.3 m<sup>2</sup>, leaf weight 6.2 g, leaf production 3.0 kg/shrub/ series, leaves water content 75.73% and 25.45% protein content.

Even if the scientifically reports regarding Kokuso 21 variety are pretty poor, the existing studies presents very encouraging data, which made the decision of planting Kokuso 21 variety in our University, very easily.

### Mulberry micropropagation

As we state in the introduction one of the goal of GCEARS-PSP is to create a reserve of *Morus spp.*, and this aim will be reached by mulberry micropropagation.

The mulberry (*Morus spp*) is an important tree in the sericulture industry because its leaves represent the only source of food for *Bombyx mori*. Qualitative and quantitative improvements in mulberry varieties play a vital role in industrial advances. Unfortunately, the perennial nature of the plants, together with the species prolonged juvenile period, slows this process. Plant tissue-culture techniques have been used extensively for the stock improvement. During the last forty years, several researchers (Chattopadhyay and Datta, 1990; Chitra and Padmaja, 2001; Hossain *et al.*, 1990; Thomas, 2002) have reported success in plant regeneration from different explant types.

Mulberry has been micropropagated from many explants sources, such as sooth tips and axillary, isolated, or winter buds. The earliest reports, from Japan, include one from Ohyama (1970), who obtained the first complete plantlets from axillary-bud tissue. The majority of the research has involved in the use of MS (Murashige and Skoog, 1962) media for nodal cuttings, and for culture of axillary and apical buds. Other authors (Sharma and Thope, 1990) observed that an MS medium supplemented with 2.5 µM BSA (bovine serum albumin) was optimum for *in vitro*-raised seedlings of *Morus alba*. Their explants exhibited

significant differences in shoot-multiplication rates, and could be stored at 4°C for at least six months without any decrease in vigor. Rooting was enhanced by activated charcoal (0.05-0.10%). Transplantation was 100% successful when the shoots were grown in a 1:1 sand-vermiculite mix in the greenhouse (Pattnaik and Chand, 1997). Thomas (2002) in a review presents the main researched on tissue culture in mulberry.

The success of shoot-culture experiments is highly influenced by the source and age of the explant, as well as the conditions under which it is cultured. Positioning of the explant also plays a crucial role in bud induction (Hossain *et al.*, 1991). The same authors showed that, in the 10 studied genotypes, the maximum response of multiple-shoot induction was obtained from subterminal buds (Hossain *et al.*, 1992). They were able to produce multiple shoots from nodal explants of a 10 year old *Morus leavigata* tree. Shoot proliferation was higher when the BA level was raised to as much as 11 µM, but further increases suppressed development. The highest percentage of explants giving rise to shoots, and the maximum length of those shoots, was observed on MS media supplemented with 4.4 µM BA, whereas the greatest number of shoots per explants was obtained from 11 µM BA.

Paul *et al.* (2001) described a rapid *in vitro*-propagation procedure, using for culture young axillaries buds, which were multiplied, rooted and transplanted to soil within 3 months.

When micropropagated plants are compared with those generated through cutting, the former have significantly higher vigor. Zaman *et al.* (1997) have assessed the field performance and biochemical analyses of both tissue culture-derived plants and plants raised according to conventional methods. Their studies confirmed that micropropagated plants had significantly greater morphogenic vigor. The biochemical analysis of the leaves revealed no significant nutritional difference between the two types.

Survival rates for acclimatized plants varied from 75% in *Morus nigra* (Yadav *et al.*, 1990) up to 90-95% in *Morus nigra* (Jain *et al.*, 1992a, 1992b); *Morus ihou*, *Morus cathayana* and *Morus serrata* (Pattnaik and Chand, 1997) and *Morus australis* (Pattnaik *et al.*, 1996). Sharma and Thrope (1990) transplanted *Morus alba* plants with 100% success.

An important progress has been made in mulberry improvement through tissue-culture techniques. Micropropagation procedures are already standardized in various *Morus* species, fact that provides good chances to GCEARS-PSP to realize new mulberry plants.

Anyway, more researches are still needed in the area of haploid and triploid plant production, somatic embryogenesis, etc. The attention should be focused on genetic engineering in mulberry. Judicious choice of the explant source, coupled with some refinements in media composition, should increase the success rate for recalcitrant genotypes. Improvements in regeneration ability and control of somaclonal variation are crucial for maximum exploitation of this genus. When more emphasis is paid to these research areas, the future of sericulture industry will be assured.

#### **Bioactive compounds of different vegetal matrices from *Mulberry* spp.**

Mulberry is exclusively used for rearing silkworm due to the presence of unique chemo-factors like morin, β-sitosterol in leaves. Plant is a potential source for curing debilitating diseases. Flavonoids, anthocyanin and alkaloids present in the leaves, bark, root and fruits of mulberry play a pivotal role in containing free radicals and prooxidants generated in the body due to metabolism and phagocytosis (Ramesh *et al.*, 2014).

*Morus alba* tree bark, fruits, and leaves have been used in conventional and natural medicine (China and India) for the treatment of diabetes, atherosclerosis, hyperlipidemia, hypertension, and more recently, some cancer and neurogenerative diseases (Butt *et al.*, 2008). Mulberry leaves have been used for years to treat hyperglycemia, inflammation, cough, hypertension, cancer and fever (Kang *et al.*, 2006). Because of its good therapeutic activity and low toxicity, *Morus alba* has been extensively used in conventional Chinese medicine (Li, 1998). *Morus alba* is reported to have neuroprotective, skin tonic, antioxidant, anti-hyperglycemic, antibacterial, antihypertensive and anti-hyperlipidemic activities (Nomura *et al.*, 1980). Tables 1-3 present the general chemical composition of mulberry leaves.

