

RESULTS OF GRAFTING OF *MORUS ALBA* L. ORNAMENTAL FORMS

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Abstract

Based on the processing of literary sources and our own research, we studied the features of grafting ornamental forms of *Morus alba*: 'Pendula', 'Globosa', 'Pyramidalis', 'Contorta', 'Macrophylla' and 'Tatarica'. It is proposed to distribute these ornamental forms according to the grafting and budding dates: winter – the second or the third decade of February – the first decade of March; spring: early spring (the second or the third decade of March); spring the average (the first – the second decade of April); late spring (the third decade of April – the first to the second decade of May); summer – the end of the first decade of July is the first decade of August. It is investigated that the best period for its holding is the first and the second decade of April. It is determined that the most effective method of winter grafting is improved copulation, which was carried out in early March – the highest average splicing of various components after grafting of these plants was observed in forms 'Tatarica' (70 %) and 'Macrophylla' (60 %). When grafting plants by splitting in these forms, the highest average splicing is also noted – compared 61 % and 54 %. The highest splicing results of grafted plants during their spring grafting were also obtained by applying the improved copulation method in forms 'Tatarica' – (88–92 %), 'Pendula' – (78–84 %) and 'Macrophylla' – compared 65–75 %. The largest number of splices of grafted plants during grafting by applying the method for behind the bark is noted in form 'Tatarica' (87–91 %, a little lower – in 'Pendula' (73–77 %) and 'Macrophylla' – compared 64–72 %. It was investigated that the wild plant (*M. alba*) and its studied ornamental forms resemble their internal structure and the grafting sites are quickly spliced and are completely covered with a protective fabric before the onset of the rest period.

Key words: bark, improved copulation, splitting, white mulberry, winter and spring grafting.

Introduction

With the development of ornamental horticulture, the demand for introduced ornamental fruit plants, including ornamental belonging to Moraceae family, greatly increases. White mulberry (*Morus alba* L.) varieties can be grown with the use of grafting (in winter and spring) or chip bud-

ding (in spring and summer).

Grafting refers to artificial methods of vegetative propagation, which is a combination of shoots or buds of one plant with another, which has a root system (Vitenko 2014, Vitenko and Shlapak 2016).

Krenke (1966) notes that the descriptions of different grafting techniques have been known since the times of Aristotle

and Theophrastus, etc. The most common among them is improved copulation, from behind the bark splitting.

Sobchenko (2005, 2008) points out that currently there are about 400 different methods of grafting various plants. Commercial grafting methods include two large groups: 1) ablactation – when different parts of different plants are joined by convergence they are not separated from the parental forms until they are completely merged; 2) grafting of one plant into a separate part of another. This compound allows the implanted parts to grow together due to the activity of living cells in the cambial zone, as a result of division the contact area between two plants is filled with calus tissue with a large number of parenchymal cells.

Kosenko et al. (2008) define grafting as a form of botanic transplantation (transplanting and joining of tissue organs of different plants), this value is widely used in gardening and consists of splicing parts of one plant with another.

Matvienko et al. (2006) recommend using the middle parts of grafted plants, because the buds in the lower ones usually develop more slowly and upper part doesn't matured.

According to Glukhov et al. (2003), during reproduction *M. alba* is used for bank grafting and in the butt of fire arms (April), bud graftin in T-like incision (August), the butt bud (August).

So far, the problem of a low degree of splicing of various parts of these ornamental plants has not been solved, and the optimal time for grafting in Ukraine has not been determined.

The aim of our work is to study the effective ways of the reproduction of forms of *M. alba* by grafting. For this, it is necessary to establish the best methods and time of grafting.

Materials and Methods

During the studing years 2006–2018 grafting of forms of *M. alba* the following materials were used: cleft-graft scion – 3–5 summer saplings (*M. alba*), which were grown on the experimental site of the den-dropark 'Sofiyivka' of the National Academy of Sciences of Ukraine and training and experimental section of the green house complex of the faculty of Forestry and Landskape Gardening of Uman National University of Horticulture.

But the cleft-graft scions shoots of the ornamental varieties of *M. alba*: '*Pendula*', '*Globosa*', '*Pyramidalis*', '*Contorta*', '*Macrophylla*', and '*Tatarica*'. The leaf cutting was cut from the medial part of one-year shoots of mother plants grown on experimental plots of the Sofiyivka arboretum and Uman National University of Horticulture of the National Academy of Sciences of Ukraine.

While grafting of forms of *M. alba*, the most effective methods were chosen: improved copulation behind the cortex and in splitting.

