Nutrient types for explants in in-vitro propagation of mulberries and microcloning indicators of mother material

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Abstract. Uzbek hybrid (♀*Oshima* I2N2 (*Morus bombycis Koidz*.)× ♂SANIIsh-25 (*Morus* multicaulis *Perr*.)) obtained by crossing the mulberry tree, obtained by selection in vitro from Jarariq-9 variety Murasige Skug (MS), Woody Plant Medium (WPM) and Driver and Kuniyuki (DKW) medium were used to obtain and develop explants. The average germination time of the explant in the WPM (standard) main nutrient medium in Uzbekistan hybrid, day-7.3 MS-6.6 days, DKW-7.6 days, but opening from the explant in WPM-56.8, MS-51.06%; DKW was observed to be 73.13%. It was observed that total shoot germination was MS-74.73 %; WPM-71.46%; DKW-78.86 %. It is determined that the indicators of Jarariq-9 variety compared to the indicators of the Uzbekistan hybrid, the average germination time of the explant in the WPM (standard) main nutrient medium is 7.6 days, MS-8.33 days, DKW-8 days, bud opening from the explant is 76.2% in WPM, MS-53.5%; and, DKW-74.4% Keywords: Mulberry, hybrids, in-vitro, sericulture.

1 Introduction

The scientific institutions of countries with developed sericulture are paying special attention to the breeding of economically efficient mulberry varieties that are adapted to various climate stress conditions, have high nutritional value, leaf productivity and protein content, and based on natural climatic conditions [1-3]. With the effective use of vegetative, marker-associated selection and microcloning methods of modern in vitro cultivation of plants, 626,000 hectares in the people of the Republic of China, 280,000 hectares in India, and more than 35,000 hectares in Thailand and Brazil are cultivated [4-6].

The Decree of the President of the Republic of Uzbekistan No. DP–4947 "On the strategy of actions for the further development of the Republic of Uzbekistan in 2017-2021" on February 7, 2017, No. RP–4411 "Additional measures for the development of deep processing in the cocoon industry" on July 31, 2019, No. RP–4567 "On additional measures for the development of the silkworm feed base in the cocoon industry" on January 17, 2020 [7]. Scientists such as M.I.Grebinskaya, K.Rakhmonberdiev, O.Polatov, R.Abdullaev, U.Kuchkarov, M.Zhorayev, D.I.Kholmatov, and M. Khibbimov have been conducting scientific research on breeding mulberry saplings in different ways in Uzbekistan [8-10].

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It should be noted that in the republic, no scientific researches have been conducted on modern in vitro reproduction of mulberry and agrotechnology of primary saplings cultivation, but several scientific researches on in vitro microclonal reproduction of potatoes, apples, grapes and cherries are conducted extensive scientific research by K.S. Sultonov, S.Kh.Abduramanova, E.G.Kholmuratov, G.B.Nasirova, and M.Sh.Sabirova [11-15].

Therefore, the nutritional value of the mulberry tree for the silkworm is high, it is great scientific and practical importance to develop the agrotechnology of primary sapling cultivation by in vitro breeding of high-yielding varieties, and to carry out scientific research on the continuous development of the nutritional base of silkworm and the increase of the volume of fertile saplings [16-18].

2 Materials and Methods

The object of research is selected Jarariq-9 and Uzbek hybrids belonging to the genus Morus of the mulberry tree widely used in local conditions.

The subject of the research is the in vitro breeding of mulberry varieties and hybrids, determination of the nutrient medium for their explants, and selection of the appropriate ones.

Agrotechnologies of mulberry tree care, cultivation of mulberry saplings by micro-grafting method, statistical analysis of experimental results (*Dospekhov*) methods were used in the research:

- it is justified that the use of the in vitro method in the cultivation of primary saplings of mulberry free from pathogens for the first time in Uzbekistan;
- it is determined that the effectiveness of taking explants from the apical meristem of the Jarariq-9 variety and Uzbekistan hybrid for in vitro cultivation in April and May;
- it is determined that Woody Plant Medium was found to be the nutrient medium that provides the maximum regeneration level for micropropagation of mulberry in vitro;
- it is selected that 16/8 day-night, light mode 2000-3000 lux, room temperature 20-22(C), relative humidity 60-70% norm was chosen for growing mulberry varieties and hybrids in vitro;
- it is determined that IMK-0.1-0.2 mg/l of growth regulators for the induction of the process of root formation of mulberry varieties and hybrids; BAP-0.1-0.01 mg/l; A moderate proportion of Gambor (V5)-2%.

