Special Issue on: Plant biology and Genetics. OPEN ACCESS https://scidoc.org/IJMBG.php

International Journal of Medical Biotechnology & Genetics (IJMBG)
ISSN: 2379-1020

Effect of Plant Growth Regulators Ivin, Methyur and Kamethur on the Organogenesis of Miniature Rose (*Rosa mini* L.) *in Vitro*

Research Article

Tsygankova V.A.[1*], Oliynyk O.O.[2], Kvasko O.Yu.[2], Pilyo S.G.[1], Klyuchko S.V.[1], Brovarets V.S.[1]

[1] Department for Chemistry of Bioactive Nitrogen-Containing Heterocyclic Compounds, V.P. Kukhar Institute of Bioorganic Chemistry and Petrochemistry, National Academy of Sciences of Ukraine, 1, Murmanskaya str., 02094, Kyiv, Ukraine.
[2] Department of Ecobiotechnology and Biodiversity, Faculty of plant protection, biotechnology and ecology, National University of Life and Environmental Sciences of Ukraine, Heroiv Oborony Str.15, 03041, Kyiv, Ukraine.

Abstract

The effect of synthetic plant growth regulators Ivin (N-oxide-2,6-dimethylpyridine), Methyur (sodium salt of 6-methyl-2-mercapto-4-hydroxypyrimidine) and Kamethur (potassium salt of 6-methyl-2-mercapto-4-hydroxypyrimidine) on the organogenesis of miniature rose (*Rosa mini* L.) *in vitro* was studied. It was shown that the effect of synthetic plant growth regulators Ivin, Methyur and Kamethur used at concentrations of 10^{-5}M, 10^{-6}M, 10^{-7}M per 1 liter of MS (Murashige and Skoog) medium on the organogenesis of shoots and roots of miniature rose (*Rosa mini* L.) *in vitro* is similar or higher than the effect of the plant hormone auxin IAA (2-(1*H*-Indol-3-yl)acetic acid) used at the same concentrations. The parameters of organogenesis of miniature rose (*Rosa mini* L.) *in vitro* (average length of shoots (cm) per explant, length of roots (cm) per explant, number of roots (pcs) per explant, and frequency (%) of shoot rooting), measured on the 21st and 28th days of cultivation, obtained on MS medium modified with Ivin, Methyur and Kamethur, varied depending on the concentrations of plant growth regulators. The synthetic plant growth regulators showed the highest effect on the organogenesis of shoots and roots of miniature rose (*Rosa mini* L.) *in vitro* when used in concentrations: Ivin at concentrations of 10^{-5}M and 10^{-6}M, Kamethur at concentrations of 10^{-5}M and 10^{-6}M, Methyur at concentrations of 10^{-5}M and 10^{-7}M. The plant hormone auxin IAA showed the highest effect on the organogenesis of shoots and roots of miniature rose (*Rosa mini* L.) *in vitro* when used at concentrations of 10^{-6}M and 10^{-7}M. Proposed molecular mechanisms of action of the synthetic plant growth regulators Ivin, Methyur and Kamethur are discussed. The results of the work confirm the promise of using synthetic plant growth regulators Ivin, Methyur and Kamethur to improve the organogenesis of shoots and roots of the miniature rose (*Rosa mini* L.) *in vitro*.

Keywords: *Rosa mini* L*; in vitro* Culture; MS Medium; Organogenesis of Shoots and Roots; IAA; Pyridine; Pyrimidine; Ivin; Methyur and Kamethur.

Introduction

The rose is rightfully considered the most elegant and incredibly beautiful flower belonging to the genus *Rosa* L. and family Rosaceae [1-3]. The genus *Rosa* L., with over 200 species and thousands of cultivars grown worldwide, includes both wild rose species and oil rose species, which are widely used as garden ornamental plants and as a raw material for the production of rose essential oils, water and alcohol extracts of rose used in the perfumery, pharmaceutical and food industries [2-5]. Rose water and alcohol extracts enriched in secondary metabolites such as flavonoids (e.g., flavones, flavonols, anthocyanins) and rose essential oils containing monoterpenes and sesquiterpenes have therapeutic properties and can be used such as a respiratory antiseptic, anti-inflammatory, mucolytic, expectorant, decongestant, antioxidant, antiviral and anticancer agents for the prevention and treatment of serious diseases [3-5]. The fruits of wild roses, called rose hips, are exceptionally rich in vitamin C, 60 times the amount of vitamin C in citrus fruit, a significant amount of iron, calcium, and phosphorus [6].

Shrub or spray miniature roses with small flowers are in great

***Corresponding Author**:
Tsygankova V.A,
Department for Chemistry of Bioactive Nitrogen-Containing Heterocyclic Compounds, V.P. Kukhar Institute of Bioorganic Chemistry and Petrochemistry, National Academy of Sciences of Ukraine, 1, Murmanskaya str., 02094, Kyiv, Ukraine.
E-mail: vTsygankova@ukr.net

Received: September 28, 2022
Accepted: October 27, 2022
Published: October 31, 2022

Citation: Tsygankova V.A., Oliynyk O.O., Kvasko O.Yu., Pilyo S.G., Klyuchko S.V., Brovarets V.S. Effect of Plant Growth Regulators Ivin, Methyur and Kamethur on the Organogenesis of Miniature Rose (*Rosa mini* L.) *in Vitro*. *Int J Med Biotechnol Genetics.* 2022;S1:02:001:1-8.

demand among florists, designers and amateur gardeners for a beautiful bush and abundant flowering [4, 7, 8]. The Lydia variety, bred by the specialists of the Dutch company in 1995, is a small plant with abundant flowering and the formation of large flower rosettes.It is one of the most popular varieties of miniature shrub roses belonging to the floribunda category [8]. The Lydia variety is divided into 3 sub-varieties: Lovely Lydia, Spray White Lydia, and Floribunda Classic Lydia. The main advantages of the Lydia variety are frost resistance, immunity to fungal pathogens and pests, lack of capriciousness in growing, flowering during the growing season [8]. Growing Lydia roses is not difficult; the main thing is to observe the regime of watering, feeding and lighting [8]. This crop is planted in the second half of spring indoors, outdoors in containers, or outdoors in the soil well lit by the spring sun, but without direct sunlight and protected from drafts and strong winds [8].

