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Research Article

Rhizogenesis in Shrub rose cultivated *in vitro*

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Abstract: The study of the reproduction characteristics of roses of the garden class Shrub, the definition of the dependence of the hormonal determination of explant rhizogenesis on the concentrations of phytohormones that are part of the nutrient medium, are relevant and has both scientific and practical interest. This study presents the results of studies of hormonal determination of rhizogenesis in explants of cultivars of roses of the garden class Shrub: Gärtnerfreude, Lavender Dream, Pomponella, Red Cascade, Sommerabend cultivated *in vitro* on nutrient medium containing growth regulators. It has been established that of the nutrient medium modified by the addition of 0.2-1.0 mg/l α-naphthylacetic acid (α-NAA), the most effective was the medium with the content of α-NAA 0.5 mg/l, the content of macro- and microelements half of the Murashige and Skoog prescriptions, and a decrease in the sucrose content to 2.0%. On this medium, the frequency of rhizogenesis averaged 61.2% for the studied cultivars. Hormonal determination of rhizogenesis and efficiency of root formation *in vitro* in the Shrub rose regenerants depended on the genotype of the plant: cv. Lavender Dream (66.0%) and cv. Sommerabend (67.0%) had the highest rhizogenesis ability. The use of the universal growth regulator Humifield in combination with 0.5 mg/l α-NAA contributed to an increase in the rooting rate of the studied rose cultivars up to 70.0-86.0%.

Özet: Çalı güllerinin üreme özelliklerinin incelenmesi ve eksplant kök oluşumunun hormonal kontrolünün kullanılan besleyici ortamın fitohormon konsantrasyonuna bağlı olduğunun tanımlanması önemlidir ve hem bilimsel hem de pratik açıdan üzerinde durulan konulardır. Bu çalışmada, büyüme düzenleyicileri içeren besin ortamı üzerinde *in vitro* olarak yetiştirilen çalı gülü kültivarlarının (Gärtnerfreude, Lavender Dream, Pomponella, Red Cascade, Sommerabend) eksplantlarında kök oluşumunun hormonal belirlenmesine yönelik denemelerin sonuçları sunulmuştur. 0,2-1,0 mg/l a-naftilasetik asit (a-NAA) ilavesiyle modifiye edilen besin ortamlarından en etkilisinin, 0,5 mg/l a-NAA içerikli, sukroz oranı %2'ye kadar düşürülmüş ortam olduğu tespit edilmiştir. Bu ortamda, çalışılan kültivarlar için kök oluşumu sıklığı ortalama %61,2 olarak belirlenmiştir. Kullanılan kültivarlardaki kök oluşumunun *in vitro* etkinliğindeki hormonal katkının kültivarların genotiplerine bağlı olduğu belirlenmiştir: Lavender Dream kültivarı (%66,0) ve Sommerabend kültivarı (%67,0) en yüksekkök oluşumu yeteneği sergilemişlerdir. Bir büyüme düzenleyicisi olan Humifield'in 0,5 mg/l α-NAA ile kombinasyon halinde kullanılması, incelenen gül kültvarlarında köklenme oranının %70,0-86,0'a kadar artmasına katkıda bulunmuştur.

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Introduction

The modern technology of plant propagation (use of plant tissue culture methods) effectively complements traditional methods, when propagation of plants by seed or vegetative means is not effective. Traditionally, self-rooted seedlings of garden roses are obtained by rooting of stem cuttings, layering, dividing the plants (Kroin 2016). An alternative to traditional methods of plant propagation is the introduction of modern technologies, among which the leading place belongs to micropropagation (Chawla 2011, Kumar & Reddy 2011, Hasnain *et al* 2022).

The method of tissue culture for micropropagation of roses using isolated meristems began to be developed in the 70-80s of the 20th century (Horn 1992). However, the issue of the induction and course of the stages of organogenesis of each plant genotype has not yet been practically studied, approaches to the selection of effective inducers of various stages of organogenesis *in vitro* have not been determined, and the mechanisms that are responsible for the effective organogenesis of roses, the cultivation of which is of great interest for green building have not been sufficiently studied.

