Efficient protocol for in vitro clonal propagation of *Rosa damascena* Mill. and its comparison with conventional propagation method

Veselina Badzhelova[1*], Violeta Bozhanova[2]
[1]Institute of Roses, Essential and Medical Cultures, Kazanlak, Bulgaria
[2]Field Crops Institute, Chirpan, Bulgaria
[*]Corresponding author: *veselina.nenova@abv.bg*

Abstract

Badzhelova, V. & Bozhanova, V. (2018). Efficient protocol for in vitro clonal propagation of *Rosa damascena* Mill. and its comparison with conventional propagation method. *Rastenievadni nauki, 55*(6), 37-44

In the present study a comparison is made between the best variants of medium for multiplication and rooting selected in the previous experiments in culture of nodal segments at two main Bulgarian oil-bearing genotypes aiming to develop effective protocol for *in vitro* clonal propagation. Furthermore the advantages of clonal in vitro propagation over the currently used conventional methods for the production of Kazanlak oil-bearing rose (*Rosa damascena* Mill. f. *trigintipetala* Dieck) seedlings are experimentally demonstrated. The results obtained confirmed the high multiplication potential of the MS medium supplemented with 3.0 mg/l BAP and the high rate of rooting of the regenerated plants in ex vitro conditions by direct rooting in a soil mixture. This combination of multiplication medium and ex vitro rooting can be successfully included in the protocol for clonal micropropagation in the Kazanlak oilseed rose, ensuring repeatability of results over different years. By comparing the conventional propagation method with in vitro method it was found that with the conventional method only 37% of all inserted cuttings form a root and grow to well-rooted plants that can be used as planting material. In applying the clonal micropropagation method, the efficiency increased to 765%, as 918 rooted plants were obtained of total 120 introduced explants, i.e. this method is about 21 times more effective than the conventional one.

Keywords: oil-bearing rose; clonal micropropagation; protocol

Abbreviations: MS – Murashige and Skoog medium, IBA – indole-3-butyric acid, BAP – 6-benzylaminopurine

INTRODUCTION

The oil-bearing rose is a traditional crop for Bulgarian agriculture. In the word the propagation of rose is realized mainly by classical vegetative techniques regardless of their main disadvantages associated with season dependence and slow multiplication rates (Pati et al., 2006).

In Bulgaria, seedlings of oil-bearing rose are only produced by conventional means mainly through rooting green cuttings into a greenhouse – technology developed in Institute of Roses and Essential Oil Crops in 1986 (Zlatev et al., 2001). This method has a number of disadvantages, the main one being the low percentage of rooting, small multiplication coefficient and a lot of labor which makes the production more expensive. All of the traditional methods for obtaining the young plants of the oil-bearing rose are not only slow and inefficient, but also can spread infection from one plant to another. These problems can be solved by using the method for clonal micropropagation. This method has become more popular over the last couple of years as an alternative to the traditional methods for vegetative propagation. Micropropagation is the propagation of plants in *in vitro* conditions, which

ensures rapid production of a large amount of plant material preserving the genetic potential of the parent plants.

Tissue culture is a tool for a rapid multiplication of the genetically identical groups of a large number of plants. Protocols for clonal micropropagation can be used not only for selection, but also for propagation, disinfection and storing genetic resources (Altman and Loberant, 1998; Cassells, 1997; Altman, 2000; Lal et al., 2015). The propagation happening in *in vitro* conditions leads to the obtaining of a larger number of plants that are better at adapting, with more developed root system (Roberts and Schum, 2003). Throughout the years micropropagation has become a successful alternative to conventional methods for vegetative propagation for various plant species. This is why it is one of the most applicable directions of plant biotechnology (Ishioka and Tanimoto, 1990; Kornova and Michailova, 1994; Ginova and Kondakova, 2013).

Thanks to the intensive research of a large number of authors on the main factors on which clonal micropropagation depends – type and decontamination of the primary explants, genotype, composition of culture medium for multiplication and rooting, in recent years the method of clonal micropropagation has begun to replace ever more successfully the classic vegetative propagation of the rose (Kornova and Michailova, 1994; Senapati and Rout, 2008).