Rose propagation methods mainly include vegetative methods such as cutting, grafting, layering, budding, and rarely seed propagation [7, 9]. As a rule, vegetative propagation and seed propagation methods are very time consuming and do not allow obtaining a healthy plant. Micropropagation through *in vitro* culture is widely used as an alternative biotechnological method to produce healthy plant clones that are genetically identical to the parent plant [9].

Rose propagation *in vitro* involves several stages such as initiation of aseptic cultures, proliferation and multiplication of shoots using apical buds, nodal stem segments, shoot tip segments, or leaf petioles through organogenesis or somatic embryogenesis, rooting of microshoots, acclimatization and establishment in the field [9-11].

The efficiency of rose organogenesis *in vitro* is influenced by various factors, including the type of plant explant, plant species, plant genotypes or cultivars, the composition of nutrient media used for plant cultivation and physical factors [10]. Plant hormones cytokinins such as BAP (6-benzyl aminopurine), 2-ip (2-isopentyladenine) used in high concentrations, auxins such as IBA (indole-3-butyric-acid), NAA (α-naphthalene acetic acid), IAA (indole-3-acetic acid), and gibberellic acid (GA3) used at low concentrations are the main factors affecting rose organogenesis *in vitro* [9-15]. At the same time, the optimization of nutrient media is carried out using plant hormones or their synthetic analogues, for example, a synthetic analogue of cytokinin TDS (Thidiazuron), which make it possible to have a high stimulating effect on the regeneration of roses *in vitro* [10, 12, 14, 15]. A topical issue is the search for new synthetic analogues of plant hormones, auxins and cytokinins, to increase the efficiency of rose organogenesis *in vitro*.

The main goal of our work is to study the effect of synthetic plant growth regulators Ivin, Methyur and Kamethur on the organogenesis of shoots and roots of miniature rose (*Rosa mini* L.) *in vitro*.

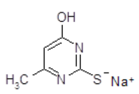
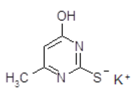
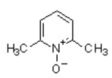
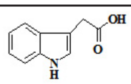
Materials and Methods

Plant growth regulators Ivin (N-oxide-2,6-dimethylpyridine), Methyur (sodium salt of 6-methyl-2-mercapto-4-hydroxypyrimidine) and Kamethur (potassium salt of 6-methyl-2-mercapto-4-hydroxypyrimidine) were synthesized in the Department for Chemistry of Bioactive Nitrogen-Containing Heterocyclic Compounds, V.P. Kukhar Institute of Bioorganic Chemistry and Petrochemistry of the National Academy of Sciences of Ukraine (Table 1). The growth regulatory activity of synthetic plant growth regulators Ivin, Methyur and Kamethur was compared with the activity of plant hormone auxin IAA 2-(1*H*-Indol-3-yl) acetic acid (Table 1).

In experiments, explants of a miniature rose (*Rosa mini* L.), grown on a hormone-free MS (Murashige and Skoog) medium [16], divided up to 0.5 cm in size with 1-2 microbuds were used.These explants were divided into segments with an axillary bud, one or two leaf blades, and a stem no larger than 1.0 cm in size.

Sterilization was carried out according to the scheme: treatment of explants in sodium hypochlorite solution - 20 min. followed by washing in sterile distilled water; sterilization with 70% ethanol (C_2H_5OH) - 4 sec. with washing in sterile distilled water; sterilization in 0.1% solution of Mercury (II) chloride ($HgCl_2$) for 7 min.

Table 1. Chemical name, structure and relative molecular weight of synthetic plant growth regulators Ivin, Methyur and Kamethur, and plant hormone auxin IAA.

Plant growth regulator or plant hormone	Chemical structure	Chemical name and relative molecular weight
Methyur		Sodium salt of 6-methyl-2-mercapto-4-hydroxypyrimidine MW=165.17
Kamethur		Potassium salt of 6-methyl-2-mercapto-4-hydroxypyrimidine MW=181.28
Ivin		N-oxide-2,6-dimethylpyridine MW=125.17
IAA		2-(1*H*-Indol-3-yl)acetic acid MW=175.19

with 3 times washing with sterile distilled water.

Further, these segments were cultivated at an illumination of 3000-4000 lux, a temperature of 24 ± 2ºC, an air humidity of ~ 70%, and a 16-hour photoperiod for 28 days on control hormone-free MS medium, or modified MS medium containing either synthetic plant growth regulators Ivin, Methyur and Kamethur or plant hormone auxin IAA used at concentrations of 10^{-5}M, 10^{-6}M, 10^{-7}M per 1 liter of MS medium. MS medium for explant cultivation was supplemented also with macro- and micro salts contained in MS basal medium [16], sucrose (0.3%), mesoinositol (100 mg/l) and agar (0.7%), medium pH – 5.7-5.8.

Parameters of organogenesis of miniature rose (*Rosa mini* L.) (average length of shoots (cm) per explant, length of roots (cm) per explant, average number of roots (pcs) per explant, and frequency (%) of shoot rooting, i.e. the average number of rooted shoots per number of explants) were measured on the 21st and 28th days of *in vitro* cultivation, respectively.

Statistical Analysis

All experiments were performed in three replicates. Statistical analysis of the data was performed using dispersive Student's-t test with the level of significance at p≤0.05, the values are mean ± Standard Deviation (± SD) [17].

Results

The conducted studies showed that the use of synthetic plant growth regulators Ivin, Methyur and Kamethur as components of the MS medium had a positive effect on the formation and growth of shoots and roots of miniature rose (*Rosa mini* L.) *in*

Figure 1. Organogenesis of shoots and roots of miniature rose (*Rosa mini* L.) *in vitro*, measured on the 28th day of cultivation: 1-control hormone-free MS medium, 2 - MS medium containing Ivin at a concentration of 10^{-5}M, 3 - MS medium-containing Kamethur at a concentration of 10^{-5}M, 4 - MS medium containing Methyur at a concentration of 10^{-5}M, 5 - MS medium containing plant hormone auxin IAA at a concentration of 10^{-5}M.