The expediency of using ornamental plants for architectural and artistic design of residential areas depends, first of all, on the ecological and biological properties of these plants including drought resistance, winter hardiness, dust and gas resistance, resistance to pests and pathogens etc. In the process of creating decorative plantings for outdoor gardening, special attention is paid to flowering shrubs that do not need systematic care and have a long bloom period. These requirements are met by roses of the garden class Shrub, which include, in particular, roses of the selection by D. Austin (English Roses), the so-called ground cover roses, etc. Observations have shown that the roses of this group are well adapted to the soil and climatic conditions of the Right-Bank Forest-Steppe Zone of Ukraine, showing high decorative qualities (Moroz *et al*. 2010, Moroz *et al*. 2012, Denysko 2022).

In vitro propagation makes it possible to juvenile a culture, heal plants from fungal and bacterial infections, preserve their species and varietal characteristics, and significantly increase the multiplication factor, which plays an important role in the development of cost-effective propagation technologies and accelerated plant cloning (Kalinin *et al*. 1992, Jain & Ishii 2003, Nikbakht *et al.* 2005, Hameed *et al.* 2006, Tytarenko & Tesliuk 2020).

The final stage of plant propagation *in vitro* is the rhizogenesis of the resulting explants, which combines many vital biochemical, physiological and histological processes, the efficiency of which further affects the viability of regenerated plants obtained *in vitro* (De Klerk 2002). The process of adventitious root formation occurs in several stages: induction, initiation, appearance and growth of roots in explants (Podwyszynska 2003).

The success of the stage of rhizogenesis depends on many factors including the genotype of the culture itself, the hormonal composition of the nutrient medium, the number of passages, the conditions of conducting the experiment (Bidabadi & Jain 2020). To achieve a high percentage of root formation, the addition of growth regulators of auxin nature to nutrient medium is used, a decrease by 1/2, and sometimes by 1/4 of the content of macro- and microelements and sucrose can be used (Arnold *et al*. 1995, Carelli & Echeverrigaray 2002).

The key role in the induction of the formation and development of roots is played by plant growth and development regulators of the auxin type of action (Kalinin *et al*. 1992, Vedmid *et al*. 2002, Rugini & Pesce 2006). They contribute to the stimulation of morphogenetic and physiological processes in explants, affect the division and growth of plant cells and provide a regular sequence of phases of individual development (Koldar *et al*. 2021). Recently, as evidenced by the results of the analysis of scientific literature, the role of plant growth and development regulators of low-toxic environmentally friendly substances that are effective when used in small quantities is increasing (Koldar 2008, Molnar *et al*. 2011, Chauhan *et al*. 2018).

A high number of studies addresses the characteristic features of reproduction, growth and development *in vitro* of different classes of roses (Dubois *et al*. 1988, Datta *et al.* 2002, Hameed *et al*. 2006, Attia *et al.* 2012). But in most cases, technologies developed for a certain genotype are not effective for other plants, and each of the next genotype under study requires the development of its own *in vitro* propagation technology.

Therefore, the study of the characteristics of regeneration, morphogenesis of Shrub class roses under *in vitro* condition, in particular, the achievement of rhizogenesis by explants is relevant and needs appropriate research. Stimulation of shoot and root formation is achieved by selecting the necessary ratios of cytokinins and auxins and their concentrations in the nutrient medium. They are external factors that contribute to the activation of competent cells capable of perceiving inducing factors, thereby excluding determination only in a certain direction (Koldar 2012, Podgajeckyj *et al*. 2018, Kosenko *et al*. 2021). Hence, the preparation of nutrient medium is carried out individually for definite plant genotypes, taking into account their species and cultivar characteristics (Nebykov *et al*. 2016, Khudolieieva *et al*. 2017, Mishchenko & Krivosheeva 2018).