In previously series of experiments we have identified appropriate combinations of main regeneration factors and have achieved high multiplication and rooting rate in culture of nodal segments at two main Bulgarian oil-bearing genotypes (Badzhelova, 2017).

In this study we repeated the experiment using the same genotypes and the best variants of medium for multiplication and rooting. In addition we compared the two propagation methods aiming to prove the advantages of *in vitro* clonal micropropagation over the currently used green cuttings method.

MATERIALS AND METHODS

The experiments were carried out in the laboratory for *in vitro* propagation of Industrial Plants OOD Kazanlak and include three main stages: introduction in culture, multiplication and rooting of the obtained plants.

Plant material

Two genotypes from Kazanlak oil-bearing rose (*Rosa damascena* Mill. f. *trigintipetala* Dieck) are included in the first experiment: Population № 5, labeled as R3 and variety Eleina, labeled as R3-4. The genotype R3 is used in the attempt of comparing the effectiveness of the in vitro method and the conventional method for propagation. Donor plants have been obtained from elite collection and selection plots of the Institute of Roses and Essential Oil Crops (IREOC) in Kazanlak.

Experiment for clonal micropropagation

The experiment is realized in 2017. Nodal segments were used as starting explants. All of the used explants were put into disinfection process. The disinfection runs in two stages: first stage – 0.2% HgCI solution for 3 minutes and rinsing in sterile distilled water for 2 minutes; second stage – disinfection in 0.5% NaClO solution for 20 minutes and rinsing in sterile distilled water for 2-3 minutes. The sterilization process continues in 0.25% NaClO solution for 30 minutes and rinsing in sterile distilled water. After each treatment the explants were washed in distilled water. The induction and proliferation growth medium was MS (Murashige and Skoog, 1962), hormones free. 20 days after culture induction the disinfected live explants were transferred in multiplication media. In the multiplication stage three variations of growth media were used, differentiating one from another by concentration of BAP – version 1 (MS+0.5 BAP mg/l), version 2 (MS+3.0 BAP mg/l) and version 3 (MS+5.0 BAP mg/l). The experiment was performed by complete randomized block design in two replications.

Two variants of roots formation have been used. First variant was in vitro rooting on MS medium with addition of 0.1mg/L IBA. Second method applied is ex vitro rooting of shoots direct in soil mixture 1:1 (perlite : peat), without passing through root formation medium. Prior to placing in the soil mixture, in vitro produced shoots are immersed in a solution of IBA – 0.1 mg/L for 1 minute with gentle shaking of the vessel where treated.

The data have been processed by analysis of variance ANOVA through the software package Statistica 8.0 (StatSoft, Inc. 2002).

Experiment for comparison between in vitro multiplication method and conventional propagation method

The experiment was held in 2018 г.

In vitro multiplication method

In vitro material of genotype R3 was used as starting explants. In vitro shoots were placed directly on a multiplication culture medium MS+3.0 mg/L BAP solidified with agar 6 g/L. The pH is set at 6.0 before sterilization, which is carried out at 121°C at 1.1 atm. for 20 min. A total of 40 explants in 5 glass vessels and 8 explants per vessel in three replications for reliability of the result were used. Explants were cultivated for a period of 35 days. At the end of the period, the number of adventive shoots formed and % of multiplication have been reported (Table 3). All regenerants have been transferred directly to fertilizer-soil mixture 1:1 /perlite : peat/ without passing through a root formation medium. Prior to placing them in the fertilizer-soil mixture, they are immersed in IBA 0.1 mg/L solution for 1 minute with gentle shaking of the vessel of treatment. Rooting takes place in a closed greenhouse under conditions of high humidity 80-90% and 30-32°C for 20 days. After adaptation of the seedlings the greenhouse is fully opened.

Conventional propagation method

The experiment was set by the technology established at IREOC for the rooting green cuttings of oil-bearing rose in a cultivation plant (Zlatev et al., 2001).