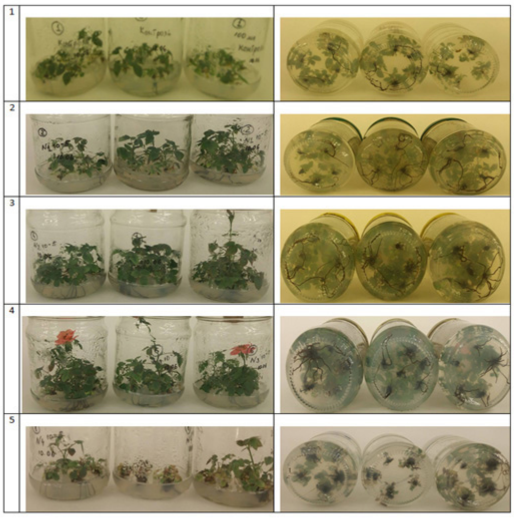
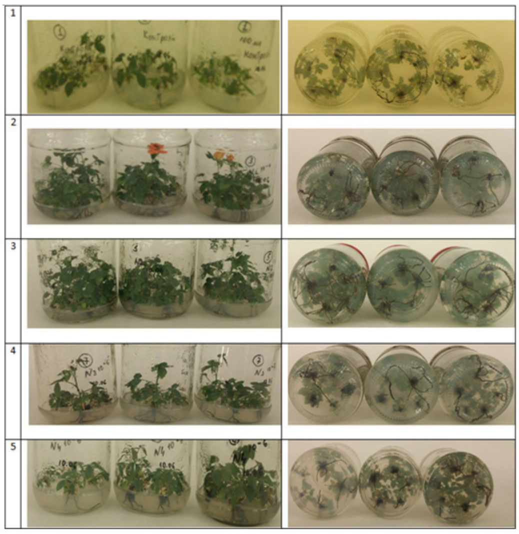


Figure 2. Organogenesis of shoots and roots of miniature rose (*Rosa mini* L.) *in vitro*, measured on the 28th day of cultivation: 1- control hormone-free MS medium, 2 - MS medium containing Ivin at a concentration of 10^{-6}M, 3 - MS medium containing Kamethur at a concentration of 10^{-6}M, 4 - MS medium containing Methyur at a concentration of 10^{-6}M, 5 - MS medium containing plant hormone auxin IAA at a concentration of 10^{-6}M.



vitro (Fig. 1 – Fig. 3).

Parameters of organogenesis of miniature rose (*Rosa mini* L.) *in vitro*, measured on the 21st and 28th days of cultivation, obtained on MS medium modified with synthetic plant growth regulators Ivin, Methyur and Kamethur at concentration of 10^{-5}M, 10^{-6}M and 10^{-7}M, were generally similar to or higher than those obtained on the control hormone-free medium MS, or on the medium MS containing auxin IAA used at the same concentrations.

The effect of synthetic plant growth regulators Ivin, Methyur and Kamethur on the organogenesis of shoots and roots of miniature rose (*Rosa mini* L.) *in vitro* varied depending on the concentrations of plant growth regulators (Table 2 – Table 4).

Figure 3. Organogenesis of shoots and roots of miniature rose (*Rosa mini* L.) *in vitro*, measured on the 28th day of cultivation: 1- control hormone-free MS medium, 2 - MS medium containing Ivin at a concentration of 10^{-7}M, 3 - MS medium containing Kamethur at a concentration of 10^{-7}M, 4 - MS medium containing Methyur at a concentration of 10^{-7}M, 5 - MS medium containing plant hormone auxin IAA at a concentration of 10^{-7}M.

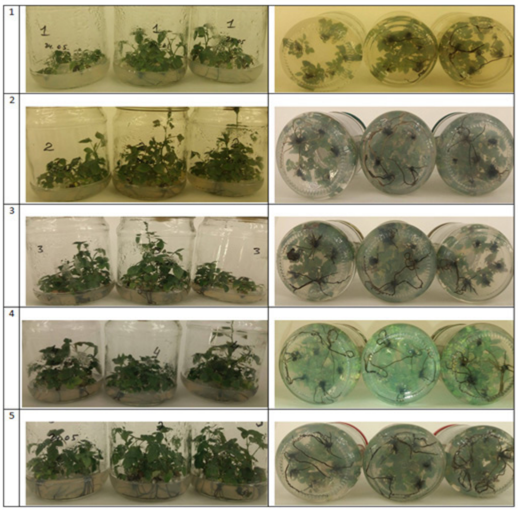


Table 2. Parameters of organogenesis of miniature rose (*Rosa mini* L.) *in vitro*, measured on the 21st and 28th days of cultivation, obtained on the control (hormone-free) MS and modified MS medium containing Ivin, Kamethur, Methyur and IAA at a concentration of 10^{-5}M.

MS medium	Length of shoots (cm) on the 21st day	Length of roots (cm) on the 21st day	Number of roots (pcs) on the 21st day	Frequency (%) of shoot rooting on the 21st day	Length of shoots (cm) on the 28th day	Length of roots (cm) on the 28th day	Number of roots (pcs) on the 28th day	Frequency (%) of shoot rooting on the 28th day
Control hormone-free MS	1.0±0.57*	1.17±0.7*	3.0±1.39*	39.33±7.99*	2.17±0.33*	2.0±0.57*	3.5±1.27*	53.17±4.84*
MS+Ivin	1.88±0.47	2.0±0.57	6.0±1.39**	67.75±0.93 **	2.83±0.33	2.38±0.25	6.33±1.30**	67.8±4.18**
MS+ Kamethur	2.1±0.37**	2.75±0.28**	5.8±1.13**	67.2±4.12**	2.75±0.28**	2.83±0.33**	6.6±1.47**	73.75±3.69**
MS+ Methyur	2.8±0.24**	1.4±0.48	7.5±1.49**	87.75±3.24**	2.75±0.63**	1.88±0.24	7.92±2.73**	87.33±5.10**
MS+IAA	1.25±0.22	0.58±0.17	3.2±1.14	47.0±2.88	1.55±0.25	0.8±0.24**	4.67±1.57	47.25±5.02

Note. **Significant differences from control values *, p ≤0.05, n =3, the values are mean ± SD

Table 3. Parameters of organogenesis of miniature rose (*Rosa mini* L.) *in vitro*, measured on the 21st and 28th days of cultivation, obtained on the control (hormone-free) MS and modified MS medium containing Ivin, Kamethur, Methyur and IAA at a concentration of 10^{-6}M.

