

Original Research Article

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Getting Sprouts from Mulberry Trees in *in vitro* conditions

Yo. Ya. Mirzaeva¹, N. A. Khujamshukurov^{2*} and D. X. Kuchkarova³

¹TSAU, ²TCTI, ³TACI (Uzbekistan)

*Corresponding author

A B S T R A C T

This article describes, which is widely used in the production of mulberry trees in Uzbekistan the hybrid of Uzbek mulberry (Oshima I₂N₂ (*Morus bombycis* Koidz.) × SANIISH-25 (*Morus multicaulis* Perr.), obtained by selection Jarariq 9 variety, grafted from Balkhi (*Morus alba* L.) and Shotut (*Morus nigra* L.) trees *in vitro* conditions in standard nutrient medium (MS, DKW, WPM) to get explant and their developing possibilities were analyzed. As a result of the research, the average values (R=0.05) of each mulberry explants were compared to the total samples in standard nutrient medium. In particular, in MS (standard) primary nutrient medium, the average budding time of the explant was 7.8 days (WPM-8.1; DKW-7.8), and the budblossoming of the explant was 46.30% (WPM-59.08; DKW-67, 73), the length of the central point of the buds, 2.92 mm (WPM-1.69; DKW-1.82), the length of the lower growing explant, 0.96 mm (WPM-1.84; DKW-1.33), the lower growing buds were observed 4.99 grains (WPM-3.5; DKW-5.05) and the overall viability of bud was 64.93% (WPM-63.17; DKW-72.74). During the observations, it was noted that the budding time of the explants vary than the blossoming of the bud. In particular, in the hybrid explant of Uzbekistan the length of the central growth point of bud was defined 2.9 mm, which budding time 8.33 hours and in the explant of Jarariq 9 variety 3.37 mm. Also, an average budding time were 7.88 hours, it was observed that the length of the central point of the bud was on average 1.58 mm in the Shotut (*Morus nigra* L.). The buddingtime of mulberry explants does not affect its central growth point length. According to the results of the analysis, to get explants of Uzbekistan hybrid and Jarariq 9 varieties and for developing them it is defined to effectively use MS, DKW, WPM standard nutrient medium. For Balkhi (*Morus alba* L.) and Shotut (*Morus nigra* L.) varieties, it is noted that the composition modify of MS, DKW, WPM standard nutrient medium.

Keywords

Mulberry (*Morus* sp.), Micro propagation, *in vitro* screening, *M. bombycis* Koidz., *M. multicaulis* Perr., *M. nigra* L., *M. alba* L.

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Introduction

In the conditions of the Republic of Uzbekistan the mulberry tree is the only feed

base of the sericulture industry, one of the strategic sources of providing the population with nutritious, rich in vitamins and medicinal properties. Mulberry trees, like other

defensive trees evaporate groundwater from their stems, also help to improve ameliorative condition of soils by lowering groundwater levels. The importance of mulberry trees against salinization and swamping. In addition, mulberry trees have been widely used as ornamental trees in urban and rural landscaping. Therefore, the Government of the Republic of Uzbekistan has developed a program for intensive development of mulberry farming mulberry trees are planted, 25720 ha in 2017, 32150 ha in 2018, 39545 ha in 2019 and it has been planned mulberry fields to plant 48244 ha in 2020, 54034 ha in 2021(1). In recent years, in Uzbekistan condition the demand is growing for Shotut (*Morus nigra* L.) and Balkhi (*Morus alba* L.) mulberries in the processing and storage of agricultural products. It is known that the founder of Eastern medicine, Abu Ali Ibn Sina, in his book "Laws of Medicine" described the healing properties of Balkhi (*Morus alba* L.) mulberry and *Morus nigra* (Shotut), their fruit is a very healing drug in the treatment of high blood pressure (2). Shotut (*Morus nigra* L.) fruit is rich in sugars, dye and pectin, carbohydrates, mineral salts, glucose, sucrose, fructose, apple, citric, phosphoric acids, as well as iron. In Uzbekistan condition dried fruit of the mulberry is stored for a long time, mulberry fruit is widely used in industry for the preparation of various confectionery products, juices, jams, vitamins and medicines. Wine made from Shotut (*Morus nigra* L.) fruit has a unique pleasant aroma and is highly valued by degustators compared to wine made from apples and plums.