It is developed that the technology of establishing mother mulberry trees in a protected and open area, which allows rapid reproduction of high-quality sprouts and saplings of new valuable varieties of mulberry.

3 Results and Discussion

It is defined that the scientific significance of the research results is to multiply explants of mulberry Jarariq-9 variety and the hybrid of Uzbekistan in vitro, determining the composition of the necessary nutrient medium for them and sterilizing of them, obtaining clean explants, propagation, rooting and explaining by the development of methods of transfer from in vitro (cultivation of material in a sterile state in an artificial nutrient medium) to in vivo (cultivation of material cultured in a sterile state in natural conditions).

Uzbek hybrid (♀*Oshima* I2N2 (*Morus bombycis Koidz.*)× ♂SANIIsh-25 (*Morus* multicaulis *Perr.*)) obtained by crossing the mulberry tree, obtained by selection in vitro from Jarariq-9 variety Murasige Skug (MS), Woody Plant Medium (WPM) and Driver and Kuniyuki (DKW) medium were used to obtain and develop explants.

The average germination time of the explant in the WPM (standard) main nutrient medium in Uzbekistan hybrid, day-7.3 MS-6.6 days, DKW-7.6 days, but opening from the

explant in WPM-56.8, MS-51.06%; DKW was observed to be 73.13%. It was observed that total shoot germination was MS-74.73 %; WPM-71.46%; DKW-78.86 %.

It is determined that the indicators of Jarariq-9 variety compared to the indicators of the Uzbekistan hybrid, the average germination time of the explant in the WPM (standard) main nutrient medium is 7.6 days, MS-8.33 days, DKW-8 days, bud opening from the explant is 76.2% in WPM, MS-53.5%; DKW-74.4% (Table 1).

Table 1. The average development of indicators of mulberry Uzbekistan hybrid and Jarariq-9 variety explants in different nutrient medium (2017-2019).

Experience samples	Experience varieties and hybrids	Food environment	Average germination time of the explant, days	Bud opening from explant, %	Total shoot germination %
1	A hybrid of Uzbekistan	MS	6.6	51.06	74.73
2		WPM	7.33	56.8	71.46
3		DKW	7.6	73.13	78.86
Sample average			**7.17**	**60.33**	**75.01**
1	Jarariq-9	MS	8.33	53.5	73.09
2		WPM	7.6	76.2	82.73
3		DKW	8	76.4	70.06
Sample average			**7.97**	**68.7**	**75.29**
Average indicator of two samples			7.57	67.67	75.15
The average indicators of the total samples, EKF-0.5			0.21	0.49	0.13

It was noted that the time of budding of the explants was different compared to the opening during the observations. It was determined that total shoot germination was MS-71.46%; WPM-82.73 %; and, DKW 70.06 % (Figure 1).

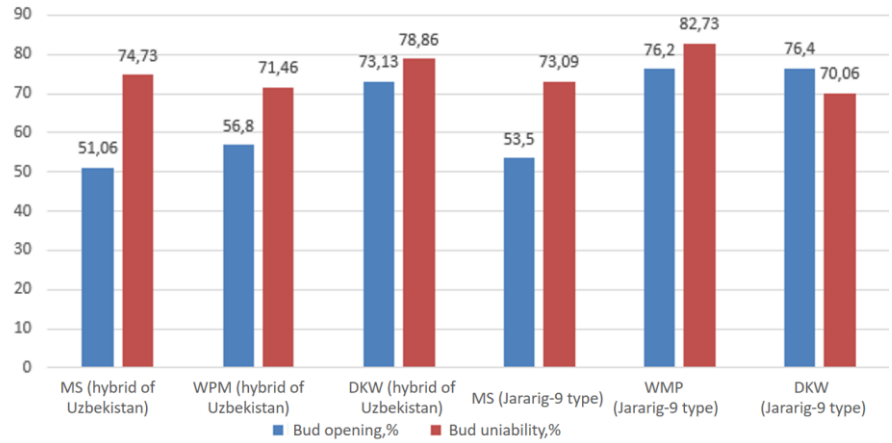

Fig. 1. Development indicators of explants of Uzbekistan hybrid and Jarariq-9 varieties of mulberry in MS, WPM, DKW nutrient medium.