This is done as follows: ground, depending on the desired height of the root-stock (150–180 cm) cut away, cutting parallel to the surface of the earth. On the center of cross section (throughout the diameter of the cut), using a garden knife, make a cut with a depth of 0.5 to 1.5 cm. The graft is taken with two kidneys from the middle part of the annual shoot growth. The average graft length is 15–20 cm. At the lower end of the graft, two incisions were made with a length of 1.5 cm and then inserted into the splitting of the root-stocks. The splicing area is treated with garden oil and it is bounded with a special film (tape). When the graft and the wild plant fused together (after 1.5–2 months), the special film was removed.

The improved copulation method was used for grafting of thin shoots if the scion and rootstock diameters were the same. For a tight connection of grafting components and their better growth in sections adnation longitudinal splitting ('tongues') is made connecting them with a tab one after the other. Using this method, smooth and straight diagonal cuts were made on the root plant and the graft. The length of the incision is 3–4 times greater than the thickness of the graft by 3–5 cm. The splicing area is also treated with garden ointment and it is bounded with a special film (tape). A special film (tape) is removed when splicing components rootstocks and graft were made.

Grafting for behind the bark is carried out in the period of active movement of juice. Transplants should be prepared shortly before grafting the graft is prepared with ordinary copulation. On the side of the stump (wild plant) in a smooth place make a longitudinal section of the bark length by 3–5 cm. The edges of this incision are slightly separated from the wood by the heel of the coping knife. The graft is placed in the incision of the bark of a wild plant. The place of attachment of the graft and the wild plant is well tied with a special tape. It was removed the tape when they are spliced.

The features of anatomical structure of the annual shoots of *M. alba* were investigated using their cross-section using the Gritsayenko's method (2003). According to this technique the typical shoots were selected at three-time repetition. Blurring and clarification of selected shoots were carried out in Javel water for two–three days. After clarification the cuts were washed with distilled water for two three days. Coloring of the shoots was done with the help of a crystal paint (a mixture of 1 ml of 5 % sulfuric acid and 1 ml of

1 % aqueous dye solution) and followed by fixing in a solution of the next composition: 30 ml of glycerol; 80 ml of water; 2 g of zinc chloride and 0.2 g of potassium iodine.

Results and Analysis

We studied the peculiarities of grafting forms of *M. alba* ('Pendula', 'Globosa', 'Pyramidalis', 'Contorta', 'Macrophylla', and 'Tatarica'). Taking into account of its long growing period the grafting terms were suggested to be divided into winter, spring and summer periods.

Winter grafting was held in February (the 2nd–3rd decades) and in early March (the 1st decade). Spring grafting of *M. alba* and its ornamental varieties was divided into 3 periods: I – early spring (the 2nd–3rd decades of March); II – mid spring (the 1st–2nd decades of April); III – late spring (the 3rd decade of April – the 1st–2nd decades of May); summer grafting took place from late July till early August.

Preparations for winter grafting of *M. alba* forms began with autumn planting of rootstocks into containers and placing them in special premises till the beginning of the process. The scions forms of *M. alba* were cut in late January–early February and preserved in a basement. A few days before the scions were moved to the premises and prepared for vaccination. Then they were carried to the open area. After 1–2 years of cultivation the plants were sold (Vitenko 2014).

The final data on the application of grafting components of various ornamental forms of *M. alba* during the winter terms of its carrying out (February – early March) is given in diagram one and two (figs 1 and 2).

The best grafting technique for win-

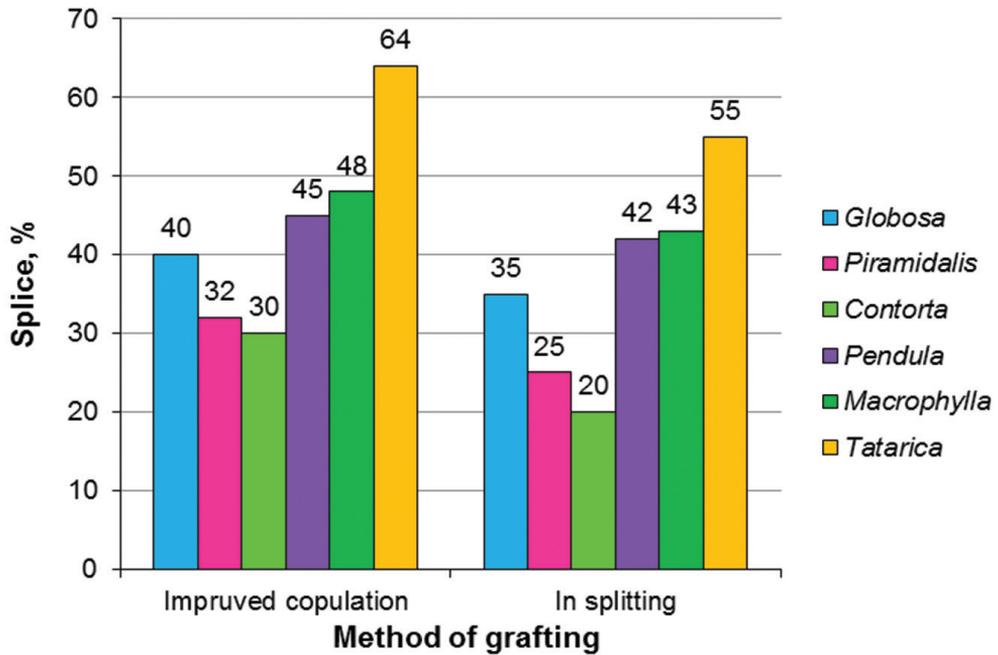


Fig. 1. Results of winter grafting forms of *M. alba* (February).