MS medium	Length of shoots (cm) on the 21st day	Length of roots (cm) on the 21st day	Number of roots (pcs) on the 21st day	Frequency (%) of shoot rooting on the 21st day	Length of shoots (cm) on the 28th day	Length of roots (cm) on the 28th day	Number of roots (pcs) on the 28th day	Frequency (%) of shoot rooting on the 28th day
Control (hormone-free) MS	1.0±0.57*	1.17±0.7*	3.0±1.39*	39.33±7.99*	2.17±0.33*	2.0±0.57*	3.5±1.27*	53.17±4.84*
MS+ Ivin	1.75±0.12**	1.5±0.36	6.16±1.37**	80.17±2.74**	2.82±0.19**	2.28±0.23	6.33±1.3**	80.83±3.05**
MS+ Kamethur	1.8±0.24**	2.08±0.39**	6.33±1.19**	73.67±2.51**	2.87±0.17**	3.15±0.33**	7.83±2.11**	99.83±0.33**
MS+ Methyur	1.42±0.16	1.67±0.33	5.95±1.25**	50.23±2.98	1.87±0.16	1.9±0.1	6.65±1.5**	60.5±1.92
MS+ IAA	1.34±0.18	1.82±0.16	2.83±0.94	60.33±1.4**	1.83±0.21	1.71±0.19	3.5±0.84	80.16±2.74**

Note. **Significant differences from control values*, p ≤0.05, n =3, the values are mean ± SD

Table 4. Parameters of organogenesis of miniature rose (*Rosa mini* L.) *in vitro*, measured on the 21st and 28th days of cultivation, obtained on the control (hormone-free) MS and modified MS medium containing Ivin, Kamethur, Methyur and IAA at a concentration of 10^{-7}M.

MS medium	Length of shoots (cm) on the 21st day	Length of roots (cm) on the 21st day	Number of roots (pcs) on the 21st day	Frequency (%) of shoot rooting on the 21st day	Length of shoots (cm) on the 28th day	Length of roots (cm) on the 28th day	Number of roots (pcs) on the 28th day	Frequency (%) of shoot rooting on the 28th day
Control (hormone-free) MS	1.0±0.57*	1.17±0.7*	3.0±1.39*	39.33±7.99*	2.17±0.33*	2.0±0.57*	3.5±1.27*	53.17±4.84*
MS+Ivin	1.73±0.66	1.03±0.17	1.75±0.33	20.67±1.49**	2.67±0.41	2.92±0.47	5.33±1.31	60.17±2.65
MS+Kamethur	1.2±0.31	1.55±0.44**	5.88±1.28**	60.67±2.61**	2.25±0.33	2.83±0.21**	6.19±1.32**	67.17±1.85**
MS+ Methyur	3.42±0.95**	0.82 ±0.16	3.83±0.94	80.33±2.51**	3.92±0.64**	2.08±0.3	3.5±0.67	87.5±1.41**
MS+IAA	2.85±0.77**	1.58±0.59	5.67±1.73	93.17±1.55**	2.93±0.32**	2.0±0.36	6.16±1.85	93.67±0.97**

Note. **Significant differences from control values*, $p \leq 0.05$, $n = 3$, the values are mean ± SD

According to the parameters of length of shoots (cm) per explant, measured on the 21st and 28th days of cultivation, synthetic plant growth regulators showed the highest activity: Ivin at concentration of 10^{-6}M (1.75±0,12 cm and 2.82±0,19 cm), Kamethur at concentrations of 10^{-5}M (2.1±0.37 cm and 2.75±0.28 cm) and 10^{-6}M (1.8±0.24 cm and 2.87±0.17 cm), and Methyur at concentrations of 10^{-5}M (2.8±0.24 cm and 2.75±0.63 cm) and 10^{-7}M (3.42±0.95 cm and 3.92±0.64 cm), respectively (Table 2 – Table 4).

Synthetic plant growth regulators showed the lowest activity in relation to the parameters of length of shoots (cm) per explant, measured on the 21st and 28th days of cultivation: Ivin at concentrations of 10^{-5}M (1.88 ± 0.47 cm and 2.83 ± 0.33 cm) and 10^{-7}M (1.73 ± 0.66 cm and 2.67 ± 0.41 cm), Kamethur at a concentration of 10^{-7}M (1.2±0.31 cm and 2.25 ± 0.33 cm) and Methyur at concentration of 10^{-6}M (1.42±0.16 cm and 1.87±0.16 cm), respectively (Table 2 – Table 4). Parameters of length of shoots (cm) per explant indicated above did not differ statistically than those obtained on the control hormone-free medium MS, measured on the 21st and 28th days of cultivation (1.0±0.57 cm and 2.17±0.33 cm), respectively (Table 2 – Table 4).

According to the parameters of length of roots (cm) per explant, measured on the 21st and 28th days of cultivation, synthetic plant growth regulator showed the highest activity: Kamethur at concentration of 10^{-5}M (2.75±0.28 cm and 2.83±0.33 cm), 10^{-6}M (2.08±0.39 cm and 3.15±0.33 cm) and 10^{-7}M (1.55±0.44 cm and 2.83±0.21 cm), respectively (Table 2 – Table 4).