**Table 1.** The average values of the main constituents in mulberry leaves (According to Flaczyk *et al.*, 2013)

Compound	Concentration g/100g	Phenolic acids	Concentration g/100g DW	Flavonols	Concentration g/100g DW
Water	5.2	Gallic	0.02	Rutin	0.91
Protein	14.70	Protocatechuic	0.16	Quercetin 3- $\beta$ -D- glucoside	0.54
Ash	22.37	p-hydroxybenzoic	0.11	Kaempferol 3- $\beta$ -D- glucopyranoside	0.32
Fat	0.11	Vanillic	0.71	Total Flavonols	1.76
Succharose	18.62	Chlorogenic	4.64		
Glucose	4.90	Caffeic	2.54		
Fructose	1.61	p-coumaric	0.27		
Xylose	0.85	Ferulic	0.39		
Galactose	0.27	Sinapic	0.21		
Other carbohydrates	17.04	Total phenolic acids	9.09		

DW = dry weigh

**Table 2.** Antioxidant compounds and activity of mulberry leaves (according to Flaczyk *et al.*, 2013)

Compound	Unit	Value
Total phenolics	g gallic acid equivalents/100g DW	14.42
Ascorbic acid	mg/100g of DW	1.76
Antioxidant activity	DPPH ( $\mu$ Mol Trolox /g DW)	214.08
	ABTS ( $\mu$ Mol Trolox /g DW)	51.76
	Chelating activity (%) for 0.002g	29.88

DW = dry weight

**Table 3.** Bioactive compounds in mulberry fruits, according to Zhang *et al.* (2008)

Phenolic compound	Concentration $\mu$ g/g FW	Flavonoid	Concentration $\mu$ g/g FW
Protocatechuic acid	14.66	Taxifolin	6.53
Chlorogenic acid	24.72	Rutin	111.38
4-CQA (CAE)	7.32	Kamepferon- hexoside	20.80
3,5-diCQA (CAE)	7.99	Quercetin	3.29
Total	54.68	Total	142
Total nonantocyanins phenolic (GAE)			653.2

FW = fresh weight



In the last period, wild food plants have become very attractive to the food industry, promoting their use as replacements for synthetic chemicals and nutraceuticals, in this way mulberry became a very important resource for its phytochemical composition, nutritional value and antioxidant properties.

As other authors done, Donno *et al.* (2014) identified mulberry as a rich source of antioxidant compounds; the observed fingerprint demonstrated that the species represent a rich source of phytochemicals, like organic acids, monoterpenes and polyphenolic compounds, especially flavonols and anthocyanins, which led to reasonably good overall fruit quality.

Among the bioactive compounds, one of the most important constituents of mulberry fruits is represented by anthocyanins (Lee *et al.*, 2005). Several studies have investigated the content of phenolics as flavonoids and anthocyanins in mulberry extracts, as well as carotenoids content (Arabshdi-Delousee and Urooi, 2007).

Due to this health-promoting compounds mulberry fruits are traditionally used as a laxative, odontalgic, anthelmintic, expectorant, hypoglycaemic and emetic agent; the traditional Chinese medicine used mulberry fruit as folk remedy to treat oral and dental diseases, diabetes, hypertension, arthritis and anaemia (Liang *et al.*, 2012).

Several researches investigated the nutraceutical properties of *Morus spp.* fruits, studying their nutritional potentials, but a complete profile with quality traits, phytochemical composition and antioxidant activity evaluation is still lacking. Previous studies have examined the total content of phenols, flavonoids, anthocyanins and antioxidant activity of *Morus spp.* grown in different regions (Chen *et al.*, 2012; Ozgen *et al.*, 2009; Uzun and Bayir, 2012), but total polyphenol content, antioxidant activity and most of the potential health-promoting agents of mulberry fruits still remain under scribed.

Our research team has already started a chemical characterisation of *Morus spp.* varieties. We started to analyze mulberry leaf and fruits, belonging to *Morus nigra* specie - Ukraina variety; using modern techniques for sugar spectrum, lipid, protein and mineral content, free amino acid profile, polyphenolic and flavones contents and the methods are presented by Bobiş *et al.*, 2018.

The high phenolic content and antioxidant activity of mulberry underline the nutritive and phyto-medicinal potential of this fruit. The results presented in this review indicate that *Morus spp.* has the potential to be further developed into a nutritionally and biotechnological valuable raw material for food and beverage application.

## CONCLUSIONS

The recovery of Romanian sericulture is possible through the GCEARS-PSP actions. Its success depends on different factors, such as the production performances of mulberry plantation, obtaining high quality leaves for *Bombyx mori* feeding and rich mulberry leaves and fruits for production diversification.

A number of 570 mulberry trees (*Morus latifolia*) were planted in USAMV CN.

The literature study presented in this review, contains the characterisation of this plant, its leaves and fruits composition, bioactivity and their therapeutically effects on human are shortly presented here, but it demonstrates that the GCEARS-PSP has the ability to reach its economical and scientifically objectives.

Further studies will be done on the components of Kokuso 21 variety leaf and fruits in order to provide to the local pharmaceutical industry a very valuable vegetal raw material and to provide to the silkworms a quality fodder base.

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