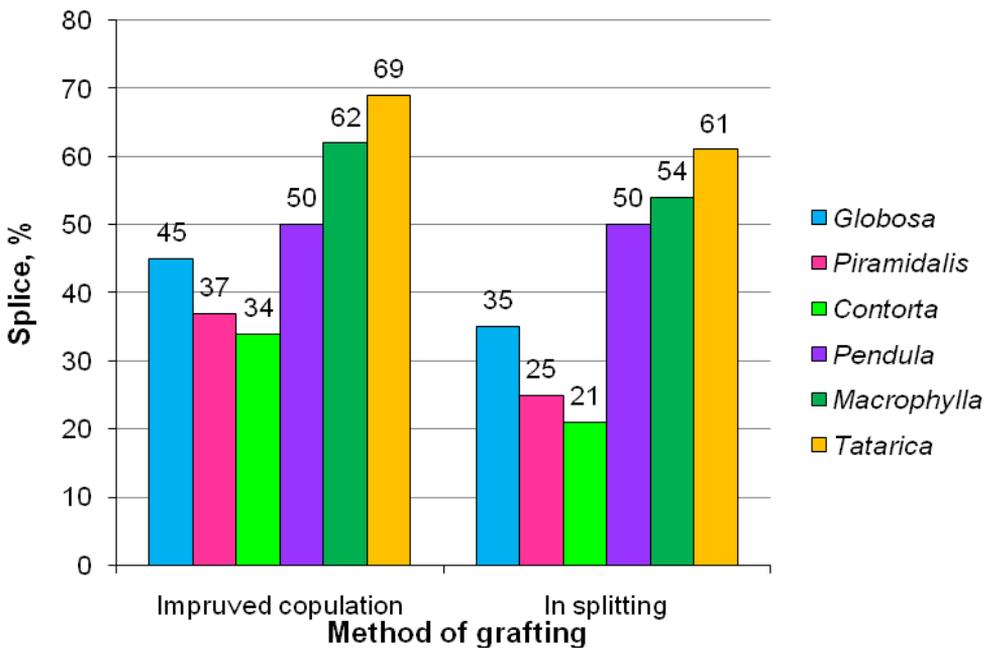


Fig. 2. Results of winter grafting forms of *M. alba* (March).

ter conditions proved to be the improved copulation, which was carried out in early March. The highest percentage of spliced components of the grafting is noted in '*Tatarica*' (70 %) and '*Macrophylla*' (60 %). The February grafting of these forms gave lower rates – 64 and 55 % accordingly. Method of the improved copulation gave the lowest rates at '*Pyramidalis*' – 37 % in early March and 32 % in February and '*Contorta*' – 35 % and 30 % accordingly.

The cleft graft technique proved less positive though the tendency stayed similar to the improved copulation. The highest average cleft grafting in success was also noted with the above mentioned forms – 61 % and 54 % accordingly. In our opinion, this rather low indicators plicing components rootstocks and graft were made. *M. alba* success on winter period can be explained by low physiological activity, when the process of growing together of scion and rootstock is still quite slow. It is also difficult to select the most viable cuttings in winter as they do not have enough time to grow in the stiff climatic conditions of Ukraine.

Spring grafting of *M. alba* forms was divided into 3 periods: early spring (the 2nd–3rd decades of March); mid spring (the 1nd–2rd decades of April); late spring (the 3rd decade of April – the 1st–2nd decades of May). Such division was grounded by a large discrepancy in the quantitative indicator of positive results.

The first period had the vastest day temperature changes and it has least favorable for grafting as the scions had been cut in winter, when all grafted material looked viable. *M. alba* and its forms ('*Pendula*', '*Globosa*', '*Pyramidalis*', '*Contorta*', '*Macrophylla*' and '*Tatarica*') have one important distinction from the native flora – anelongated period of linear shoot growth (the 1stdecade of autumn). It is the

reason why their apical and partially medial parts do not have time to grow stiff before the resting period. It should also be noted that it's difficult to recognize the improper scions from the one-year shoots.

The second period is characterized by the intense sap flow in studied plants. During this (especially in the 2nd decade of April) all annual shoots start budding, which allows the quality scion for further grafting. We think this is the best spring period for the effective growing of good planting material (ornamental forms of *M. alba*).