Synthetic plant growth regulators showed the lowest activity inrelation to the parameters of length of roots (cm) per explant, measured on the 21st and 28th days of cultivation: Ivin at concentrations of 10^{-5}M (2.0±0.57 cm and 2.38±0.25 cm), 10^{-6}M (1.5±0.36 cm and 2.28±0.23 cm) and 10^{-7}M (1.03±0.17 cm and 2.92±0.47 cm), and Methyur at concentrations of 10^{-5}M (1.4±0.48 cm and 1.88±0.24 cm), 10^{-6}M (1.67±0.33 cm and 1.9±0.1 cm) and 10^{-7}M (0.82±0.16 cm and 2.08±0.3 cm), respectively (Table 2 – Table 4). Parameters of length of roots (cm) per explant indicated above did not differ statistically than those obtained on the control hormone-free medium MS, measured on the 21st and 28th days of cultivation (1.17±0.7 cm and 2.0±0.57 cm), respectively (Table 2 – Table 4).

According to the parameters of number of roots (pcs) per explant, measured on the 21st and 28th days of cultivation, synthetic plant growth regulators showed the highest activity: Ivin at concentrations of 10^{-5}M (6.0±1.39 pcs and 6.33±1.30 pcs) and 10^{-6}M (6.16±1.37 pcs and 6.33±1.3 pcs), Kamethur at concentrations of 10^{-5}M (5.8±1.13 pcs and 6.6±1.47 pcs), 10^{-6}M (6.33±1.19 pcs and 7.83±2.11pcs) and 10^{-7}M (5.88±1.28 pcs and 6.19±1.32 pcs), Methyur at concentrations of 10^{-5}M (7.5±1.49 pcs and 7.92±2.73 pcs) and 10^{-6}M (5.95±1.25 pcs and 6.65±1.5 pcs), respectively (Table 2 – Table 4).

Synthetic plant growth regulators showed the lowest activity in relation to the parameters of number of roots (pcs) per explant, measured on the 21st and 28th days of cultivation: Ivin at a concentration of 10^{-7}M (1.75±0.33 pcs and 5.33±1.31 pcs) and Methyur at a concentration of 10^{-7}M (3.83±0.94 pcs and 3.5±0.67 pcs) (Table 2 – Table 4). Parameters of number of roots (pcs) per explant indicated above did not differ statistically than those obtained on the control hormone-free medium MS, measured on the 21st and 28th days of cultivation (3.0±1.39 pcs and 3.5±1.27 pcs), respectively (Table 2 – Table 4).

According to the parameters of the frequency (%) of shoot rooting, measured on the 21st and 28th days of cultivation, synthetic plant growth regulators showed the highest activity: Ivin at concentrations of 10^{-5}M (67.75±0.93 % and 67.8±4.18 %) and 10^{-6}M (80.17±2.74% and 80.83±3.05 %), Kamethur at concentrations of 10^{-5}M (67.2±4.12 % and 73.75±3.69 %), 10^{-6}M (73.67±2.51 % and 99.83±0.33 %) and 10^{-7}M (60.67±2.61% and 67.17±1.85%), Methyur at concentrations of 10^{-5}M (87.75±3.24% and 87.33±5.10 %) and 10^{-7}M (80.33±2.51% and 87.5±1.41%), respectively (Table 2 – Table 4).

Synthetic plant growth regulators showed the lowest activity in relation to the parameters of frequency (%) of shoot rooting, measured on the 21st and 28th days of cultivation: Ivin at concentration of 10^{-7}M (20.67±1.49% and 60.17±2.65%) and Methyur at concentration of 10^{-6}M (50.23±2.98 % and 60.5±1.92 %), respectively (Table 2 – Table 4). Parameters of frequency (%) of shoot rooting indicated above did not differ statistically or were less than those obtained on the control hormone-free medium MS, measured on the 21st and 28th days of cultivation (39.33±7.99 % and 53.17±4.84%), respectively (Table 2 – Table 4).

It has been shown that the effect of synthetic growth regulators

Ivin, Methyur and Kamethur on the organogenesis of shoots and roots of miniature rose (*Rosa mini L.*) *in vitro* is similar or higher than the effect of the plant hormone auxin IAA used in the same concentrations 10^{-5}M, 10^{-6}M, 10^{-7}M per 1 liter of MS medium (Table 2 – Table 4).

The plant hormone auxin IAA showed the highest activity in relation to the parameters of shoot and root organogenesis, measured on the 21st and 28th days of cultivation, according to the length of shoots (cm) per explant at concentration of 10^{-7}M (2.85±0.77cm and 2.93±0.32 cm) and for the frequency (%) of shoot rooting at concentrations of 10^{-6}M (60.33±1.4 % and 80.16±2.74 %) and 10^{-7}M (93.17±1.55% and 93.17±1.55%), respectively (Table 2 – Table 4).

The plant hormone auxin IAA showed the lowest activity in relation to the parameters of shoot and root organogenesis, measured on the 21st and 28th days of cultivation, in relation to the length of shoots (cm) per explant at concentrations of 10^{-5}M (1.25±0.22 cm and 1.55±0.25 cm) and 10^{-6}M (1.34±0.18 cm and 1.83±0.21cm), length of roots (cm) per explant at concentrations of 10^{-5}M (0.58±0.17 cm and 0.8±0.24 cm), 10^{-6}M (1.82±0.16 cm and 1.71±0.19 cm) and 10^{-7}M (1.58±0.59 cm and 2.0±0.36 cm), number of roots (pcs) per explant at concentrations of 10^{-5}M (3.2±1.14 pcs and 4.67±1.57 pcs),10^{-6}M (2.83±0.94 pcs and 3.5±0.84 pcs) and 10^{-7}M (5.67±1.73 pcs and 6.16±1.85 pcs), and frequency (%) of shoot rooting at concentrations of 10^{-5}M (47.0±2.88 % and 47.25±5.02 %), respectively (Table 2 – Table 4). Parameters of organogenesis indicated above did not differ statistically or were less than those obtained on the control hormone-free medium MS, measured on the 21st and 28th days of cultivation in relation to the length of shoots (cm) (1.0±0.57 cm and 2.17±0.33 cm), length of roots (cm)(1.17±0.7 cm and 2.0±0.57 cm), number of roots (pcs)(3.0±1.39 pcs and 3.5±1.27 pcs), and frequency (%) of shoot rooting (39.33±7.99 % and 53.17±4.84 %), respectively (Table 2 – Table 4).