In Uzbekistan, by propagating Shotut (*Morus nigra* L.) and Balkhi (*Morus alba* L.) mulberry are created large plantations, mainly by getting cuttings from existing trees in residential areas and farms, and selecting grafts adapted to specific soil conditions. In Uzbekistan, the grafting time of Shotut

(*Morus nigra* L.) and Balkhi (*Morus alba* L.) mulberry is different, depending on climatic conditions and seasonal changes in Tashkent, Syrdarya, Samarkand, Jizzakh, Fergana, Andijan, Namangan and Navoi regions in early April, Kashkadarya, Surkhandarya and Bukhara regions (southern regions) in March, in the Autonomous Republic of Karakalpakstan and Khorezm region (Northern regions) in the middle of April, it is grafted from the beginning the movement of liquid in the stem of mulberry. In Uzbekistan condition, Balkhi (*Morus alba* L.) mulberry is propagated by vegetative way due the seeds of fruits are not formed and are hollow. When arranging mulberry orchards, first Shotut (*Morus nigra* L.) and Balkhi (*Morus alba* L.) mulberry cuttings are prepared. Cuttings are prepared from annual branches of Shotut (*Morus nigra* L.) and Balkhi (*Morus alba* L.) mulberry in autumn, winter and early spring (before the onset of liquid movement in the tree). For grafting, annual mature sprouts of the plant are cut to a length of 40-50 cm, banded in 50-100 pieces and stored in special rooms at a temperature of 4°C, relative humidity of 70-80% (3).

It is known that the microclonal development method is primarily applied to plants that are very difficult to develop in other methods. This method allows you to grow in a short time, in large quantities, disease-free plant seedlings. In practice, almost all plants, decorative and room flowers, decorative shrubs and trees, fruit and vegetable crops, potatoes, fruit trees, and many medicinal plants are grown through microclonal development (4 - 8).

Although in Uzbekistan, large-scale cultivation of decorative flowers, fruit trees (9), vines (10), potatoes (11) by microclonal development has been established, no practical research has been conducted on mulberry trees.

The purpose of the work

Obtaining seedlings from mulberry trees and determining the importance of standard nutrient medium during their initial development.

Materials and Methods

Research methods (including equipment),
Research methods (including equipment).

Objects of research

Jarariq-9, Balkhi (*Morus alba* L.), Shotut (*Morus nigra* L.) and Uzbek hybrids of mulberry were spread, which are widely used in Uzbekistan.

The agronomic characteristics of Shotut (*Morus nigra* L.) are described as follows: high, i.e. the vigintidiaploid has $2n = 22 \times = 308$ chromosomes, and a plant equal to Shotut (*Morus nigra* L.) in terms of the sum of chromosomes is not found in the world. The beginning of the development period is middlelate. The deciduous trees are spherical, short, the diameter of the branch is large (thick.) The branch is large (thick) weakly clasped, dark brown, very large, longer, light brown, covered with bumps. The distance of joint is 4.0 cm. Varieties differ from varieties by slow growth. Sprout-oblong-round, very large, size 8.5 x 5.5 mm, dark brown, almost black.

Characteristic features are the stickiness of the bud and the absence of side buds. It is large bud among Central Asian varieties. Bisexual, single, mostly female. Fruit - broad oval, black, very short, thick, hard to cut fruit. The average weight of 1000 seeds is 2560 mg on average. Some seeds grow, but require 30°C heat and 80-90 days (late growing) for growing. Freshly harvested seeds grow in 42-45 days. The leaves are broadly heart-shaped,

whole or indented, glossy (light), rough-edged, with thick hairs on the underside, which give it a greenish-gray.