The aim of the study is to define the dependence of hormonal determination of rhizogenesis in explants of roses of the Shrub class on the concentrations of α-naphthylacetic acid (α-NAA) and the universal growth regulator Humifield in nutrient medium under *in vitro* propagation.

Materials and Methods

Description of the study site

The hormonal determination of rhizogenesis by rose regenerants was carried out in the laboratory of microclonal propagation of the Department of Ornamental and Fruit Plants of the Sofiyivka National Dendrological Park of the National Academy of Sciences of Ukraine (hereinafter NDP "Sofiyivka"). NDP "Sofiyivka" is located in the northern part of the city of Uman, Cherkasy region (Ukraine).

Plant material for in vitro studies

Plant material of Shrub roses (Gärtnerfreude, Lavender Dream, Pomponella, Red Cascade, Sommerabend) from the NDP "Sofiyivka" collection fund was used for the study (Fig. 1).

Cultivar (cv.) Gärtnerfreude (W. Kordes Söhne, 1991). The flowers are carmine-red, diameter 3-4 cm, cup-shaped, double, no fragrance, in inflorescences. The leaves are dark green, glossy. The plant is spreading, branched, 0.5-0.7 m high. The bloom is abundant, repeated (June, August).

cv. Lavender Dream (G. P. Ilsink, 1984). The flowers are pinkish-lilac, diameter 3-4 cm, cup-shaped, semi-double, mild fragrance, in large inflorescences. The

Fig. 1. The Shrub roses from the NDP "Sofiyivka" collection fund. **a.** Gärtnerfreude, **b.** Lavender Dream, **c.** Pomponella, **d.** Red Cascade, **e.** Sommerabend.

leaves are light green, matte. The plant is spreading, 1-1.5 m high. The bloom is abundant, repeated (June, August).

cv. Pomponella (W. Kordes Söhne, 2005). The flowers are pink, diameter 4-6 cm, spherical, double, mild fragrance, collected in inflorescences. The leaves are dark green, semi-glossy. The plant is upright, branched, 0.8-1.5 m high. The bloom is abundant, repeated (June, August).

cv. Red Cascade (R. S. Moore, 1976). The flowers are dark red, cup-shaped, diameter 2-3 cm, double, no fragrance, in large inflorescences. The leaves are green, semi-glossy. The plant is creeping, up to 0.5 m high with prickly stems 0.8-1.2 m long. The bloom is abundant, almost ceaseless throughout the season.

cv. Sommerabend (W. Kordes Söhne, 1995). The flowers are dark red, cup-shaped, diameter 4-6 cm, double, no fragrance, in large inflorescences. The leaves are green and glossy. The plant is creeping, up to 0.4 m high with prickly stems up to 4 m long. The bloom is abundant, ceaseless throughout the season.

Cultivation conditions and determinants of stimulating root formation in explants

The single nodes (5.0-15.0 mm) taken from anually shoots from 6-14-years-old paternal rose plants was the source of explants for an introduction into *in vitro* culture. Pretreatment of roses explants was carried out using a disinfectant BTC 885 containing ammonium chloride salts in a concentration of 21.7% (IPAX CLEANOGEL, USA), and the main treatment was carried out with the treatment of mercury dichloride ($HgCl_2$) with the addition of the emulsifier Tween-80 (Scharlau Chemie, Spain) with an exposure of 1.5 min. After two-stage sterilization, aseptic and viable explants were cultivated on nutrient medium supplemented with phytohormones of the auxin (0.01 mg/l β-IBA) and cytokin (2.0 mg/l 6-BAP) groups (Kosenko *et al*. 2021).

The plant material was cultivated at a temperature of 24 ± 1°C, a 16-hour photoperiod, an illumination intensity of 3000 lx, and a relative humidity of 70.0%. Nutrient medium, materials and instruments were prepared according to the methodological recommendations by Pierik (1997), Kunakh (2005) and Chawla (2011).