In addition, in the Uzbekistan hybrid explant, the average budding time was 8.33 hours, while in the Jarariq-9 explant, the average budding time was 7.6 hours. It depicts that the budding time of mulberry tree explants is not affected by the length of its central growing point.

According to the results of the analysis, it was noted that WPM, MS, DKW, standard nutrient medium can be effectively used for obtaining and developing explants from Uzbekistan hybrid and Jarariq-9 varieties.

It was noted that the average germination time of the explant obtained from the Uzbekistan hybrid is 6.6 days, and 8.3 days for the Jarariq-9 variety in MS nutrient medium, which is widely used in scientific research.

If the time of budding of Uzbekistan hybrid explants was observed to start 2 days earlier than Jarariq-9 explants, the indicators of budding of explants were also different (51.06% in Uzbekistan hybrid and 53.5% in Jarariq-9 variety)

It was found that the average germination time of Uzbekistan hybrid is 7.33 days, Jarariq-9 and explant is 7.6 days in WPM medium. The indicators of budding of the explants were also different, it was found that it was 56.8% in the Uzbekistan hybrid and 76.2% in the Jarariq-9 variety. It was observed that the average germination time of Uzbekistan hybrid explants is 7.6 days in DKW-food medium, and 8 days in Jarariq-9 variety.

It was observed that the indicators of budding of the explants differed from each other and were 73.13% in Uzbekistan hybrid and 76.4% in Jarariq-9. The highest indicator was obtained in WPM in our nutrient medium selected for in vitro propagation of Uzbekistan hybrid and Jarariq-9 variety (Figure 2). In the course of research, it was noted that hybrid cultures are easier to propagate in vitro than mulberry cultivars. In our further studies, only the most effective nutrient medium in the propagation of mulberry cultivars was carried out in WPM. In Table 2, young saplings grown from Uzbekistan hybrid seeds on the 14th day of observation were 4.16 cm tall, 0.23 cm root length, 2 roots, and according to the average results obtained on the 24th day of observation, the height was 9.16 cm.

Fig. 2. Development of selected research facilities in the WMP feed environment under “inti vintro” conditions and adaptation to the substrate. A-view of the grown explants without preliminary root dressing in the WMP nutrient environment of the Jarariq-9 variety (B) and Uzbekistan hybrid (C) explants and adaptation to the natural substrate.

Table 2. Selection of the first maternal material obtained from the Uzbekistan hybrid of mulberry from among the samples (2017-2019).

Materials selected for the experiment	Revision	Planting time	Initial state, cm	Result of 14 days			Result of 24 days		
				Height, cm	Height of root, cm	The number of roots	Height, cm	Height of root, cm	The number of roots
A young sprout from a seed	1	April	1.5	4	0.2	2	10	1.6	4
	2	April	1.5	3.5	0.3	3	8	1.1	5
	3	April	1.5	5	0.2	2	9.5	0.8	5
Average				4.16	0.23	2.33	9.16	1.16	4.6
2-3 years old mulberry sapling	1	April	1.5	6	1.5	4	11	2.6	7
	2	April	1.5	7	0.9	3	10	3.1	5
	3	April	1.5	6.5	0.7	5	10.5	2.0	8
Average				6.5	1.03	4	10.5	2.56	6.6
10-15 years old mulberry tree	1	April	1.5	6	0.3	3	10	1.5	5
	2	April	1.5	5	0.5	2	8.7	1.0	3
	3	April	1.5	4.8	0.7	2	8	0.9	3
Average				5.2	0.5	2.3	8.9	1.13	3.6
The average indicator of sample				5.28	0.58	2.87	9.52	1.61	4.9
The average indicators of the total samples, EKF is 0.5				1.04	0.11	0.64	1.71	0.27	0.83