For the production of cuttings, vegetative material of young shoots with well preserved leaves was used. The cuttings were cut early in the morning and kept in water containers for transporting to the place where they were prepared for placement in a greenhouse. The plant material is cut into stem cuttings 10-12 cm long, leaving four leaves (Atanasova and Nedkov, 2004). Immediately before being placed in the substrate, the base of the cuttings is treated with a root stimulant to achieve a higher rate of rooting (Lambev, 2011). IBA was used in this experiment for a treatment period of 10-15 seconds. The solution is prepared by dissolving 1g of IBA in 0.5 l of 96% ethyl alcohol and adding 0.5 l distilled water.

The substrate reaction is established at pH 6-7. The experiment is set in containers on pre-shaped beds with a width of 1.30-1.40 m. A total of 40 cuttings have been prepared in three replications. During the rooting in the greenhouse, optimal water and air regime were maintained, 85-100% humidity and 32-35°C. These conditions were controlled throughout the entire rooting period, while observing all plant protection measures foreseen in the technology. Subtraction of the rooted roses was done manually, in autumn.

RESULTS AND DISCUSSION

In the first experiment three versions of growth medium are included for multiplication, with application of BAP in different concentrations in which the same genotypes of Kazanlak oil-bearing rose show best results (Badzhelova, 2017). The results in Table 1 show that the three versions demonstrated high potential for multiplication. The percentages of the multiplicated explants vary from 85 to 100% for each version and genotype. In both genotypes, the biggest number of axillary shoots (75 pcs for R3 and 68 pcs for R3-4) and the biggest average number of shoots from one explant (3.75 for R3 and 3.58 for R3-4) were induced in the second medium (version 2) with a supplement of 3.0 mg/l BAP. The lowest number of axillary shoots and the lowest average number of shoots from one explant from the two genotypes were obtained in version 1 which contains the lowest concentration of BAP. Both genotypes react identically to the medium versions but genotype R3-4 has lower capability for multiplication. The analysis of the variance presented in Table 2 shows that the genotype and multiplication medium variants have a statistically significant influence on the variation in explant multiplication expressed by the total number of induced adventive shoots. Percentages of total sums of squares accounted for genotype, medium and G×M interaction were used to indicate the variation attributed to each component. The variation in the total number of induced adventive shoots is mostly due to medium composition (72.9% from the total variation), followed by genotype (19.98 % from the total variation). The interaction between both studied factors – genotype and medium does not have a statistically significant impact on overall variation of the total number of induced shoots. This fact explains the identical response of the two genotypes to the medium variants used.

Table 1. Study on the effect of different concentrations of BAP on the multiplication process

Genotype	Variant of medium	Explants placed Nr.	Reacted explants %	Induced shoots Nr.	Average number of shoots per explant
R3	1. MS+0.5 BAP mg/l	20	95	54	2.84
	2. MS+3.0 BAP mg/l	20	100	75	3.75
	3. MS+5.0 BAP mg/l	20	100	57	2.85
R3-4	1. MS+0.5 BAP mg/l	20	90	40	2.22
	2. MS+3.0 BAP mg/l	20	95	68	3.58
	3. MS+5.0 BAP mg/l	20	85	44	2,59

Table 2. Analysis of variance for the effect of the studied factors on the total number of multiplied adventive shoots

Source of variation	df	SS	MS	F	Strength of influence (η, %)
Genotype	1	385.33	385.33***	21.81	19.98
Medium	2	1404.67	702.33***	39.76	72.9
Interaction (G x M)	2	28.67	14.3	0.811	
Error	6	106.	17.67		