The objects of the study were explants of the second and further passages, which reached a height of 2.0-4.0 cm with 2-3 pairs of leaves (Fig. 2). The authors used the method of induction of rhizogenesis in explants by adding α-NAA of various concentrations to the nutrient medium (0.2, 0.5, 0.8, 1.0 mg/l). The cultivation of explants was carried out on a universal basic agar nutrient medium according to the prescription of Murashige and Skoog (MS) with the addition of a half dose of macro- and microelements (Murashige & Skoog 1962). To increase the percentage of rooting of explants, the universal plant growth regulator Humifield (potassium humate) was used — a natural product manufactured by Humintech GmbH (Germany) from leonardite, a special type of brown coal with a high content of humic acids. The stimulant (coal humate) contains about 80.0% humic acids and a full range of microelements with 100% solubility. In order to optimize the rhizogenesis of explants of the studied rose cultivars, the study was made of the complex effect of α-NAA at a concentration of 0.5 mg/l and Humifield — 5.0; 10.0; 15.0 and 20.0 mg/l.

Fig. 2. Explants of roses were selected for cultivation in rooting medium.

Experimental design, data collection and statistical analysis

Percentage of rooted explants and quantitative parameters of their development were defined within 30-40 days. For quantitative analysis, at least 25 explants were taken per experimental variant. Data on the parameters of the development of rooted explants, in particular the number of roots, the root length, and the shoot height were determined as the average of the measurements of 5 separate plants. All experiments were performed in triplicate. Results were presented as mean value (x) ± standard deviation (SD). Data were subjected to ANOVA and the means were compared by Duncans multiple range test ($p \leq 0.05$).

Results

When cultivating explants of different cultivars of roses on nutrient medium for 10-18 days in the basal part of the explant, the initial stage of rhizogenesis determination was observed — the appearance of callus in the explants. Depending on the cultivar and the concentration of auxin in nutrient medium, root primordia appeared with different growth rates, from which roots formed within 28-36 days. In the course of the study, the dependence of the efficiency of rhizogenesis on the plant genotype and auxin concentration was established. Root formation was not observed on a hormone-free medium (control) (Fig. 3).

Of the nutrient medium modified by the addition of 0.2–1.0 mg/l α-NAA, the most effective was the medium containing 0.5 mg/l α-NAA, during cultivation in which the frequency of rhizogenesis for the studied cultivars averaged 61.2% of the number of explants planted for rooting. The susceptibility of each cultivar to the studied concentrations of α-NAA showed that regenerated plants of the cv. Lavender Dream (66.0%) and cv. Sommerabend (67.0%) had the highest percentages for rhizogenesis (Fig. 4). The number of rooted explants decreased by an average of 8% with an increase in concentration of α-NAA up to 0.8 mg/l. An increase in the concentration of α-NAA to 1.0 mg/l did not activate rhizogenesis more; instead, it inhibited it, whereupon the number of rooted plants by variety was in the range of 23.0-43.0%. The proportion of rooted plants was relatively low with a decrease in the concentration of α-NAA to 0.2 mg/l, which resulted in the decrease of the rhizogenesis of regenerated plants to 4.0-18.0%.

Fig. 4. Regenerated plant cv. Sommerabend.

The cultivars differed in the number of roots, the root length, and the shoot height (Table 1).