Thus, the obtained results showed both cytokinin- and auxin-like effects of synthetic plant growth regulators Ivin, Methyur and Kamethur on the processes of elongation, division and differentiation of the isolated plant cells, which are the main processes of organogenesis of shoot and root meristems of miniature rose (*Rosa mini* L.) *in vitro*.

Analysis of growth regulating activity showed that synthetic plant growth regulators have a more powerful effect on the organogenesis of shoots and roots of miniature rose (*Rosa mini* L.) *in vitro* when used as components of the MS medium in concentrations: Ivin at concentrations of 10^{-5}M and 10^{-6}M, Kamethur at concentrations of 10^{-5}M and 10^{-6}M, Methyur at concentrations of 10^{-5}M and 10^{-7}M.

The plant hormone auxin IAA showed the highest effect on the organogenesis of shoots and roots of miniature rose (*Rosa mini L.*) *in vitro* when used as component of the MS medium at concentrations of 10^{-6}M and 10^{-7}M.

Discussion

Rose micropropagation through *in vitro* culture refers to a method of asexual propagation that produces healthy plant clones that are genetically equal plants to the parent plant and to each other [9]. Since the 1970s, rose micropropagation protocols have been developed [18].

The apical buds, nodal stem segments, shoot tip segments, or leaf petioles are used as explants for proliferation and multiplication of shoots and roots [9-11]. To enhance the organogenesis of stems, leaves and roots, MS basal medium [16] supplemented with plant hormones cytokinins and auxins is used [9-15, 18]. Cytokinins BAP, 2-ip, and TDS, and auxins IAA, NAA, and IBA are most widely used for shoot and root organogenesis [9-15, 18].

The ratio of plant hormones auxins and cytokinins affects organogenesis towards the proliferation of shoots or roots. Usually, high concentrations of cytokinins and low concentrations of auxins or gibberellic acid GA3 promote shoot proliferation through direct organogenesis or somatic embryogenesis [9-15, 18]. To promote root organogenesis, modified MS medium with a high content of mineral salts, with or without the addition of auxins at low concentrations, is used [9-15, 18].

Synthetic plant growth regulators Ivin, Methyur and Kamethur were previously studied by us in tissue culture of potato, tobacco and cherry to intensify plant organogenesis *in vitro* [19, 20].

In our earlier published work [19], it was found that Ivin (N-oxide-2,6-dimethylpyridine) and its derivatives, which were used as components of culture media in concentration range from 0,05 mg to 10 mg per 1 liter, exhibit predominant cytokinin-like activity, affecting the processes of increasing the growth of callus biomass, delaying cell ageing, activating photosynthesis in plant cells and promoting organogenesis of potato and tobacco plants *in vitro*. The effect of Ivin and its derivatives on plant organogenesis was similar or higher than that of the plant hormone cytokinin Kinetin (6-furfurylaminopurine) used at the same concentrations. In our work [20] it was shown that the use of Methyur and Kamethur at concentration of 10^{-5}M - 10^{-8}M as components of the MS medium improved the development of the root system on cherry microshoots. The plant growth regulating activity of Kamethur and Methyur was similar or higher than that of the auxin IBA, cytokinin BAP, and gibberellic acid.

This work is aimed at searching for new effective plant growth regulators capable of initiating organogenesis of miniature rose (*Rosa mini* L.) *in vitro* on MS medium without the addition of plant hormones auxins and cytokinins. The obtained results showed that synthetic plant growth regulators Ivin (N-oxide-2,6-dimethylpyridine), Methyur (sodium salt of 6-methyl-2-mercapto-4-hydroxypyrimidine) and Kamethur (potassium salt of 6-methyl-2-mercapto-4-hydroxypyrimidine) revealed both cytokinin - and auxin-like activity on the organogenesis of shoots and roots of miniature rose (*Rosa mini* L.) *in vitro*. Their growth regulating effect is similar to or exceeds that of the plant hormone auxin IAA. The physiological and molecular mechanisms of action of the synthetic plant growth regulators Ivin (N-oxide-2,6-dimethylpyridine), Methyur (sodium salt of 6-methyl-2-mercapto-4-hydroxypyrimidine) and Kamethur (potassium salt of 6-methyl-2-mercapto-4-hydroxypyrimidine) on plant development are of the considerable interest. The results of our previous studies with synthetic low molecular weight heterocycles, derivatives of pyridine, pyrimidine, pyrazole, isoflavones, oxazolopyrimidine and oxazole showed their auxin- and cytokinin-like effects on the processes of elongation, division and differentiation of plant cells [21-30], which are the main processes of organogenesis of plant shoot and root meristems *in vivo* and *in vitro*, which are controlled by plant hormones [31, 32].

Our earlier studies of the molecular mechanisms of action of plant growth regulators Ivin and Methyur demonstrated their stimulating effect on gene expression at the level of transcription and translation of genetic information, due to which the time of plant ontogenesis is almost halved [33]. In addition, we came to the conclusion that synthetic plant growth regulators Ivin and Methyur affect plant growth indirectly, through the endogenous pool of plant hormones [34].

The proposed mechanism of action of synthetic plant growth regulators directly, through a network of plant hormone signaling pathway [35, 36], or indirectly, i.e. affecting the biosynthesis of plant hormones, their stability and metabolism [36], was discussed in the works of other authors [37-47].

Therefore, our further work will focus on study of molecular mechanism of action of synthetic plant growth regulators Ivin, Methyur and Kamethur.