*** Significant at $p < 0.001$

Shoot multiplication of explants is a key phase for the success of clonal micropropagation (Pati et al., 2006). The results from that experiment confirm the significance of BAP as exogenously added cytokinin to the medium for the best course of in vitro multiplication of nodal explants in various plant species, incl. different types of roses (Vijaya et al., 1991; Pati et al., 2006). It is known that the positive effect of BAP on rose multiplication is related to its ability to reduce apical dominance and to induce the development of axillary buds (Kapchina et al., 2000). According to Roberts and Schum (2003) applying BAP in concentration of 0.45 mg/l (2 mM) is suitable for multiplying most rose species and varieties, which is confirmed by the current study and our previously studies with oil-bearing rose and white rose (Badzhelova, 2017; Badzhelova et al., 2018). Adding BAP into the multiplication media as an only cytokinin with a concentration of 3.00 mg/l is very appropriate for obtaining a good amount of micro shoots when researching the genotypes of the Kazanlak oil-bearing rose. The increasing of the concenctration of this growth regulator starts to suppress the formation of micro shoots. Our results are in line with the findings by a number of authors that with increasing the BAP concentration above a certain level reduction of multiplication ability in rose occurs (Hameed et al., 2006; Mitrofanova et al., 2016).

The results obtained from this experiments are a confirmation to our previous researches with the same genotypes (Badzelova, 2017) and they show that the growth medium for multiplication – MS with a supplement of 3.0mg/l BAP is effective and can be added in the protocol for clonal micropropagation for Kazanlak oil-bearing rose, ensuring repeatability of results over different years.

According to a number of authors one of the main problems of in vitro clonal micropropagation of different plant species, incl. the rose, is the rooting of the regenerated plants (Pati et al., 2006; Ginova et al., 2012). In this experiment we investigate the influence of two rooting conditions - *in vitro* and *ex vitro*. In *in vitro* conditions we used medium for rooting based on MS with a supplement of IBA (MS+0.1mg/l IBA). In a previous experiment with the same genotypes the biggest percentage of rooted

plants were obtained in this medium (Badzhelova, 2017). The results shown in Table 3 demonstrate the rooting of the young shoots not only in *in vitro*, but also in direct rooting in fertilizer-soil mixture in two genotypes. However, the better rooting of the multiplication shoots was made in *ex vitro* conditions. The percentage of the rooted plants in direct rooting in fertilizer-soil mixture is much higher – 100% in genotype R3 and 95% in genotype R3-4. In *in vitro* conditions 90% of regenerated plants from genotype R3 and 70% from genotype R3-4 were rooted. The two genotypes do not differ significantly in their rooting capability, but R3 genotype is superior to R3-4. All plantlets of R3 95% set under both conditions have been successfully rooted, whereas in R3-4 they are 82.5%.

This results are an affirmation for our previous experiment and show that the rooting of the young plant directly into the fertilizer-soil mixture without first going through an *in vitro* medium, can be added into the protocol of the clonal micropropagation of the Kazanlak oil-bearing rose. This allows the whole procedure of clonal micropropagation to be shortened and it will lead to saving money and time in comparison to using *in vitro* rooting. Because of these obvious advantages, many rooting studies have been carried out so far in *ex vitro* conditions, which prove the high efficacy of this method in other types of roses, too (Pittet and Moncousin, 1982; Pati et al., 2006).

As a result of this experiment the higher multiplication potential of the version of growth medium MS with supplement of 3.0 mg/l and the high percentage of rooting of the regenerated plants directly into fertilizer-soil mixture was confirmed. Therefore this combination of version of medium for multiplication and rooting is effective and can be added in the protocol for clonal micropropagation for Kazanlak oil-bearing rose, ensuring repeatability of results over different years.

In additional experiment the effectiveness of the method of clonal micropropagation (based by many experiments and confirmed by the results shown in this article) and the conventional method for propagationg of Kazanlak oil-bearing rose Population № 5 were compared.