The number of formed roots per explant on average ranged from 1.9 (at a concentration of 1.0 mg/l α-NAA) to 3.2 (0.5 mg/l α-NAA) pcs., while the highest number

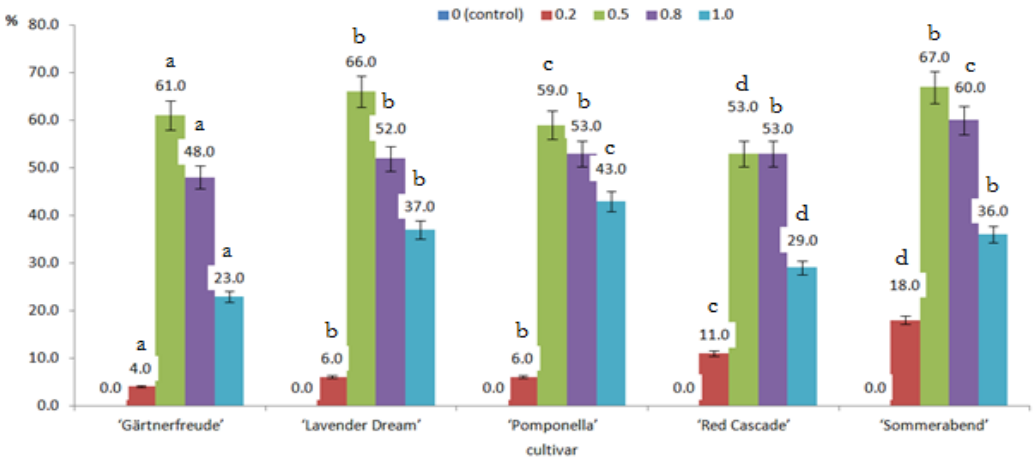

Fig. 3. Rooted rose plants on MS nutrient medium containing α-NAA (mg/l).
Note: The letters (**a-d**) define homogeneity groups (Duncan test, $p < 0.05$).

of roots in explants of Gärtnerfreude (3.1 pcs.) were formed on nutrient medium with a concentration of 0.8 mg/l α-NAA, and in explants of Lavender Dream, Pomponella, Red Cascade, Sommerabend (respectively 2.8; 3.9; 3.7; 3.5 pcs.) they were formed by concentration of 0.5 mg/l α-NAA. The shortest roots in all cultivars of roses were noted on the nutrient medium with the addition of 0.2 mg/l and 1.0 mg/l of α-NAA (with an average of 2.6 and 2.2 cm). When 0.5 mg/l of α-NAA was added to the nutrient medium, the roots reached a length from 3.2 cm (Gärtnerfreude) to 5.3 cm (Lavender Dream).

The main shoots continued to grow in the explants, and within 25-38 days, they reached 2.5-4.8 cm in height and formed three to four pairs of well-developed leaves. With a change in the concentration of the hormonal component of the nutrient medium, simultaneously with root formation, the growth of the vegetative part of plants was different (Fig. 5). At 0.5 mg/l concentration of α-NAA, vegetative shoots reached a maximum height in cv. Pomponella which became 4.8 cm, in cv. Red Cascade — 4.6 cm, and in cv. Lavender Dream, cv. Sommerabendand cv. Gärtnerfreude —4.4, 4.3 and 3.6 cm, respectively.

Fig. 5. Rooted rose plants

In order to increase the rhizogenes potential of regenerated plants of the studied culrivars, the MS nutrient medium containing 0.5 mg/l α-NAA was optimized by adding Humifield growth regulator. The composition of the regulator includes humic acids, which have a versatile effect on plant growth processes and carry out their regulation throughout the entire period of vegetation, promote the enzymatic activity of cells, and stimulate vital processes (Rugini & Pesce 2006, Kashyap *et al.* 2017).

The complex use of the growth regulators resulted in the activation of the initiation of root primordia and the growth of roots. The highest frequency of rhizogenesis was observed in explants cultivated on a medium containing α-NAA 0.5 mg/l and Humifield 10 mg/l (Fig. 6).

The highest proportion of rooted plants was obtained for the cv. Lavender Dream (86.0%), which is 20.0% higher than when using α-NAA only. A high percentage of rooting was also noted for the cv. Red Cascade (80.0%). Rooting percentages for cultivars Pomponella and Sommerabend with the addition of 10 mg/l Humifield were slightly lower, and were 78% and 70%, respectively (Fig. 7). With the content of 5 mg/l of Humifield the number of rooted roses explants was 9.0-27.0% higher than the control.