It was noted that the root length was 1.16 cm, and the number of roots was 4.6. Also, the height of the 2-3-years old mulberry sapling of Uzbekistan hybrid was 6.5 cm, the root length was 1.03 cm, the number of roots was 4, and on the 24th day of observation, the height was 10.5 cm, the root length was 2.56 cm, and the number of roots was 6.6. The average result obtained from a 10-15-year-old mulberry tree of Uzbekistan in 14 days is 5.2 cm in height, 0.5 cm in root length, 2.3 roots, and in 24 days the average result is 8.9 cm in height, 1 root length. 13 cm, the number of roots was 3.6. According to the results of the study, it was determined that it is appropriate to take the maternal explant from the apical meristem of 2-3 years old mulberry saplings. The average result of 2-3 years old mulberry saplings of Jarariq-9 variety in 14 days was 7 cm, root length, 1.3 cm, the number of roots was 4.3, and the average result in 24 days was 11.06 cm, root length, 3. 1 cm, the number of roots was 7.3 (Table 3). The average result of 10-15-year-old mulberry tree of Jarariq-9 variety in 14 days is 5.4 cm height, root length, 0.6 cm, number of roots is 2.6 and the average result obtained in 24 days is 9.36 cm height, root length, 1.53 cm, the number of roots was 4.3. In the course of research, it was noted that the most optimal sample for in vitro reproduction should be taken from 2-3 years old mulberry trees with an active growing season. Therefore, further studies were conducted on the basis of 2-3 years saplings of mulberry Jarariq-9 variety and Uzbekistan hybrid.

Table 3. The selection of the first maternal material from the samples of Jarariq-9 variety of mulberry (2017-2019).

Materials selected for the experiment	Revision	Planting time	Initial state, cm	Result of 14 days			Result of 24 days		
				Heght, cm	Height of root, cm	The number of roots	Height, cm	Height of root, cm	The number of roots
A 2-3 years old mulberry sapling	1	April	1.5	6.8	1.6	5	12	2.8	8
	2	April	1.5	7.3	1.3	3	11	3.3	7
	3	April	1.5	6.6	1.7	5	10,5	2.1	7
Average				7	1.3	4.3	11.1	2.7	7.3
10-15 years old mulberry tree	1	April	1.5	6.1	0.5	3	11	1.7	6
	2	April	1.5	5.2	0.7	3	8,9	1.1	4
	3	April	1.5	4.9	0.7	2	8,2	1.8	3
Average				5.4	0.6	2.6	9.36	1.53	4.3
The average of two samples				6.2	0.95	3.45	10.23	2.11	5.8
The average indicators of the total samples, EKF-0.5				0.17	0.08	0.83	0.16	0.71	1.03

In the first microcloning of the 2-3 years old mulberry saplings of Uzbekistan hybrid, the growth rate of the maternal explant was 10.16 cm on average, the root length was 2.56 cm, and the number of roots was 6.6 (Table 4).

In the second microcloning, the plant length of the explants adapted to the nutrient medium was 11.1 cm, the root growth was 7.9 cm, and the number of roots was 8.56. Compared to the first 10 days in the second cloning, the plant grew 2 cm faster, the root was 1.23 cm, and the number of roots was 1.33, and in the second 10 days, the plant was 1 cm longer, the root growth was 5.6 cm, and the number of roots was 2 more.

Table 4. The indicators of microcloning of maternal explants selected from 2-3 years old mulberry saplings of the Uzbekistan hybrid (2017-2019).

Materials selected for the experiment	Revision	Planting time	Initial state, cm	Result of 14 days			Result of 24 days		
				Heght, cm	Height of root, cm	The umber of root	Heght, cm	Height of root, cm	The number of roots
A 2-3 years old mulberry sapling	1	April	1.5	6	1.5	4	10	2.6	7
	2	April	1.5	7	0.9	3	10	3.1	5
	3	April	1.5	6.5	0.7	5	10.5	2.0	8

Average				6.5	1.03	4	10.16	2.56	6.6
A 2-3 years old mulberry sapling	1	May	1.5	8	2.9	5	10.9	8	8
	2	May	1.5	9	1.8	6	11	7.9	9
	3	May	1.5	8.5	2.1	5	11.5	8	9
Average				8.5	2.26	5.33	11.1	7.9	8.66
The average indicator of sample				7.5	1.64	4.66	10.63	5.23	7.63
The average indicators of the total samples, EKF-0.5				0.74	0.19	0.48	1.32	1.04	0.82

It was found that the growth index of the maternal explant selected on the 2-3 years old mulberry saplings of the Jarariq-9 variety on the 24th day of the first microcloning was 11.06 cm in height, root length was 3.1 cm, and the number of roots was 7.3 units (Table 5).

