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Comparison of in vitro callus induction between *Rosa damascena* and *Rosa alba*

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Abstract. *The potential for callus induction in the local population of Rosa damascena and Rosa alba was studied, and the influence of the different compositions of the main nutrient media and the hormones added to them was established. The results show that the induction and proliferation of callus mass are highly dependent on both the type of nutrient medium and the genotype. The highest total number of responding explants was obtained in Rosa alba, respectively 423 of 720 pre-placed and the highest percentage of explants that formed callus in both genotypes were reported on nutrient medium A24 - from 95% in Rosa damascena to 100% in Rosa alba.*

Keywords: *Rosa damascena* Mill., *Rosa alba*, tissue culture, nutrient medium, proliferation

Abbreviations: MS - Murashige and Skoog medium, RMS – modified MS medium, BAP - 6-Benzylaminopurine, TDZ-Thidiazuron, NAA - 1-Naphthaleneacetic acid,

Introduction

Rose production in Bulgaria has a centuries-old tradition. The world-famous *Rosa Kazanlak* is a source of income for many families in the Rose Valley. In Bulgaria, there are mainly two types of oil-bearing rose - *Rosa damascena Mill.f.trigintipetala Dieck*, represented by an improved local population-„Population №5" and the local population of *Rosa alba* L. Over the years, many researchers have worked tirelessly to study the genesis, peculiarities of reproduction, and agronomic requirements of this important culture for our country (Kovacheva, 2005; Lambev, 2011; Ginova and Kondakova, 2013; Badzhelova et al., 2018). Modern biotechnological methods, included in various breeding programs, made the possible achievement of biological diversity in a large number of important economic plant species. As a result of the influence of various biotic and abiotic factors during the cultivation process, the plants develop unorganized cell mass - callus. After the groundbreaking discovery that callus can be generated artificially in vitro and that the balance between two plant hormones, auxin, and cytokinin, determines the state of cell differentiation and dedifferentiation, callus has been widely used in plant biotechnologies (Ikeuchi et al., 2013).

The processes of cell dedifferentiation in plant explants and the subsequent formation of callus tissue are directly dependent on the interaction of the explant with the conditions for in vitro cultivation (Verpoorte et al., 2002).

Ginova et al. (2012) in their review for evaluation of the propagation methods of *Rosa damascena* Mill cite results of research for plant regeneration capability from Bulgarian rose callus by Ishioka and Tanimoto (1990).

In vitro study was carried out on the *Rosa hybrida* L. *cv. Babylon* to standardize the hormonal concentration for the callus induction using leaf bits as explant on Murashige and Skoog (MS) medium supplemented with different concentrations of auxins and cytokinins (Darsini and Anitha, 2008). The diverse results of the mentioned studies do not give an idea about the possibilities of callus induction in the Bulgarian oil-bearing rose. There are no known published data on callus initiation from white oil-bearing rose to date. This gives us reason to conduct a more in-depth study of the problem.

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This experiment aims to study the possibilities for callus induction in vitro, in two oil-bearing species, from the Rose Valley in Bulgaria.

Material and methods

Plant material

In this research *„Population №5“* of *Rosa damascena* Mill. *f. Trigintipetala Dieck* has been given a laboratory number (R3), and the *Rosa alba* population cultivar a laboratory number (R2), respectively. The plant material has been obtained from elite experimental fields of the Institute of Roses and Essential Oil Crops in the town of Kazanlak, situated in the Valley of Roses, Bulgaria. In vitro material obtained from clonal in vitro propagation of the two genotypes was used (Badzhelova, 2017; Badzhelova et al., 2018). The starting explants were leaf stalks with adjacent leaf segments.

Callus induction

The potential for callus induction on different basic nutrient media and the influence of the combination and concentration of plant hormones added to them have been studied.

Two series of nutrient media have been prepared, with six variants for each series.

- series (A1) basal medium is standard MS (Table 1).
- series (A2) is an RMS medium with reduced ammonium ion content (Table 2).