With the content of 5 mg/l of Humifield in the medium, the rooting rates were similar to the control and amounted to 65.0-73.0%, and an increase in its amount to 15 mg/l contributed to raising the percentage of rooting increased by 3.0-5.0% for the cv. Gärtnerfreude, Lavender Dream, Red Cascade, Sommerabend. Slightly lower rates of rhizogenesis were obtained by adding 20 mg/l of Humifield to the nutrient medium, on which the rooting rates for all the cultivars became 46.0-59.0%.

Fig. 7. Rhizogenesis in cv. Red Cascade explants

Discussion

The rooting of explants *in vitro* of most taxa has always been problematic, especially after long-term cultivation in isolated culture. Many scientists noted that for the formation of roots, it was necessary to completely remove cytokinins from the composition of the nutrient medium and add increased concentrations of auxins. Preparations of this group of phytohormones are the main ones for inducing rhizogenesis (Kalinin *et al.* 1992, Arnold *et al.* 1995, Carelli & Echeverrigaray 2002, Kunakh 2005, Figas *et al.* 2016, Zapolsky 2021).

The successful use of different auxins for root induction in rose *in vitro* has been reported, in particular, indole-3-butyric acid (IBA) for *Rosa ×centifolia* L. and cv. Gruss an Teplitz (Baig *et al*. 2011) *R. hybrida* Vill. Al-Taif (Attia *et al*. 2012); *R. × damascena* Herrm. Isfahan and Kashan (Saremi-Rad & Mohammadi 2020), for *R. pisiformis* (Christ.) D. Sosn. (Özel *et al*. 2023); α-NAA for *R. canina* L. (Davoudi Pahnekolayi *et al*. 2016), *R. hybrida* (Oo *et al*. 2021); or a combination thereof for *R. canina* та *R. beggeriana* Schrenk ex Fisch. & C.A.Mey. (Moradian & Bagheri, 2019), hybrid tea rose

Table 1. Morphometric indicators of the development of rose during rooting *in vitro.*

Cultivar	α-NAA concentrations to the nutrient medium (mg/l)			
	0.2	0.5	0.8	1.0
Number of roots				
Gärtnerfreude	1.8 ± 0.2 a	2.1 ± 0.1 a	3.1 ± 0.1 a	1.7 ± 0.1 a
Lavender Dream	2.3 ± 0.2 b	2.8 ± 0.2 b	2.7 ± 0.1 b	1.6 ± 0.1 b
Pomponella	1.8 ± 0.1 a	3.9 ± 0.1 c	3.1 ± 0.1 a	2.4 ± 0.1 c
Red Cascade	2.7 ± 0.1 c	3.7 ± 0.1 d	3.4 ± 0.1 c	2.6 ± 0.1 d
Sommerabend	2.1 ± 0.1 d	3.5 ± 0.2 e	2.8 ± 0.2 b	1.4 ± 0.1 e
x ± SD	2.1 ± 0.4	3.2 ± 0.7	3.0 ± 0.3	1.9 ± 0.5
Root length (cm)				
Gärtnerfreude	2.4 ± 0.2 a	3.2 ± 0.2 a	2.6 ± 0.2 a	2.3 ± 0.3 a
Lavender Dream	2.7 ± 0.2 b	5.3 ± 0.2 b	4.8 ± 0.2 b	2.8 ± 0.2 b
Pomponella	2.3 ± 0.2 a	4.2 ± 0.2 c	3.8 ± 0.2 c	2.5 ± 0.3 ab
Red Cascade	2.8 ± 0.2 b	4.1 ± 0.2 c	3.2 ± 0.2 d	2.2 ± 0.2 a
Sommerabend	2.9 ± 0.2 b	3.6 ± 0.2 d	2.7 ± 0.2 a	1.4 ± 0.2 c
x ± SD	2.6 ± 0.3	4.1 ± 0.8	3.4 ± 0.9	2.2 ± 0.9
Shoot height (cm)				
Gärtnerfreude	2.9 ± 0.17 a	3.6 ± 0.21 a	4.1 ± 0.13 cd	2.5 ± 0.18 a
Lavender Dream	3.4 ± 0.16 b	4.4 ± 0.09 b	4.6 ± 0.15 ac	3.7 ± 0.14 bc
Pomponella	3.4 ± 0.11 bc	4.8 ± 0.7 b	4.1 ± 0.15c	2.7 ± 0.12 ad
Red Cascade	2.9 ± 0.9 ac	4.6 ± 0.16 b	3.9 ± 0.12 c	3.1 ± 0.15 cd
Sommerabend	2.6 ± 0.09 a	4.3 ± 0.15 b	3.8 ± 0.17 db	2.7 ± 0.16 a
x ± SD	3.0 ± 0.4	4.3 ± 0.5	4.1 ± 0.3	2.9 ± 0.5