With trying the clonal in vitro propagation of Kazanlak oil-bearing rose Population № 5 the best versions of medium for multiplication and rooting were applied, described in the experiment above. As exit explants micro shoots were used, obtained with clonal in vitro micropropagation of the studied genotype. The micro shoots were placed directly into the growth medium for multiplication. The results from the clonal micropropagation are shown in Table 4. All of the used exit explants reacted with

Table 3. Study of different conditions of rooting at in vitro regenerated plants

Genotype	Rooting conditions/ growth regulators		Placed shoots, pcs	Rooted plants, pcs	Rooting, %
R3	in vitro	MS+0.1mg/l IBA	40	36	90%
	ex vitro	Peat-soil mixture	40	40	100%
R3-4	in vitro	MS+0.1mg/l IBA	40	28	70%
	ex vitro	Peat-soil mixture	40	38	95%

Table 4. Induced and rooted plants at in vitro multiplication

Repetition	Number of explants	Number of induced shoots	Average number of shoots per explant	Number of rooted plants	Rooting, %
1	40	314	7.9	311	99.04%
2	40	301	7.5	294	97.67%
3	40	313	7.8	313	100%
Total	**120**	**928**	**7.7**	**918**	**98.90%**

multiplication. From 120 micro shoots obtained 928 axillary shoots were multiplicated. The average number of adventive shoots per explant is 7.73, but this result varies insignificantly between each repetition. After transferring the micro shoot directly into the fertilizer-soil mixture 98.9% of them were rooted successfully and 918 young rooted roses were obtained.

For the conventional propagation of Population № 5 of *Rosa damascena* the method of green cuttings is used and all of the necessary requirements are preserved into the three consecutive stages of the technology – selection and preparation of the exit material, preparation of the greenhouse and subtraction process and sorting of the rooted roses, described in chapter Material and Methods. The results shown in Table 5 demonstrate that from placing 120 green cuttings into three repetitions only 45 of them (37.5%) were successfully rooted. The percentage of the rooted plants varies from 20 to 55 between each repetition. After subtraction and sorting the rooted roses by height of the part that is above the ground, type of rooting system, number of roots and thickness of the top part of the roots, it was established that 84.4% of all of the rooted cuttings correspond to the requirements of Bulgarian State Standard (BSS) and can be used in production (Table 6). Based on these results a conclusion can be made that the effectiveness of the method of green cuttings is too low. Only around 37% of all of the placed cuttings developed to well-rooted small roses. When they were tested whether they were compatible with the requirements of BSS this percentage dropped to 31.7%.

The comparison between the propagation results of Population № 5 oil-bearing rose and number of obtained plants by the both used in this study methods is given in Figure 1. By comparing the conventional and in vitro method, clonal micropropropagation has been found to be much more effective. In applying the green cuttings method, only about 37% of all cuttings form roots and grow to well-rooted small roses that can be used as planting material. In applying the clonal micropropagation method efficiency is increased to 765%. Of the 120 explants used a total of 918 rooted plants have been obtained, i.e. this method is about 21 times more effective than the conventional one. Furthermore the conventional method can be applied once a year within three to four months and immediately after the obtaining of plants they must be planted in a permanent place. Rooting is done in an unheated greenhouse, making it impossible young roses to be stored until next year. However, the most important drawback of the method is the fact that in this case we do not have multiplication as in the in vitro method. We have a

Table 5. Efficiency of rooting in conventional propagation method

Repetition	Placed cuttings pcs	Efficiency of rooting	
		pcs	%
1	40	8	20.00
2	40	15	37.50
3	40	22	55.00
Total	120	45	37.50

Table 6. Standardization of rooted cuttings under BDS

Repetition	Rooted cuttings Nr.	Outside the standard Nr.	Standard Nr.	% of rooted standard roses
1	8	0	8	100%
2	15	2	13	86.6%
3	22	5	17	77.27%
Total	45	7	38	84.4%