The leaves are thick, brittle (breaks quickly). The tubes are narrowly deep, sometimes intertwined. The edge is large serrated, the upper teeth are thick, gradually sharpened, about 20.0 mm long. The average size of leaves on growing varieties of uncut trees is 14.2 × 12.7cm. The stem of the leaf - short-haired, light green. The average length of the leaf's stem is 2.7 cm. Characteristic features - the fruit is stored for a long time, sweet (sour) sour, the fruit is sweet-juicy, the seeds are large. The variety is very resistant to late evening frosts. Yields in unbroken Shotuts (*Morus nigra* L.) are 10-20 kg per bush, and 6.3-12.6 tons of mulberry leaves per hectare (12).

Balkhi mulberry (*Morus alba* L.) was created by grafting and is also known as quail among the locals (13). It is characterized agronomically as follows: mulberry of 3 × ($2n = 42$) variety with three ploids. The onset period of development is long. Cutting trees consist of dense, flexible twigs with medium branches. One of its main features is the curvature of the twig, which is conical, brownish-brown, thick at the bottom and thin at the top. The bulbs are very large and sparse, light brown, the distance of joint is 4.4 cm on average. The sprout is small, oval, plump, dark brown, the average size is 4.7 × 4.0 mm. Gender - bisexual, mostly females.

Fruits are round or oval, white, average size 21.8 × 14.6 mm, outpouring with a beak. The fruit is smooth, so the size will also vary. The fruits stem is short, average 2.7 mm in length. The fruit is seedless, and at the bottom there are unripe seeds. The fruits are juicy, sweet, early ripening. The structure of the fruit is soft, shedding, slightly crumbly. Less fruitful on cut trees. The leaf stem -green, slender,

sparsely hairy, with an average length of 4.7 cm. The variety is resistant to frost. Yields -1 bush - an average of 7.1 kg, yielding an average of 86-90 ts / ha.

Jarariq 9 variety was selected by breeders of the Uzbek Sericulture Research Institute in 2000 from a selection nursery-garden at the Jarariq Experimental Farm. The stem is a pruned tree that grows strong and has many branches. Annual varieties reach an average height of 242.8 cm at the end of the vegetation period. Branch - round, gray. The length of the joint distance is 2-2.5 cm. The buds are large triangular, brown, with side buds. Gender - male. The leaves are large, whole, heart-shaped, elongated, the base is deeply incised, the tip of the leaf is of medium length. The leaf surface is smooth, dark green, the leaf edge is serrated, the average size is 16.3 × 12.7 cm in spring and 19.4 × 17.3 cm in summer. It differs from other varieties by strong growth of the shoot, the size of the leaf, dark green. The average yield of shrub mulberry is 72.71 kg / ha (14).

Uzbek hybrid mulberry Oshima I₂ N₂ (*Morus bombycis* Koidz.) × SANIISH-25 (*Morus multicaulis* Perr.) was created at the Jarariq Experimental Farm of the Uzbek Sericulture Research Institute. This hybrid is reminiscent of varietal mulberries with the integrity, size and appearance of the leaf, even in the periods of sprouts, seedlings and adulthood. The body is erect, strongly developed, there are many branches, the growth period is long. Branch-round, long, straight-growing, vigorous, gray in color, various forms, the distance of joint average 3-5 cm, erect.

The buds are medium-large, brown, the average side buds are 3-4 mm in size. The leaves are wholly, large, ovate-oblong, medium-thick, medium-veined, the surface is rough, the average size of a summer leaf is 22.4 × 16.1 cm. The average weight of a

summer leaf is 6.86 g. Gender - female. Fruit - cylindrical, dark black, fruitful, seeds are dark. Mulberry seeds are grown on the farms of Uzbekistan from the parental varieties of this hybrid mulberry. The yield is 1.825 kg per 1 bush of mulberry bush and 91.25 kg / ha per 1 ha (15).