The last 3 periods where characterized by the high activity of all physiological processes in plants. The optimal grafting is the so-called from plant to plant by blanks of grafting elements and their maintenance in a humid environment before the onset of grafting. In this period, there is a process of rapid growing together of scion and rootstock, but there is a number of obstacles for grafting itself as high air temperature, rapid growth of so-called sleeping buds on the rootstock, etc.

The initial preparation for the spring graft was begun with the sap flow start indicated by the active bark detachment. The above mentioned condition of the scion plant gave a signal to the start of plants grafting. In case of insufficient soil moisture a few days before the grafting, the rootstocks were well watered. Preparation of ornamental varieties cuttings began just before the graft procedure. The scions had to have three buds.

Taking into account of many years experience of spring tree grafting in the 1st–2nd decades of April, we chose two techniques that gave the most positive results – the improved copulation and grafting for behind the bark.

Grafting for behind the bark was implemented during a period of active sap

movement. This propagation technique of the forms differs greatly from the improved copulation. Cuttings (scions) were cut just before the grafting of the basal and medial parts of the shoots. Grown seedlings of *M. alba* were used as stocks.

Further studies proved the effectiveness of 3-bud scion used for the graft. We left 2–3 cm of 'stump', above plant top bud which prevented its drying. The shoot was cut across at the desired stem height.

The cut was made smoothly which contributed to a better occluding of the wound and good wound healing. The cuttings were prepared as the ordinary grafting. On the side of the stump (wild plant) on a place grafting we made a longitudinal bark section of 3–5 cm to the wood. The edges of this section were slightly separated from the wood by the end of the knife.

The cutting was placed behind the bark. The obtained data during the graft-

ing (by the two above-mentioned techniques) of the *M. alba* forms data can be seen on the diagrams (figs 3 and 4).

The data in Figure 3 show that the best results of the scion and rootstock growing together during spring graft technique was observed of using the improved copulation method '*Tatarica*' – 88–92 %. High rate of graft was also noted '*Pendula*' (78–84 %) and '*Macrophylla*' (65–75 %). The lowest percentage of joining was observed with '*Contorta*' – from 54 to 62 %, and at engraftment of '*Globosa*' – 58–62 %. The '*Pyramidalis*' had some higher level plic-ing components wild and grafting plant taking rate than that of '*Globosa*': minimum – 61 %, average – 64 %, and maximum – 68 %.

Data analysis shows (Fig. 4) that the best taking of rootstocks with scions using the bark inlay method was obtained with '*Tatarica*' (87–91 %). A high percentage of

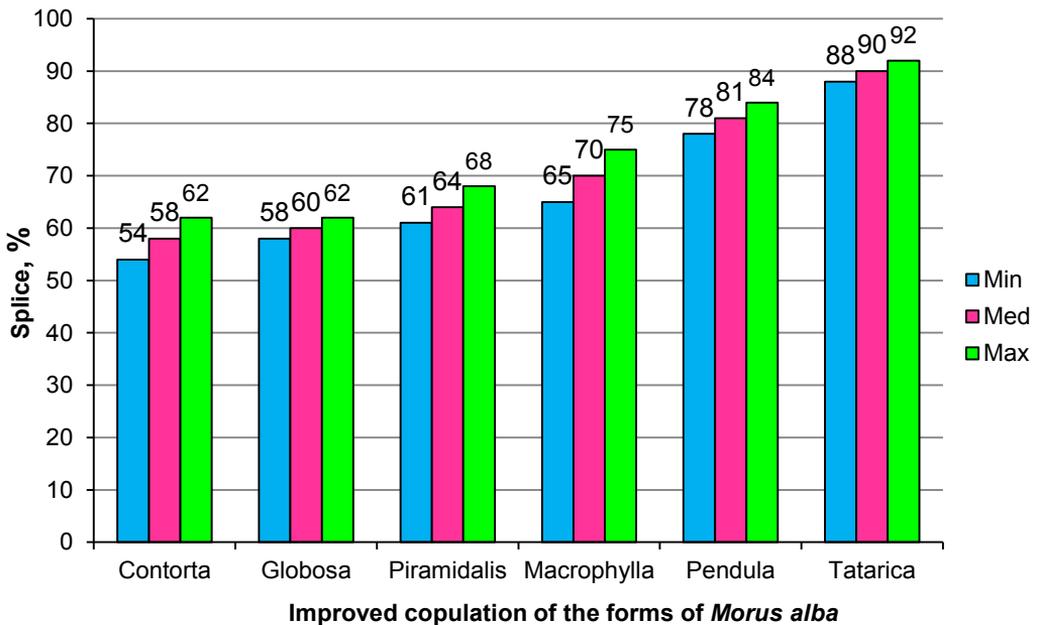


Fig. 3. Results of plicing indices graft forms of *M. alba* for the average spring grafting (the 1st–2nd decades April) by the way of improved copulation (average 2005–2017).