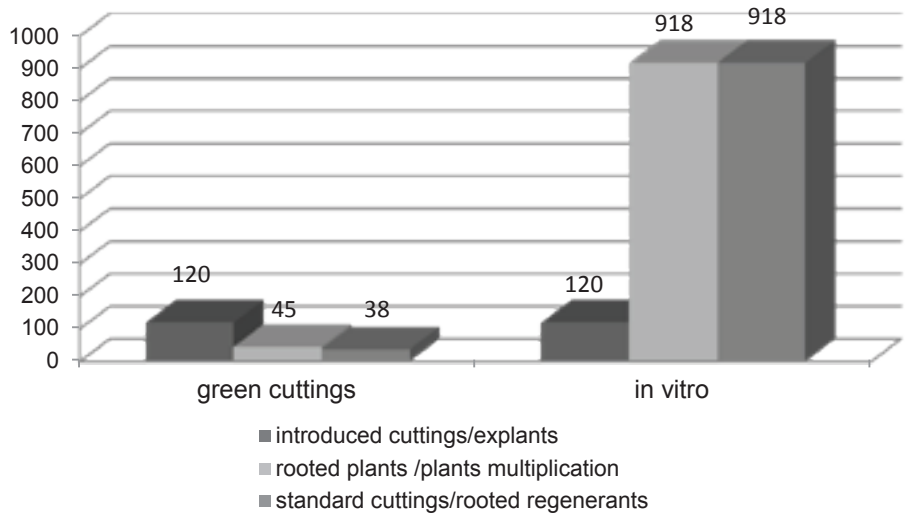

Fig. 1. Comparison between the number of obtained plants by conventional and in vitro propagation methods

certain amount of cuttings set and only a small percentage of them (37% in this experiment) are rooted and form young plants. This means that in any case at the end of the process we will get less rooted plants than the pre-set ones.

With the use of in vitro method, insignificant amounts of starting material are needed, easy for decontamination and multiplication according to the established protocol. The resulting propagation material is biologically clean, aligned, with the relevant parameters and high plant potential. This method allows to work all year-round without taking into account climatic conditions. The most important advantage is that there is a multiplication of the starting material (918 pcs. obtained from 120 explants set). The multiplication process takes place in laboratory conditions until a sufficient amount of micro shoots are obtained, and the rooting and adaptation process can be controlled in the cultivation facility. At planting in vitro seedlings in field conditions almost 100% relive was obtained. This is due to the fact that the root system is placed in a soil pack and retains its integrity when transferring from the greenhouse to the field. This enables long-term storage and long-distance transportation with no risk of drying and damage. With the conventional method the seedlings are removed from the soil with naked roots and transported to the field. This leads to root rupture and drying of the top parts, as a result of which a large number of the plants die after planting.

The results from the comparison of the effectiveness of conventional and in vitro clonal propagation of the Kazanlak oil-bearing rose are supporting the common opinion that the propagation in *in vitro* conditions lead to obtaining of a larger amount and much more adaptive plants, with better developed rooting system and with higher quality (Roberts and Schum, 2003; Uzunova, 2015).

CONCLUSIONS

The conduction of the first experiment of this research confirmed the high multiplication potential of the version of growth medium MS with supplement of 3.0 mg/l BAP and the high percentage of rooting of regenerated plants directly into the fertilizer-soil mixture from two Bulgarian genotypes Kazanlak oil-bearing rose. The successful ex vitro rooting allows for the whole procedure of the clonal propagation to be shortened and it is linked to saving money and time in comparison to using in vitro rooting. Therefore this combination of medium for multiplication and ex vitro rooting can be used successfully into the protocol for clonal micropropagation of Kazanlak oil-bearing rose, ensuring repeatability of results over different years. The comparison of the conventional propagation method and in vitro method showed that clonal micropropagation method is much more effective. The most important advantage of this method is that there is a multipli-

cation of the starting material. The propagation in in vitro conditions leads to not only the obtaining of a bigger number, but also to better adapting plants, with better rooting system and higher quality of the exit product.

REFERENCES

Altman, A. (2000). Micropropagation of plants, principles and practice. In: *Encyclopedia of Cell Technology*. New York: John Wiley & Sons, pp. 916-929.

Altman, A., & Loberant, B. (1998). Micropropagation: clonal plant propagation in vitro. In: *Agricultural biotechnology*. New York: Marcel Dekker, pp. 19-42.

Atanasova, M., & Nedkov, N. (2004). *Essential oil and medicinal crops*. Kamea, Sofia.

Badzhelova, V. (2017). In vitro propagation of oil-bearing rose (*Rosa damascena* Mill.). *Agricultural Science and Technology*, *9*(3), 194-197.