Timerosal 0.0005-0.001 mg / l, hypochlorite (Na or Ca salt) 1-10% solution, Silver nitrate (AgNO₃) 0.001-0.005% and ethyl alcohol 70-A 96% solution was used as a sterilizer. The following nutrients were used for *in vitro* culture: Murasige and Skug (MS) (16), Woody plant medium (WPM) (17), DKW-Driver and Kuniyuki (17). The thermolabile substances were filtered through a Millipor filter in a SWIN device. Growing conditions: photo period - 16/8 hours day and night, light level 4000-6000 lux, 24-26°C.

Relative humidity 60-70%. The process of introduction of mulberry into *in vitro* culture was carried out in different nutrient compositions, and this process was carried out in several variants. The calculation of statistical error, mean, reliability intervals and standard deviations to the experimental data was performed using the computer program STATISTICA 6.0 and standard methods. The statistical significance of the results was determined using the Student t-criterion.

Results and Discussion

During the research was studied the development of mulberry tree seedlings in a wide variety of nutrient medium, where plants are widely used *in vitro*. The results of the research determined that in the MS nutrient medium, which is widely used in scientific research, the average budding time of Uzbek hybrids was 6.6 days, Jarariq 9 and 8.33 Balkhi (*Morus alba* L.) mulberry - 8.6 and Shotut (*Morus nigra* L.) 7.88 days (Table 1). In the comparative analysis, it was noted that

the budding time of explant of hybrid plantations of Uzbekistan, 2 days earlier than Jarariq 9 and Balkhi (*Morus alba* L.) mulberry plantations, one day earlier than Shotut (*Morus nigra* L.). It was also observed that the rate of bud opening of the explant varied. In particular, in the hybrids of Uzbekistan hybrid and Jarariq 9 were 51.06%, 53.5%, respectively, in Balkh and Shotut were 47.36 and 33.3%.

During the observations, it was noted that the budding time of the explants varied relative to the bud blossoming. In particular, in the hybrid explant of Uzbekistan the length of the central growth point was 2.9 mm, while the budding time was 8.33 hours, it was determined 3.37 mm in Jarariq 9. It was also observed that the length of the central point of the bud was 1.58 mm on average in the Shotut (*Morus nigra* L.) expansion, with an average budding time of 7.88 hours. This suggests that the budding time of mulberry tree seedlings does not affect its central growth point length. The same indicators can be observed in the WPM nutrient medium (Table 2). In particular, the hybridization of Uzbekistan had a ripening time of 7.33 days, the length of the central point of the bud was 1.25 mm, and the budding time was 7.6 days, and in the Jarariq 9 explant it was 3.59 mm.

In the standard nutrient medium studied, a cross-correlation of the number of lower-growing shoots of the seedlings was also observed. In particular, the average number of lower growing shoots in the hybrids of Uzbekistan in the MS medium was 5.7, while in the WPM medium it was 3.5, and in the DKW 7.6 (Table 1-3). It was also found that the bud viability of Uzbekistan hybrids was 75.01% on average in the study nutrient medium, 74.75% in Jarariq 9, 60.48% in Balkhi (*Morus alba* L.) and 57.54% in Shotut (*Morus nigra* L.).

During the research, the development of explants of mulberry tree seedlings studied in different standard nutrient medium were analyzed. The results showed that the budding time of the explants averaged 8.1 days in the WPM medium and 7.8 days in the MS and DKW medium.

Blossoming bud in the explants was observed in an average of 59.08% in the WPM nutrient medium, 46.30% in the MS nutrient medium, and an average of 67.73% in the DKW nutrient medium. When the viability of the explant buds was studied in terms of nutrient media, it was observed that DKW was 72.74% in the nutrient medium, WPM was 63.17% in the nutrient medium, and MS was 64.93% on average in the nutrient medium. However, an inverse correlation can be observed, including the fact that in the MS medium the explant buds of Uzbekistan hybrids showed a survival rate of 74.73% in the MS medium, 71.46% in the WPM medium and 78.86% in the DKW medium. In Jarariq 9 variety explants, MS was found to have a survival rate of 71.46% in the nutrient medium, 82.73% in the WPM medium, and 70.06% in the DKW medium. This situation can be explained by the fact that when obtaining explants from mulberry trees depends on their varieties and exactly how they are propagated (seeds, grafts) (18).