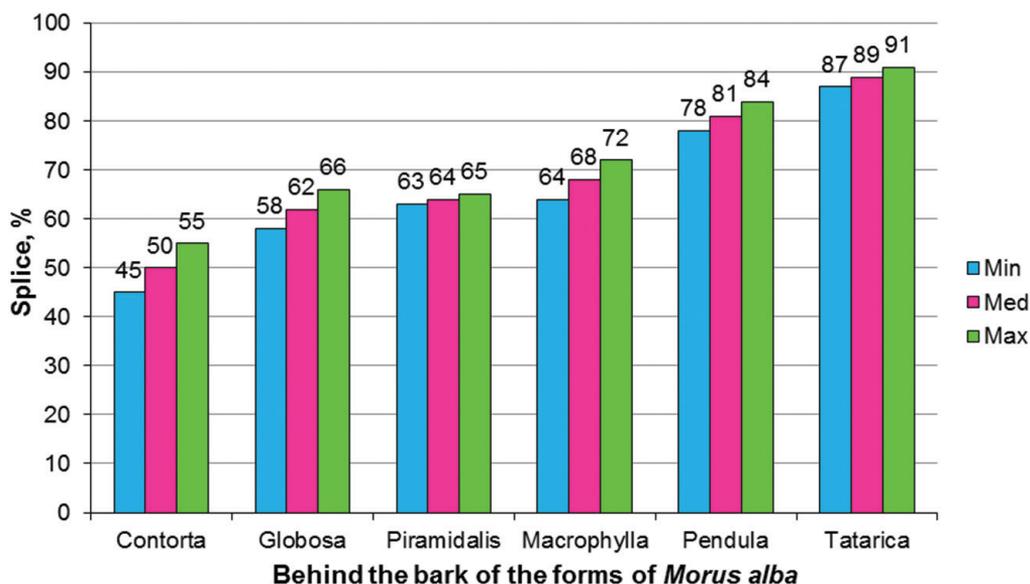


Fig. 4. Results of splicing indices graft forms of *M. alba* for the spring grafting of behind the bark (average 2005–2017).

growing together was noted with '*Pendula*' (73–77 %) and '*Macrophylla*' (64–72 %). Higher rate of success in lay method for bark of the improved copulation method was recorded at '*Globosa*' (58–66 %). The form '*Pyramidalis*' showed even slightly higher indices results of the scion and rootstock growing (63–67 %).

The lowest percentage of successful grafting growing scene was observed with '*Contorta*' (45–55 %). Behind the bark of forms *M. alba*.

Splicing graft components of rootstocks and forms are shown in Figure 5 where there is an increase in the percentage of splices of grafting forms during the medium spring grafting compared to early spring.

Among the *M. alba* varieties the best results were obtained in '*Tatarica*' (87 %), '*Pendula*' and '*Macrophylla*' (73–79 %). The lowest result was obtained with '*Contorta*' – 61 % of all grafts of '*Pyramidalis*'

and '*Globosa*' proved 65 % and 63 % respectively.

While grafting the aforementioned ornamental *M. alba* varieties in the late spring period, we obtained lower taking rates than those of the mid spring, but some what higher results than in the early spring. Among various methods of spring grafting the most effective proved to be the improved copulation (see Table 1).

Table 1 shows that the best result (92 %) was obtained on the grafting method of improved copulation forms '*Tatarica*' and then '*Pendula*' – 84 %; '*Macrophylla*' – 75 %; '*Pyramidalis*' – 68 %; '*Globosa*' – 65 %; and '*Contorta*' – 62 %.

Grafting the above-mentioned forms using the bark method also showed the best result in '*Tatarica*' – 91 %. The rest are arranged in this order: '*Pendula*' – 75 %; '*Macrophylla*' – 72 %; '*Pyramidalis*' – 65 %; '*Globosa*' – 66 %; '*Contorta*' – 55 %.