Table 5. The indicators of microcloning of maternal explant selected in 2-3 years old mulberry saplings of Jarariq-9 variety of mulberry (2017-2019).

Materials selected for the experiment	**Revision**	**Planting time**	**Initial state, cm**	**Result of 14 days**			**Result of 24 days**		
				Height, cm	**Height of root, cm**	**The umber of root**	**Height, cm**	**Height of root, cm**	**The umber of root**
A 2-3 years old mulberry sapling	1	April	1.5	7.1	1.6	4	11.2	2.8	8
	2	April	1.5	7	1.1	4	11.1	3.3	6
	3	April	1.5	6.9	1.3	5	10.9	3.2	8
Average				7	1.3	4.3	11.06	3.1	7.3
A 2-3 years old mulberry sapling	1	May	1.5	10	2.6	7	11.9	9	11
	2	May	1.5	10	3.1	5	12	8.9	9
	3	May	1.5	10.5	2.0	8	12.5	10	8
Average				10.5	2.56	6.6	12.13	9.3	9.33
The average indicator of sample				8.75	1.93	5.45	11.59	6.2	8.3
The average indicators of the total samples, EKF-0.5				0.33	0.46	0.87	1.34	0.12	0.48

In the second microcloning, it was noted that the length of the explants adapted to the nutrient medium was 12.13 cm, the root growth was 9.3 cm, and the number of roots was 9.33. It was found that the difference in the first 10 days of the second cloning was that the plant grew 3.5 cm tall, 1.26 cm root, 2.3 roots and in the second 10 days, the plant grew 1.07 cm tall, 6.2 cm root and 2.03 roots to be higher than grain.

In the next researches, research was carried out on the selection of the most optimal parts of mulberry for obtaining explants from the Uzbekistan hybrid and Jarariq-9 variety.

In the process of micropropagation of mulberry, while studying of the characteristics of the variety and the development of each microclone, the use of microcuttings from the lowest part in the preparation of quality saplings allows us to obtain high-yielding primary saplings

in a short period of time. Observing the growth of mulberry saplings, adapting them to non-sterile conditions, is the most important step in the system of plant propagation by the method of isolated tissue and organ culture.

In the microclonal reproduction of mulberry varieties and hybrids, the branching and rooting processes of microplants are controlled under the influence of growth regulators.

In the microclonal reproduction of mulberry varieties and hybrids, the branching and rooting processes of microplants are controlled under the influence of growth regulators.

If the period of transplanting sterile plants falls on April and May, it is advisable to transplant well-developed saplings with a root system of 12-14 cm and 5-6 roots.

4 Conclusions

It was determined that Thimerosal 0.0005 mg/l, Hypochlorite (Na or Sa salt) 10% solution, Silver nitrate ($AgNO_3$) as sterilizing agents for in vitro micropropagation of selected mulberry cultivars and hybrids - for introduction into culture and obtaining plant material capable of growth - 0.005% and 70% solutions of ethyl alcohol were used and the most effective sterilization agent was found to be Thimerosal 0.0005 mg/l, Silver nitrate ($AgNO_3$) -0.005%.

It was justified that WPM (Woody Plant Medium) - NH_4NO_3 (ammonium nitrate) 400.0 mg/l, $Ca(NO_3)_2$ (calcium nitrate) 471.26 mg/l, $CaCl_2$ (calcium chloride)-72.50 mg/ scientifically proven that it is effective. It was noted that the use of explants from 2-3 years old mulberries in the process of propagation of mulberry cultivars and hybrids by micrografting is highly effective. It was determined that in the results of micrografting, the micrograft close to the 3rd nutrient medium grows faster than the apical meristem and the middle part.

It was determined that WPM medium was found to be an efficient medium for in vitro microclonal propagation of mulberry cultivars and hybrids. It was found that the branch length of plants in WPM (Woody plant medium) nutrient medium is equal to 82.73% in culture, which is the highest indicator.

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