Note: The letters (a-e) define homogeneity groups (Duncan test, $p < 0.05$)

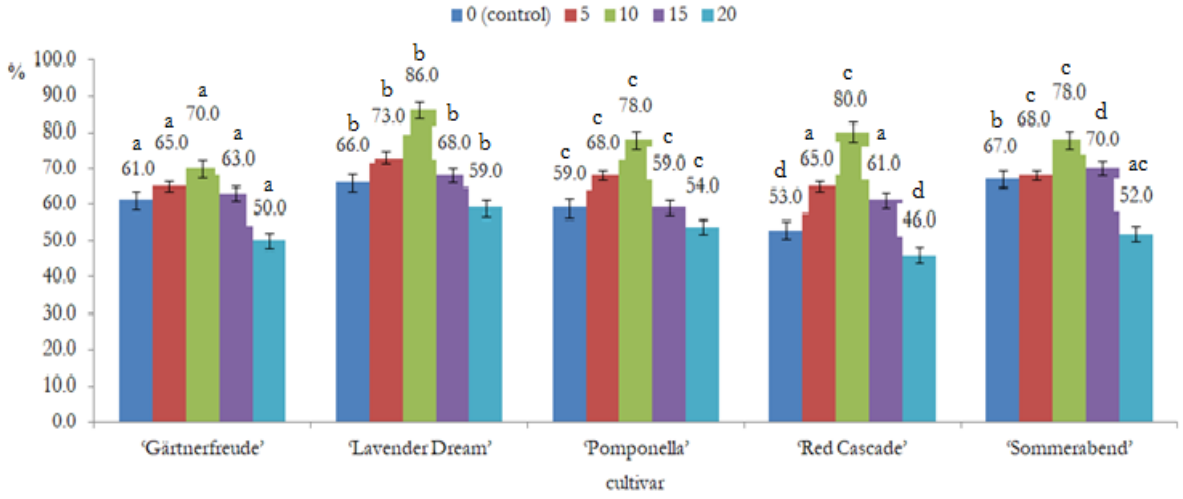

Fig. 6. Rooting of the Shrub rose depending on the content of growth regulators α- NAA (0.5 mg/l) and α-NAA + Humifield (mg/l) in the nutrient medium.
Note: The letters (a-d) define homogeneity groups (Duncan test, $p < 0.05$).

cv. Raktagandha (Kumari *et al*. 2017) and *R. hybrida* (Afrin *et al*. 2022); indole-3-acetic acid (IAA) for *R. mini* L. (Tsygankova *et al*. 2022). The result of *in vitro* rooting depended primarily on the concentration of the stimulator and, of course, it was a species-specific reaction. In earlier reports, it was noted that the ability to in vitro root formation was lower in the old world rose species *R. canina* and *R. ×damascena* compared to *R. hybrida* cultivars (Khosh-Khui & Sink 1982, Rezanejad *et al*. 2023). Rezanejad *et al.* (2023) demonstrated the efficacy of pretreatment on solid MS medium for a duration of two weeks, containing 3 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D), for the *in vitro* rooting of miniature rose Modern Hybrid and *R. × damascena*. Then, explants were transferred to a half strength MS liquid medium containing 0.05 mg/l IAA and without IAA, respectively (Rezanejad *et al*. 2023). Reports of successful rooting of roses without auxins are rare (Ozel & Arslan 2006, Šiško 2011).