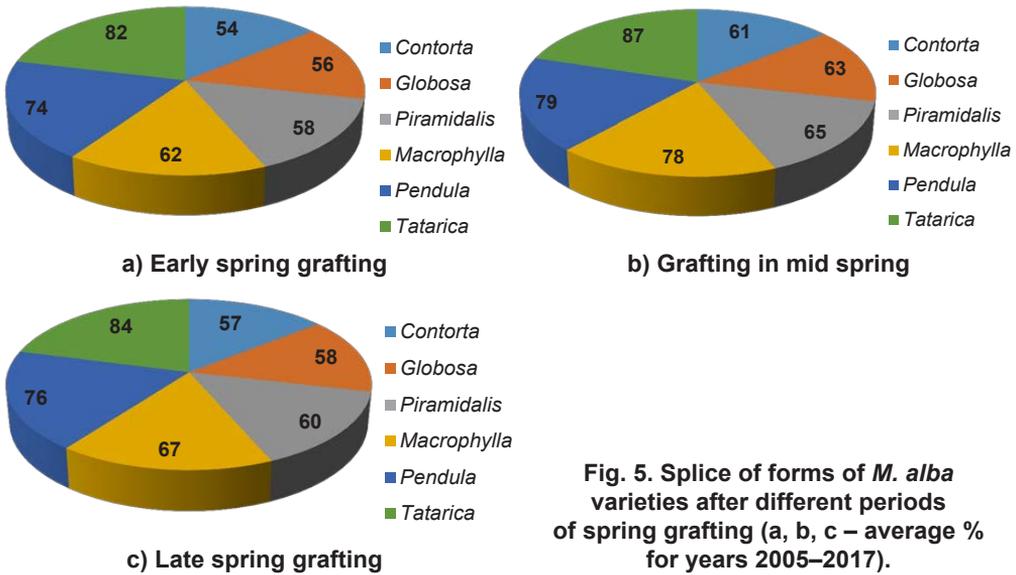


Fig. 5. Splice of forms of *M. alba* varieties after different periods of spring grafting (a, b, c – average % for years 2005–2017).

Table 1. Spring grafting results of *M. alba* ornamental forms (average for years 2005–2017).

No	Ornamental forms	Method technique					
		Improved copulation			Graft method		
		Splice, %					
		min	med	max	min	med	max
1	<i>Contorta</i>	54.0	58.0	62.0	45.0	50.0	55.0
2	<i>Globosa</i>	58.0	61.5	65.0	58.0	62.0	66.0
3	<i>Pyramidalis</i>	61.0	64.0	68.0	63.0	64.0	65.0
4	<i>Macrophylla</i>	65.0	70.0	75.0	64.0	68.0	72.0
5	<i>Pendula</i>	78.0	81.0	84.0	73.0	75.0	77.0
6	<i>Tatarica</i>	87.0	89.0	92.0	87.0	89.0	91.0

Was grafting forms of *M. alba*, it is very important to study the characteristics of the further growth of the grafting components in the place of their connection.

It is very important to study the characteristics of the further growth grafting forms of *M. alba* of the inoculative components in the place of their connection. Despite the scientifically established facts of higher compatibility of grafting components within one species, these studies

are highly required for the forms of *M. alba*, which have phenological growth and development differences from the native plants of Ukraine.

As a rootstock for forms of *M. alba*, plants of seed and vegetative origin, which are most identical in biochemical composition, were used. The years of researches have shown that the best grafting method of forms is the improved method of copulation (Fig. 6).

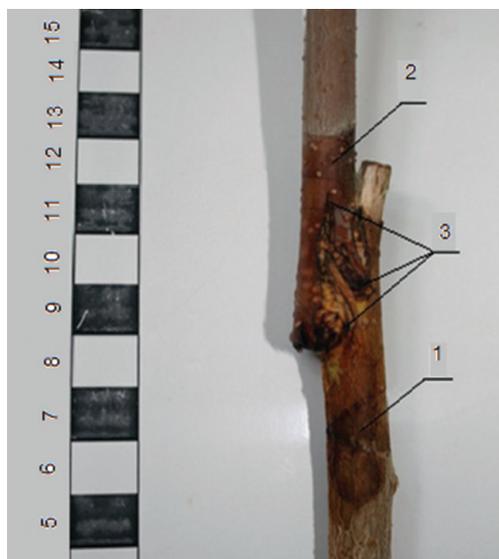


Fig. 6. Splice of form '*Macrophylla*', where: 1 – rootstock; 2 – scion; 3 – places of splice after the first year of growth.

It can be seen from Figure 6 that before the start of the rest period, the grafting sites of forms with wild plants are completely covered with protective cloth. This is due to the activity of physiological processes inside the plants.

The dynamics of the process of splice grafted plants over a two-year period can be studied using the example of '*Globosa*' form (Fig. 7), where it is evident that during two years of growth, by means of the rapid division of the cells of the productive tissue the forms undergo a period of complete splicing with a wild plant at the place of their connection using the improved copulation method.

By conducting a longitudinal section of the rootstock and the scions, we studied the structure of the tissue inside the plants to compare. The results are shown in Figure 8.

On the basis of the cross sectional cutting (Fig. 8) of the scion form '*Tatarica*' and the rootstock of *M. alba*, it can be



Fig. 7. Grafting of one-year (a) and two-year (b) *M. alba* '*Globosa*', where: 1 – rootstock, 2 – graft, 3 – place of graft.