Conclusion of the study is as follows:

In the result of research have determined that the obtained by hybrid of mulberry tree from Uzbekistan hybrid (Oshima I₂N₂ (*Morus bombycis* Koidz.) × SANIISH-25 (*Morus multicaulis* Perr.)) and Jarariq 9 variety, obtaining explants by *in vitro* condition and their development in MS, DKW and WPM nutrient medium was found to be usable. An average survival rate of 75.01% of hybrid plantations in Uzbekistan can be achieved in the studied standard nutrient medium.

Table.1 Developing indicators, the explants of mulberry tree in MS (standard) primary nutrient medium

Experiment Examples	Experiment Variety	Nutrient medium	Average budding time of explant, Day	Blossoming of bud in the explant, %	The lengths of central point of buds, mm	The lengths of lower growing buds, mm	The number of lower growing buds, piece	Viability of common buds, %
1	Uzbekistan hybrid	MS	6	35,6	2,81±0,88	0,7±0,13	3,7±0,14	61,4±3,41
2			7	42,4	2,72±0,74	0,9±0,08	5,2±0,45	74,2 ±0,95
3			7	75,2	3,36±0,36	1,3±0,18	8,2±0,19	88,6 ±1,24
Average indicator per examples			6,6	51,06	2,96	0,96	5,7	74,73
1	Jarariq 9 variety	MS	8	53,6	3,01±0,18	0,66±0,46	4,8±0,25	68,8±0,31
2			8	54,1	3,24±0,11	0,92±0,32	5,1±0,11	72,2 ±0,11
3			9	52,8	3,86±0,42	0,82±1,14	5,8±0,25	73,4 ±1,23
Average indicator per examples			8,33	53,5	3,37	0,8	5,23	71,46
1	Balkhi (<i>Morus alba</i> L.)	MS	9	35,3	2,11±1,28	1,4±0,85	4,6±1,34	57,6±1,41
2			8	48,2	3,24±1,14	0,9±1,27	5,3±0,27	63,7 ±1,28
3			9	58,6	6,01±0,32	2,4±1,44	5,7±1,23	78,6 ±0,33
Average indicator per examples			8,6	47,36	3,78	1,56	5,2	66,63
1	Shotut (<i>Morusnigra</i> L.)	MS	8	28,3	1,21±0,18	0,4±0,23	2,1±0,24	33,4±1,12
2			7	28,3	1,41±0,21	0,3±0,11	4,1±0,11	39,1 ±0,88
3			9	43,3	2,13±0,11	0,9±0,22	5,3±0,13	68,2 ±1,44
Average indicator per examples			8,0	33,3	1,58	0,53	3,83	46,9
Average indicator according to common examples, P=0,05			7,8	46,30	2,92	0,96	4,99	64,93

Table.2 Developing indicators, the explants of mulberry tree in WPM (standard) primary nutrient medium

Experiment examples	Experiment Variety	Nutrient medium	Average budding time of explant, Day	Blossoming of bud in the explant, %	The lengths of central point of buds, mm	The lengths of lower growing buds, mm	The number of lower growing buds, piece	Viability of common buds, %
1	Uzbekistan Hybrid	WPM	7	52,2	1,12±1,21	0,6±0,11	2,8±0,41	68,5±1,14
2			8	59,6	0,92±0,36	0,9±0,28	4,6±1,14	77,4 ±1,33
3			7	58,8	1,72±0,12	0,6±1,13	3,1±1,23	68,5 ±0,56
Average indicator per examples			7,33	56,8	1,25	0,7	3,5	71,46
1	Jarariq 9 variety	WPM	9	68,6	3,11±0,23	4,6±0,33	6,6±1,11	68,6±0,44
2			7	76,6	4,21±1,13	5,3±0,41	8,2±1,24	92,8 ±1,22
3			7	83,4	3,46±1,33	4,9±0,12	7,4±0,38	86,8 ±0,33
Average indicator per examples			7,6	76,2	3,59	4,93	7,4	82,73
1	Balkhi (<i>Morus alba</i> L.)	WPM	9	48,4	1,21±1,28	1,2±0,14	1,2±0,24	33,2±1,41
2			9	56,3	0,93±0,11	0,9±0,11	2,3±1,14	38,3 ±1,28
3			8	61,2	1,34±0,42	1,4±0,32	1,6±0,33	48,2 ±0,33
Average indicator per examples			8,66	55,3	1,16	1,16	1,7	39,9
1	Shotut (<i>Morusnigra</i> L.)	WPM	9	43,3	0,48±1,42	0,46±0,12	1,2±1,33	45,5±0,11
2			9	52,2	0,96±1,24	0,62±0,42	1,8±1,14	68,1 ±0,42
3			9	48,6	0,86±1,32	0,72±0,33	1,2±0,36	62,2 ±0,34
Average indicator per examples			9	48,03	0,76	0,6	1,4	58,6
Average indicator according to common examples, P=0,05			8,1	59,08	1,69	1,84	3,5	63,17