Conclusion

Our study showed that the use of synthetic plant growth regulators Ivin (N-oxide-2,6-dimethylpyridine), Methyur (sodium salt of 6-methyl-2-mercapto-4-hydroxypyrimidine) and Kamethur (potassium salt of 6-methyl-2-mercapto-4-hydroxypyrimidine) as components of the MS medium improves the organogenesis of shoots and roots of miniature rose (*Rosa mini* L.) *in vitro*. The growth regulatory activity of Ivin, Methyur and Kamethur was compared with the activity of plant hormone auxin IAA (2-(1*H*-Indol-3-yl)acetic acid). The use of synthetic growth regulators Ivin, Methyur and Kamethur had a positive effect on the formation and growth of shoots and roots, measured on the 21st and 28th days of cultivation. The parameters of average length of shoots (cm) per explant, length of roots (cm) per explant, number of roots (pcs) per explant, and frequency (%) of shoot rooting generally increased depending on the concentration of regulators. The effect of Ivin, Methyur and Kamethur, used at concentrations of 10^{-5}M, 10^{-6}M, 10^{-7}M per 1 liter of MS medium, on the organogenesis of shoots and roots of a miniature rose is similar or higher than that of the plant hormone auxin IAA used at the same concentrations. The application of synthetic plant growth regulators Ivin, Methyur and Kamethur for micropropagation of the miniature rose (*Rosa mini L.*) through *in vitro* culture is proposed.

References

[1]. Vosman B, Barendrecht CJ, Esselink D, Jones H, Scott E, Spellerberg B, et al. A European reference collection of rose varieties.Plant Research International; 2006.

[2]. Datta SK. Breeding of new ornamental varieties. Curr Sci. 2018 Mar 25;114(6):1194-206.

[3]. Mileva M, Ilieva Y, Jovtchev G, Gateva S, Zaharieva MM, Georgieva A, et al. Rose Flowers-A Delicate Perfume or a Natural Healer? Biomolecules. 2021 Jan 19;11(1):127. PubMed PMID: 33478154.

[4]. Das P, Nanda S. Medicinal Efficacy of Rose Plant: A Mini Review. PharmaTutor. 2015 Oct 1;3(10):23-6.

[5]. Chahar S. Rose: Ornamental As Well As Medicinal Plant. J Res Agric Anim Sci. 2016;4(1):8-10.

[6]. Stern KR. Introduction to Plant Biology. McGraw-Hill Higher Education. 8th Ed.256 p.

[7]. Adhikary K, Sarkar MM. Varietal evaluation of miniature rose cultivars under the plains of West Bengal, India. J Pharmacogn Phytochem. 2019;8(4):1618-21.

[8]. De Hoog J. Handbook for modern greenhouse rose cultivation.Applied Plant Research.Wageningen University and Research.Pp 220.

[9]. Gholami M, Khorshidi M, Agrawal V. The Effect of Benzyladenine and Indolebutyric Acid on Shooting and Rooting of Miniature Rose. COJ Biomed Sci Res. COJBSR. 2021.

[10]. Pati PK, Rath SP, Sharma M, Sood A, Ahuja PS. In vitro propagation of rose - A Review. Biotechnology Adv. 2006 Jan 1;24(1):94-114.

[11]. Nak-Udom N, Kanchanapoom K, Kanchanapoom K. Micropropagation from cultured nodal explants of rose (Rosa hybrida L. cv.'Perfume Delight'). Songklanakarin J Sci Technol. 2009 Nov 1;31(6):583-6.

[12]. Oliynyk OO, Kluvadenko AA, Melnychuk MD.Optimization of Culture-Media Content for Acceleration of Growth and Cultivation of Rosa DamascenaMill. in In Vitro Culture. Scientific bulletin of the National Forestry University of Ukraine.26(7):134–139.

[13]. Stone M. Propagation of miniature roses by plant tissue culture. O'Donnell MA eds. Tested studies for laboratory teaching. 2006;27:239-63.

[14]. Ozel CA, Arslan O. Efficient micropropagation of English shrub rose "Heritage" under in vitro conditions. Int J Agri Biol.2006;8(5):626-9.

[15]. Chhalgri MA, Khan MT, Nizamani GS, Yasmeen S, Khan IA, Aslam MM, et al. Effect of Plant Growth Hormones on Shoot and Root Regeneration in Rose under In Vitro Conditions. Adv Life Sci. 2020 Nov 27;8(1):93-7.

[16]. Murashige T, Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol plant. 1962 Jul;15(3):473-97.

[17]. Bang H, Zhou XK, Van Epps HL, Mazumdar M, editors. Statistical methods in molecular biology. Totowa, NJ, USA:: Humana press; 2010;3(620):636.

[18]. Canli FA, Kazaz S.Biotechnology of roses: progress and future prospects. Turk J For. 2009;10(1):167-183.

[19]. Tsygankova VA, Blume YaB. Screening and peculiarity of the biological action of synthetic plant growth regulators. Biopolym Cell. 1997;13(6):484-492.

[20]. Tsygankova V, Medvedieva T, Natalchuk T, Udovychenko K, Andrusevich Ya, Kopich V, et al. Study of the impact of pyrimidine derivatives on rooting microshoots of cherry (Prunus cerasus L.) under in vitro culture conditions. Materials of the 5th International scientific and practical conference "Scientific achievements of modern society". Cognum Publishing House. Liverpool, United Kingdom.2020;1063-1076.

[21]. Tsygankova VA, Bayer OO, Andrusevich Ya V, Galkin AP, Brovarets VS, Yemets AI, et al. Screening of five and six-membered nitrogen-containing heterocyclic compounds as new effective stimulants of Linum usitatissimum L. organogenesis in vitro. Int J Med Biotechnol Genetics S. 2016;S2(001):1-9.

[22]. Tsygankova V, Andrusevich YaV, Shtompel O, Romaniuk O, Yaikova M, Hurenko A, et al. Application of synthetic low molecular weight heterocyclic compounds derivatives of pyrimidine, pyrazole and oxazole in agricultural biotechnology as a new plant growth regulating substances. Int J Med Biotechnol Genetics. 2017;S2(002):10-32.

[23]. Tsygankova V, Andrusevich Y, Shtompel O, Kopich V, Solomyanny R, Bondarenko O. Phytohormone-like effect of pyrimidine derivatives on regulation of vegetative growth of tomato. Int J Botany Stud.2018;3(2):91-102.