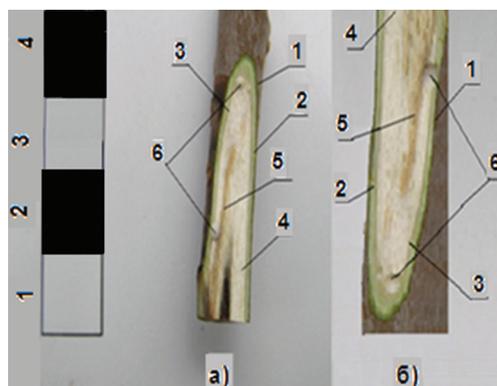


Fig. 8. Longitudinal section of shoots of *M. alba* '*Tatarica*' (a) and *M. alba* (b), where: 1 – bark, 2 – bast, 3 – cambium, 4 – wood, 5 – protective tissue, 6 – cellular sap.

concluded that their internal tissue structure is similar. Taking this into account we can state their compatibility, which is other beneficial key factor to the rapid splice of grafted components.

By carrying out a cross-section of the

grafting site, the internal structure was examined (for comparison). The results of the research induced in Figure 9.

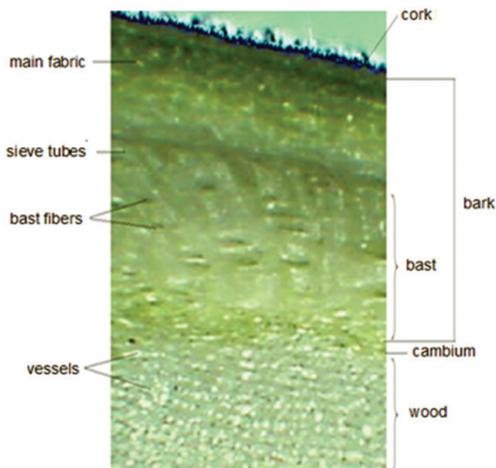


Fig. 9. A cross-section of a one-year *M. alba* shoot (photo by Sergiy Koval and Oleh Lazariev).

On the basis of the cross-section in the fusion zone of 'Tatarica' form from *M. alba*, one can conclude that their internal structure of tissues is similar. Given this aspect, it can be argued that there is compatibility between them, which, combined with other favorable factors is the key to a quick splice grafted components.

The peculiarity of the anatomical structure of annual shoots of *M. alba* was investigated with the help of their cross-section by the method of Gritsayenko (2003). According to this technique, 20 typical shoots were selected at three-time repetition blurring and clarification of the selected shoots was carried out in Javel water for two–three days. After clarification, the sections were washed (three–fourtimes change) in distilled water for two three days.

Further coloring of *M. alba* shoots was done with the help of a crystal violet dye solution (a mixture of 1 ml of 5 % sulfuric

acid and 1 ml of 1 % aqueous dye solution) and followed by fixing in a solution of this composition: 30 ml of glycerol; 80 ml of water; 2 g zinc chloride and 0.2 g potassium iodine. Due to this factor the light gray parts of the tree shoots were well colored violet-blue (Fig. 9).

It was clearly seen that the exterior of the escape is covered with a layer of cork tissue – a tissue consisting of dead and dying cells. They perform a protective function. The bark, in turn, consists of conductive, mechanical and main tissue. Tubes are part of the conductive tissue and the mechanical one is represented by bast fibers, which give the stem of this plant strength and flexibility. The main fabric performs the function of accumulation of nutrients and fills the gaps between two above-mentioned tissues. The next tissue is the cambium which function is to create new layers of wood and bast. Between the bark and the core is a solid fabric – wood, which makes up the bulk of the shoot. The main structural unit of wood is fiber, which at its initial stage of development has a rather elastic and pervious for aqueous solutions skin.

The peculiarities of splice of the forms by the example of form 'Pyramidalis' were studied with the help of a cross-section (Fig. 10).

We should also note the importance of callus in the process of rapid splice of the grafted elements. Under the pressure of newly growing cells in the insulating layer between the scion and the rootstock gaps emerge, through which callus comes to the surface of connects and helps the graft components to grow together. Simultaneously the differentiation process takes place (forming of a prior callus), which connects with the cambium of the rootstock and the scion and creates elements of xylem and phloem. After that the



Fig. 10. Longitudinal incision at the place of graft, where: 1 – bark, 2 – bast, 3 – cambium, 4 – wood.

places of grafting grow rather quickly during the growing season.