Badzhelova, V., Bozhanova, V., & Chokov, G. (2018). In vitro propagation of oil-bearing rose (*Rosa alba* L.). *Agricultural Science and Technology*, *10*(2), 90-95.

Cassells, A. C. (1997). Pathogen and microbial contamination management in micropropagation: an overview. In *Pathogen and microbial contamination management in micropropagation* (pp. 1-13). Springer, Dordrecht.

Ginova, A. & Kondakova, V. (2013). New approaches for in vitro propagation of oil-bearing rose. *Bulgarian Journal of Agricultural Science*, *19*(6), 1198-1203.

Ginova, A., Tsvetkov, I. & Kondakova, V. (2012). *Rosa damascena* Mill.- An overview for evaluation of propagation methods. *Bulgarian Journal of Agricultural Science*, *18*(4), 545-556.

Hameed, N., Shabbir, A., Ali, A., & Bajwa, R. (2006). In vitro micropropagation of disease free rose (Rosa indica L.). *Mycopath*, *4*(2), 35-38.

Ishioka, N., & Tanimoto, S. (1990). Plant regeneration from Bulgarian rose callus. *Plant Cell, Tissue and Organ Culture*, *22*(3), 197-199.

Kapchina-Toteva, V., van Telgen, H. J., & Yakimova, E. (2000). Role of phenylurea cytokinin CPPU in apical dominance release in in vitro cultured Rosa hybrida L. *Journal of Plant Growth Regulation*, *19*(2), 232-237.

Kornova, K. M., & Michailova, J. (1994). Study of the in vitro rooting of Kazanlak oil-bearing rose (Rosa damascena Mill.). *Journal of Essential Oil Research*, *6*(5), 485-492.

Lal, M., Tiwari, A. K., & Gupta, G. N. (2015). Commercial scale micropropagation of sugarcane: constraints and remedies. *Sugar Tech*, *17*(4), 339-347.

Lambev, H. (2011). Application of biostimulators in root cuttings of oil rose (*Rosa damascena* Mill.) in greenhouse. *Science & Technologies*, *1*(6), 114-117 (Bg).

Mitrofanova, I., Brailko, V., Lesnikova-Sedoshenko, N., & Mitrofanova, O. (2016). Clonal micropropagation and some physiology aspects of essential oil roses valuable cultivars regeneration in vitro. *Agriculture & Forestry/ Poljoprivreda i Sumarstvo*, *62*(4), 73-81.

Murashige, T., & Skoog, F. (1962). Revised medium for rapid growth and bioassay with tobacco micro-propagated. *Physiologia Plantarum*, *15*, 473-479.

Pati, P. K., Rath, S. P., Sharma, M., Sood, A., & Ahuja, P. S. (2006). In vitro propagation of rose—a review. *Biotechnology Advances*, *24*(1), 94-114.

Pittet, H., & Moncousin, C. (1982). Rose in vitro micropropagation. *Rev Hortic Suisse*, *55*(3), 67-69.

Roberts, A. V., & Schum, A. (2003). Cell, Tissue and Organ Culture (Micropropagation). In: *Encyclopedia of rose science*. Elsevier Academic Press, 57-66.

Senapati, S. K., & Rout, G. R. (2008). Study of culture conditions for improved micropropagation of hybrid rose. *Hort. Sci.(Prague)*, *35*(1), 27-34.

Uzunova, K. (2015). Comparative analysis of different rose cultivars (Rosa hybrida L.) rooting using conventional and biotechnology approaches. *Annuaire de l'Université de Sofia "St. Kliment Ohridski" Faculte de Biologie*, *100*(4), 191-199 (First National Conference of Biotechnology, Sofia 2014).

Vijaya, N., & Satyanarayana, G. (1991). Effect of culture media and growth regulators on in vitro propagation of rose. In *Horticulture: New Technologies and Applications* (pp. 209-214). Springer, Dordrecht.

Zlatev, S., Margina, A., & Tsvetkov, R. (2001). *Growing of Kazanlak oil-bearing rose*. Kazanlak.