Table 1. Composition nutrient media - Series A1/ basic nutrient medium is MS

Nutrition medium MS+	TDZ (mg/l)	NAA (mg/l)	BAP (mg/l)
A11	1.5		
A12	1.0	1.0	
A13	1.0	0.5	
A14	1.0	0.2	
A15		0.5	0.5
A16		0.2	1.5

Table 2. Composition nutrient media - Series A2/ basic nutrient medium is RMS

Nutrition medium RMS+	TDZ (mg/l)	NAA (mg/l)	BAP (mg/l)
A21	1.5		
A22	1.0	1.0	
A23	1.0	0.5	
A24	1.0	0.2	
A25		0.5	0.5
A26		0.2	1.5

Different types and concentrations of plant hormones from the group of auxins and cytokinins are added to the basal media. The medium is solidified with 6 g/l agar, and the pH is set at 6.0 before sterilization, which takes place at 121°C at 1.1 atm. for 20 minutes. The experiment was performed by complete randomized block design in three replications. Each replication consists of five glass vessels, containing four explants each. The glass vessels with explants are cultured in a phytostat room with a temperature of 21°C, and an illumination of 2000 lux. cool white, period light/dark - 10/14 for 35 days. The callus is monitored for 6 months, with the explants transferred to fresh medium every 4 weeks and the result is reported.

The number of reacted explants and the onset of callus mass formation is determined, as well as the influence of the type, concentration, and combination of hormones added to the induction medium.

Results and discussion

Callus formation started 30 days after the experiment starts. The initial callus induction was observed first at Rosa damascena, on nutrient medium A13 of series A1.

Callus formation was observed on most of the induction media except the A11 and A21 variants.

Table 3. Callus formation on nutrient medium series A1 (*Rosa damascena)*

Variant	Used explants (pcs)	Callus induction started at (day)	Total explants, formed callus (pcs)	Variant success (%)
A11	60	-	0	0.0 %
A12	60	40	2	3.3%
A13	60	30	22	36.6%
A14	60	37	32	53.3%
A15	60	45	5	8.3%
A16	60	34	11	18.3%

Table 4. Callus formation on nutrient medium series A2 (*Rosa damascena*)

Variant	Used explants (pcs)	Callus induction started at (day)	Total explants, formed callus (pcs)	Variant success (%)
A21	60	-	0	0.0%
A22	60	45	8	13.3%
A23	60	35	44	73.3%
A24	60	35	57	95.0%
A25	60	38	11	18.3%
A26	60	34	28	46.6 %

Table 5. Callus formation on nutrient medium series A1 (*Rosa alba*)

Variant	Used explants (pcs)	Callus induction started at (day)	Total explants, formed callus (pcs)	Variant success (%)
A11	60	-	0	0.0%
A12	60	40	18	30.0%
A13	60	35	54	90.0%
A14	60	32	58	96.6%
A15	60	42	25	41.6%
A16	60	34	43	71.6%

Table 6. Callus formation on nutrient medium series A2 (*Rosa alba*)

Variant	Used explants (pcs)	Callus induction started at (day)	Total explants formed callus (pcs)	Variant success (%)
A21	60	-	0	0.0%
A22	60	42	31	51.6%
A23	60	34	59	98.3%
A24	60	32	60	100.0%
A25	60	41	23	38.3%
A26	60	31	51	85.0%

Callus formation on different nutrient media (*Rosa damascena*)

Callus formation on different nutrient media (*Rosa alba*)

From the results presented above, obviously on variants of nutrient media A11 and A21, no callus is formed, regardless of the genotype or main nutrient medium composition.

This is probably due to the high concentration of TDZ- 1.5 mg/l, respectively, or its single use.

The obtained callus on the rest of the media has a burly, grainy structure and deep green color, probably regenerative.

It is noteworthy that the callus formation is mainly on the leaf stalk and on a very small part of the leaf segment.