Figure 11 shows the dynamics of linear growth of grafted form of *M. alba* in winter

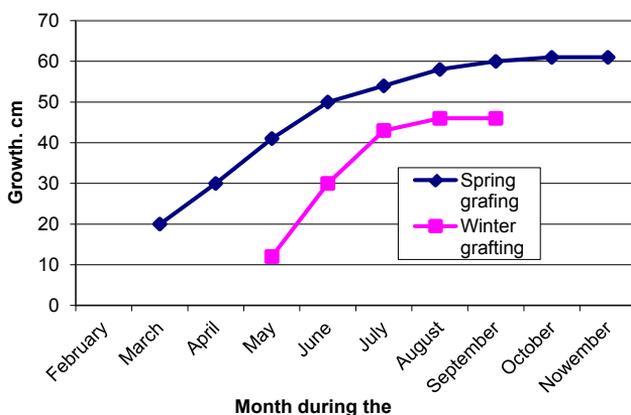


Fig. 11. Dynamics of linear growth of the grafted forms during the growing period.

(a container plant of indoor keeping during the whole growing period) and in spring (an open soil keeping during the whole growing period) that allow to compare its duration at different temperature and humidity conditions.

Analysing Figure 11 we can note a long period (February–November) of the linear growth of shoots in carrying out the winter grafting of *M. alba* forms (in containers), followed by the maintenance of these plants in green house conditions during the entire vegetation period. That allows the grafted plants to end their vegetation with a sufficient level of aging of one-year shoots. Large differences are observed in the formation of the crown forms. Winter graft of these plants in containers greatly shortens the forming period.

Conclusions

Based on the research of vaccination forms of *M. alba*, we have determined that:

- The best way of winter grafting is the improved capulation, which is carried out in early March, where the best results observed in vaccination forms '*Tatarica*' (70 %) and '*Macrophylla*' (60 %). The February grafting of the same forms turned less effective – 64 % and 55 % accordingly. The lowest results in vaccinated method of improved capulation recorded in form '*Pyramidalis*' – 37 % in early March and 32 % in February and in form '*Contorta*'. The lowest results in vaccinated method of improved capulation recorded in form '*Pyramidalis*' – 37 % in early March and 32 % in Feb-

ruary and 'Contorta', accordingly 35 % and 30 %. The greatest success in these forms was when vaccinated with a method of splitting – 61 % and 54 % accordingly.

- In spring grafting by improved copulation, the best result was in 'Tatarica' – 88–92 %. The high percentage was also registered in 'Pendula' (78–84 %) and 'Macrophylla' –65–75 % accordingly. The lowest rates were observed in 'Contorta' – from 54 % to 62 %.

- The vaccination for the bark gave a high result in 'Tatarica'– from 87 % to 91 %, high percentage of taking as in the improved copulation –in 'Pendula' (73–77 %) in 'Macrophylla' (64–72 %). And the lowest success rates were noted in 'Contorta'– from 45 % to 55 %.

- It was discovered that *M. alba* and tested forms have similar internal structure; the places of inosculation grow together rapidly enough and enter the resting period completely covered with protective tissue.

References

- GLUKHOV O.Z., KOSTYRKO D.R., MITINA L.V. 2003. Fruit mulberry *Morus alba* L. in the southeast of Ukraine (introduction, biomorphology, use). Reply. Kosenko I.S. (Ed.). Donetsk, Swan: 111–117 (in Ukrainian).
- GRITSAYENKO Z.M., GRITSAYENKO A.O., KARPENKO V.P. 2003. Methods of biological and agrochemical studies of plants and soil. Kyiv, Zat 'Novolava'. 320 p. (in Ukrainian).
- KOSENKO I.S., OPALKO A.I., OPALKO O.A. 2008. Hazelnut: Applied Genetics, Selection, Technology of Reproduction and Production: Teaching. Manual. Kyiv: Naukova Dumka: 164–168 (in Ukrainian).
- KRENKE N.P. 1966. Plant transplantation. Moscow: Nauka. 336 p. (in Russian).
- MATVIENKO V.M., BABICH R.D., KONDRATENKO P.V. 2006. Pear in Ukraine. Kyiv: Agrarna Dumka: 95–98 (in Ukrainian).
- SOBCHENKO V.F. 2005. Propagation of maple by vaccination. Collection of scientific works of Uman State Veterinary Academy. Uman: View of Uman State Forest Enterprise 61, part 1: 536–549 (in Ukrainian).
- SOBCHENKO V.F. 2008. Vaccination in the early-fall period of deciduous plants and its modification. Scientific Bulletin of National Forestry University of Ukraine. Lviv. Collection of scientific and technical works 18(1): 46–48 (in Ukrainian).
- VITENKO V.A. 2014. Features of winter grafting of ornamental forms *Morus alba* L. Krasnodar. International Journal of Natural and Humanitarian Studies 4(6): 39–44 (in Russian).
- VITENKO V.A., SHLAPAK V.P. 2016. Theoretical and applied aspects of spring vaccination of forms *Morus alba* L. Lviv, Ukraine. Bulletin of National Forestry University of Ukraine 26(3): 18–24 (in Ukrainian).