Table.3 Developing indicators, the explants of mulberry tree in DKW (standard) primary nutrient medium

Experiment examples	Experiment Variety	Nutrient medium	Average budding time of explant, Day	Blossoming of bud in the explant, %	The lengths of central point of buds, mm	The lengths of lower growing buds, mm	The number of lower growing buds, piece	Viability of common buds, %
1	Uzbekistan hybrid	DKW	8	68,4	3,12±1,18	1,8±0,32	6,6±0,38	72,6±0,44
2			7	72,2	2,48±1,36	2,6±0,42	7,4±0,64	77,4±0,12
3			8	78,8	2,24±1,32	3,4±0,13	8,8±0,44	86,6±0,42
Average indicator per examples			7,6	73,13	2,61	2,6	7,6	78,86
1	Jarariq 9 variety	DKW	8	78,4	2,14±0,11	0,8±0,42	6,4±0,11	69,4±1,11
2			8	72,4	1,66±0,33	1,2±0,11	5,2±0,33	72,2±0,18
3			8	78,6	2,28±0,24	1,1±0,24	6,2±1,12	68,6±1,14
Average indicator per examples			8	76,4	2,02	1,03	5,93	70,06
1	Balkhi (Morus alba L.)	DKW	8	68,2	1,4±1,14	1,1±1,22	4,6±1,01	78,4±0,34
2			8	66,4	1,6±1,12	0,9±1,04	3,8±0,96	72,2±0,28
3			8	62,6	1,1±1,33	0,8±1,23	3,2±0,48	74,2±1,12
Average indicator per examples			8	65,73	1,36	0,93	3,86	74,93
1	Shotut (Morus nigra L.)	DKW	8	58,2	1,38±0,14	0,86±0,22	2,6±0,31	68,4±1,24
2			7	54,6	1,22±0,11	0,72±0,18	3,1±0,44	66,2±1,33
3			8	54,2	1,34±0,42	0,78±0,11	2,8±0,25	66,8±0,86
Average indicator per examples			7,6	55,66	1,31	0,78	2,83	67,13
Average indicator according to common examples, P=0,05			7,8	67,73	1,82	1,33	5,05	72,74

In Jarariq 9 variety explants, the indicator is 74.75%. It was found that in these nutrient medium it is possible to provide survival on average 60.48% in Balkhi (*Morus alba* L.) mulberry plantations and 57.54% in Shotut (*Morus nigra* L.) plantations.

In standard nutrient medium (MS, DKW, WPM) in the explants of Uzbekistan hybrids were 5.6 piece in low-growing buds, while in the explants of Jarariq 9 variety this indicator was 6.1 piece. In this standard nutrient medium, the average number of lower-growing buds of *Morus alba* L.(Balkhi) mulberry explants was 3.5, and in the *Morus alba* L. (Shotut) explants was 2.6 piece. Therefore, it was determined that in obtaining mulberry explants to achieve 100% results should be modified the composition of nutrient medium (MS, DKW, WPM).

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