The highest percentage of explants forming callus was obtained on variant A24 - from 95% in *Rosa damascena* to 100% in *Rosa alba*, respectively, where all explants react with forming a callus. A slightly lower percentage was reported in nutrient medium A14, from series A1 - 53.3% for *Rosa damascena* and 96.6% for *Rosa alba*, which is

the same as medium A24 in type and concentration of added hormones, but differs in the composition of the basic nutrient medium.

In the research of Darsini and Anitha (2008), the best results for rapid callus proliferation is with the hormonal concentration of 1.5mg/L 2,4-D and 0.1mg/L BAP on base medium MS. Our results presented in Tables 3,4 and Tables 5,6 show that the combination 1.0 mg/l TDZ + 0.2 mg/l NAA is particularly suitable for both genotypes used, regardless of the composition of the basic nutrient medium.

Also in Iran's study on callus induction of rose damascena with different types of explants, a group of hormones was studied and the effect of their concentrations and combinations was determined. This study found that NAA and BAP had the greatest impact on callus formation. The most suitable induction medium in the particular study was NAA 0.1 mg/l and BAP 5 mg/l (Farhangi-sabet and Behboodi, 2004).

In our case, the highest percentage of responded explants was obtained in the combination of 1.0 mg l TDZ + 0.2 mg / l NAA in both genotypes studied.

As can be seen in nutrient variants A16 and A26, the influence of genotype on callus formation is the most pronounced. In *Rosa alba*, the number of explants which reacted with callus formation, ranged from 43 in the A1 series to 51 in the A2 series, and in *Rosa damascena* this number was significantly lower, 11 in the A1 series and 28 in the A2 series. In the other nutrient medium variants, there is no clear distinction between the two genotypes, but in all variants Rosa alba demonstrates better results than Rosa damascena. The total number of explants that reacted with callus mass formation for *Rosa alba* is 423, out of a total of 720 explants or 58.75%, and only 220 in *Rosa damascena* - 30.55% of all explants. These results allow us to summarize that in this experiment, *Rosa alba* demonstrated a higher callus induction potential than *Rosa damascena*.