[24]. Kornienko AM, Brovarets VS. Study of auxin-like and cytokinin-like activities of derivatives of pyrimidine, pyrazole, isoflavones, pyridine, oxazolopyrimidine and oxazole on haricot bean and pumpkin plants.Int J Chem Tech Res.2018;11(10):174-190.

[25]. Hurenko AO, Solomyanny RM, Mrug GP, Frasinyuk MS, Pilyo SG, Kornienko AM, et al. Auxin-like effect of derivatives of Pyrimidine, Pyrazole, Isoflavones, Pyridine, Oxazolopyrimidine and Oxazole on acceleration of Vegetative growth of Flax. Int JPharmTech Res. 2018;11(3):274-86.

[26]. Tsygankova V, Andrusevich YV, Shtompel OI, Kopich VM, Panchyshyn SY, Vydzhak RM, et al. Application of pyrazole derivatives as new substitutes of auxin IAA to regulate morphometric and biochemical parameters of wheat (*Triticum aestivum* L.) seedlings. JAdv Agric. 2019;10:1772-86.

[27]. Mohilnikova IV, Tsygankova VA, Solomyannyi RM, Brovarets VS, Bilko NM, Yemets AI. Screening of growth-simulating activity of synthetic compounds-pyrimidine derivatives. Dopov Nac akad nauk Ukr. 2020;10:62-70.

[28]. Mohilnikova IV, Tsygankova VA, Gurenko AO, Brovarets VS, Bilko NM, Yemets AI, et al.Influence of pyrazole derivatives on plant growth and development in vivo and in vitro.Dopov Nac akad nauk Ukr. 2021;(6):108-19.

[29]. Tsygankova VA, Voloshchuk IV, AndrusevichYa V, Kopich VM, Pilyo SG, Klyuchko SV, et al. Pyrimidine Derivatives As Analogues Of Plant Hormones For Intensification Of Wheat Growth During The Vegetation Period. life.J Adv Biol. 8:9.

[30]. Tsygankova VA, Andrusevich YV, Shtompel OI, Solomyanny RM, Hurenko AO, Frasinyuk MS, et al. New Auxin and Cytokinin Related Compounds Based on Synthetic Low Molecular Weight Heterocycles. In Auxins, Cytokinins and Gibberellins Signaling in Plants. Springer Nature Switzerland AG;2022. pp. 353-377.
[31]. Tsygankova VA. Genetic Control and Phytohormonal Regulation of Plant Embryogenesis. Int J Med Biotechnol Genetics (IJMBG). 2015;3(1):9-20.
[32]. Cheng ZJ, Shang BH, Zhang XS, Hu YX. Plant hormones and stem cells. Hormone Metabolism and Signaling in Plants. 2017:405-30.
[33]. Tsygankova VA, Zayetz VN, Galkina LA, Prikazchikova LP, Blume YB. An unusual minor protein appearing in embryonic axis cells of haricot bean seeds following germination process stimulated by 6-methylthiouracil.Biopolym Cell. 1998;14(5):438-448.
[34]. Tsygankova VA, Zayets VN, Galkina LA, Blume YB. The phytohormone-mediated action of the synthetic regulators on cell extension growth in higher plants. Biopolym Cell.1999;15(5):432.
[35]. Blázquez MA, Nelson DC, Weijers D. Evolution of Plant Hormone Response Pathways. Annu Rev Plant Biol. 2020;71:327-353. PubMed PMID: 32017604.
[36]. Fàbregas N, Fernie AR. The interface of central metabolism with hormone signaling in plants.Curr Biol. 2021;31(23):R1535-R1548. PubMed PMID: 34875246.
[37]. Tandon P, Arya HC. Association of auxin protectors, peroxidase, indoleacetic acid oxidase and polyphenol oxidase in Zizyphus gall and normal stem tissues grown in culture. Biochemie und Physiologie der Pflanzen. 1982;177(2):114-24.
[38]. Liu ZH, Hsiao IC, Pan YW. Effect of naphthaleneacetic acid on endogenous indole-3-acetic acid, peroxidase and auxin oxidase in hypocotyl cuttings of soybean during root formation. Bot Bull Acad Sin. 1996;37(4):247-253.
[39]. Murthy BN, Murch SJ, Saxena PK. Thidiazuron-induced somatic embryogenesis in intact seedlings of peanut (Arachis hypogaea): Endogenous growth regulator levels and significance of cotyledons. Physiol Plantarum. 1995;94(2):268-76.
[40]. Hutchinson MJ, Saxena PK. Role of purine metabolism in thidiazuron-induced somatic embryogenesis of geranium (Pelargonium× hortorum) hypocotyl cultures. Physiol Plantarum. 1996;98(3):517-22.
[41]. Guo B, Abbasi BH, Zeb A, Xu LL, Wei YH. Thidiazuron: a multi-dimensional plant growth regulator. Afr J Biotechnol. 2011;10(45):8984-9000.
[42]. Ahmad N, Faisal M, editors. Thidiazuron: From urea derivative to plant growth regulator. Singapore: Springer; 2018.
[43]. Rigal A, Ma Q, Robert S. Unraveling plant hormone signaling through the use of small molecules. Front Plant Sci. 2014;5:373. PubMed PMID: 25126092.
[44]. Serrano M, Kombrink E, Meesters C. Considerations for designing chemical screening strategies in plant biology. Front Plant Sci. 2015;6:131. PubMed PMID: 25904921.
[45]. Dejonghe W, Russinova E. Plant Chemical Genetics: From Phenotype-Based Screens to Synthetic Biology. Plant Physiol. 2017;174(1):5-20. PubMed PMID: 28275150.
[46]. Raggi S, Doyle SM, Robert S. Auxin: At the crossroads between chemistry and biology. The Chemical Biology of Plant Biostimulants.Book SeriesWiley Series in Renewable Resources. 2020:122-52.
[47]. Pařízková B, Pernisová M, Novák O. What Has Been Seen Cannot Be Unseen-Detecting Auxin In Vivo. Int J Mol Sci. 2017;18(12):2736. PubMed PMID: 29258197.