In the course of our research, rooting of the researched cultivars of roses was not observed on a hormone-free MS medium. The determinants of stimulating root formation in explants were different ratios of α-NAA in the nutrient medium. The use of this external factor contributed to the activation of rhizogenesis processes and the formation of up to 61.2% of rooted explants.

Putrescine (Musavi Ahmadabadi *et al.* 2023), chitosan (Yegorova *et al*. 2023) and phloroglucinol (Deltalab *et al*. 2023) in conjunction with phytohormones were used to enhance the root formation of certain rose cultivars under *in vitro* conditions. Numerous studies proved the effectiveness of using humic substances to stimulate root growth, which was depended on the growth regulator's source, the rate of application and, to a lesser extent, on the plant's type and growing conditions (Chen & Aviad 1990, Rose *et al*. 2014, Nardi *et al*. 2021). According to the results of research by scientists from different countries of the world, the use of humic acid was effective for shoot and root growths of pea plants (Gawlik *et al*. 2014), rooting azaleas (Elmongy *et al*. 2018), enhancing Sorbonne lily bulb and root growth under *in vitro* conditions (Wu *et al*. 2016), rooting and acclimatization of pears (Marino *et al*. 2009), *ex vitro* acclimatization of strawberry (Neri *et al*. 2022). During the Date palm (*Phoenix dactylifera* L.) micropropagation, the addition of humic acid and zinc oxide nanoparticles to the medium increased the level of macronutrients Nitrogen (N), Phosphorus (P), Potassium (K), Sulfur (S), and the micronutrient Zinc (Zn) in the shoots, and showed high effectiveness at the stages of callus formation, shoot reproduction and rooting *in vitro*, compared to other treatments (Al-Mayahi 2021).

For this reason, we investigated the effect of plant growth regulator Humifield with a high content of humic acid on rhizogenesis in Shrub rose regenerants cultivated *in vitro*. Its effectiveness was previously proven under *ex vitro* conditions. The incorporation of Humifield in a mixture with fertilizers was instrumental in augmenting the grain yield of blue lupine (Kotelnytska *et al*. 2021). In wheat, the combination of herbicides and Humifield provided the best performance in weed control, including perennial ones (Korotkova *et al*. 2021).

The use of 0.5 mg/l α-NOC in combination with 10 mg/l Humifield increased the frequency of rhizogenesis up to 70-86%, depending on the rose genotype. *In vitro* hormonal determination in Shrub class rose regenerants contributed to a significant increase in the frequency of rhizogenesis and accelerated plant cloning, what is the basis for obtaining mass planting material necessary for green building.

Conclusions

Hormonal determination of rhizogenesis and efficiency of *in vitro* root formation in regenerants of the Shrub class roses depended on the plant genotype (cultivar) and concentrations of α-NAA in nutrient medium. The addition of 0.5 mg/l of α-NAA to the nutrient medium contributed to the production of 61.2% of rooted explants. The cultivars with the highest rhizogenesis ability were Lavender Dream (66.0%) and Sommerabend (67.0%)

The complex use of growth regulators containing 0.5 mg/l α-NAA and 10 mg/l Humifield contributed to the rooting of 70.0 to 86.0% of roses of the studied cultivars. The highest rhizogenic activity was shown by the cv. Lavender Dream, in which 86% of rooted regenerated plants were obtained.

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