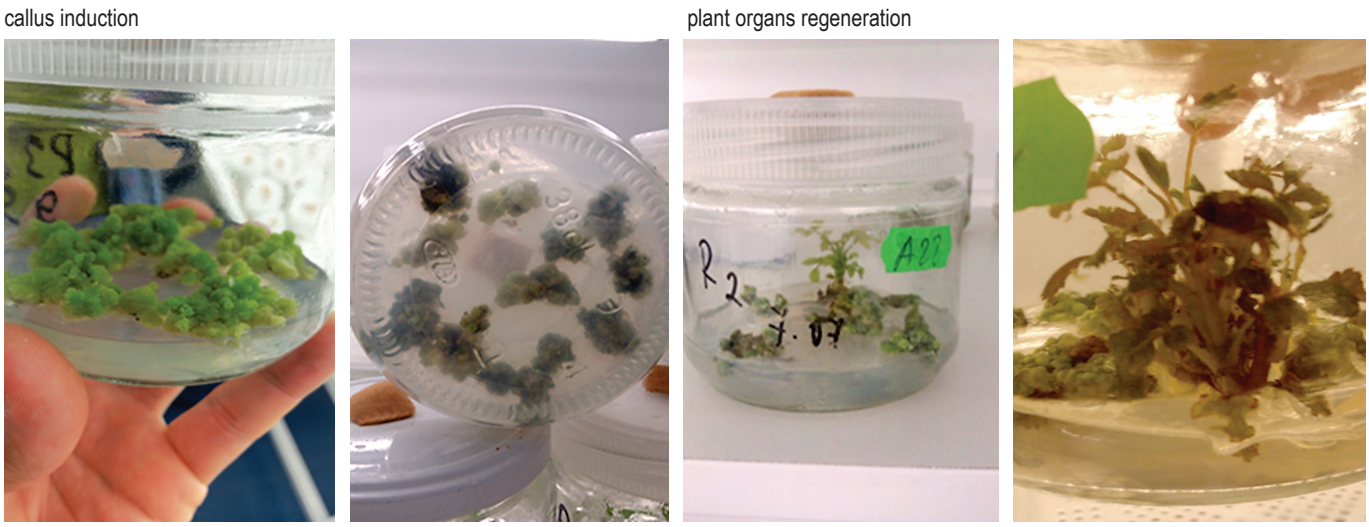

Figure 1. Callus induction and plant organs regeneration

One and a half months after the beginning of the experiment, in two of the explants of *Rosa alba*, on medium A22, regeneration of plant organs from the callus mass in the area of the leaf petiole was obtained. Probably the combination of TDZ and NAA has a synergistic effect on the indirect regeneration of plant organs in the oil-bearing rose. This result was accepted as accidental and was not discussed because it is not the subject of this experiment. On the other hand, in a study from 1990 Ishioka and Tanimoto obtained indirect regeneration on Bulgarian rose callus on the medium without ammonium nitrate and contained indoleacetic acid and benzyl adenine (Ishioka and Tanimoto, 1990).

All this gives reason for additional studies, including the same species with similar conditions.

Conclusion

For the first time, a study related to the potential for callus induction in two species of oil-bearing rose from Bulgaria was conducted. In many of the studies related to callus induction and indirect regeneration of roses, certain similarities in the approach of work were noticed. Usually, the main nutrient medium is MS, or its variations are obtained by changing the amount of ammonium nitrate. In this study, the best results

were reached on a modified MS basic medium with a reduced content of ammonium ions. From that, we can conclude that in order to achieve a high callus induction in different types of rose, it is recommended to use a basic nutrient medium MS with a reduced content of ammonium ions. In a number of studies, as an addition to the induction medium, a certain set of hormones from the group of auxins and cytokinins are included. It turns out that most parts from the successful experiments were obtained using mainly BAP, TDZ, NAA, and 2,4-D in different concentrations and combinations between them. Our study reported the highest results on the induction medium containing TDZ and NAA. The high concentration of TDZ when used alone, does not lead to the formation of callus mass, regardless of the composition of the basic nutrient medium and genotype. Apparently, the appropriate combination and optimal concentration of the used hormones, influence the speed and volume of the callus induction process, also found that, under equal conditions, this process is faster and more successful in Rosa alba. This experiment showed that the induction of callus in both species of oil-bearing rose from Bulgaria directly depends on the genotype and the composition of the basic nutrient medium and the hormones added to it.

References

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

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

Darsini DTP and Anitha J, 2008. *In vitro* callus induction of *Rosa hybrida* L. Cv. Babylon. Biosciences, Biotechnology Research Asia, 5, 857-860.

Farhangi-sabet M and Behboodi B SH, 2004. The study of biotechnology and callus formation on Rosa damascena Mill. in the Kashan Region, Fourth International Iran & Russia Conference.

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

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

Ikeuchi M, Sugimoto K and Iwase A, 2013. Plant Callus: Mechanisms of induction and repression. The Plant Cell, 25, 3159-3173.

Ishioka N and Tanimoto S, 1990**.** Plant regeneration from Bulgarian rose callus. Plant Cell Tiss. Organ Cult, 22, 197-199.

Kovacheva N, 2005. Research for oil-bearing rose population (*Rosa alba* L.). Jubilee scientific conference with international participation "State of the art and problems of agricultural science and education", 19-20 October, Plovdiv, 277-283.

Lambev H, 2011. Application of biostimulators in root cuttings of oil rose (*Rosa Damascena* Mill.) in greenhouse. Science and Technologies, 6.

Murashige T and Skoog F, 1962. Revised medium for rapid growth and bioassay with tobacco tissue culture. Physiologia Plantarum, 15, 473-479.

Verpoorte R, Contin A and Memelink J, 2002. Biotechnology for the production of plant secondary metabolites. Phytochemistry Reviews, 1, 13-25.