

Screening of Five and Six-Membered Nitrogen-Containing Heterocyclic Compounds as New Effective Stimulants of *Linum Usitatissimum* L. Organogenesis *in Vitro*

Research Article

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Abstract

Linum usitatissimum L. (flax or linseed) is one of the oldest economically important crops used in biotechnology as a source for bioactive compounds. Flax fiber and seed have industrial significance as a source of cellulosic fiber for textile and paper industry and seed oil for pharmaceutical, cosmetic and food industry. The elaboration of new effective techniques for *in vitro* regeneration of *Linum usitatissimum* L. is very actual problem today. The influence of low molecular weight five and six-membered nitrogen-containing heterocyclic compounds on organogenesis of *Linum usitatissimum* L. cultivar heavenly *in vitro* has been studied. It was found that heterocyclic compounds derivatives pyridine, pyrimidine, pyrazole and isoflavones revealed high stimulating effect on direct shoot organogenesis of *Linum usitatissimum* L. *in vitro*. The maximal shoot regeneration frequency was obtained on the MS media containing heterocyclic compounds at the concentration 10⁻⁸ M/l as compared to lower regeneration frequency obtained on the control MS medium containing 1 mg/l BAP and 0.05 mg/l NAA. The maximal index of explants with regeneration (in %) that exceeded almost twice the same index in control MS medium was obtained on the MS medium containing heterocyclic compound derivative of isoflavones. At the same time, the index of explants with regeneration (in %) that exceeded one and a half times the same index in control MS medium and the maximal index of shoots elongated more than 1 cm (in %) that exceeded almost twice the same index in control MS medium were obtained on the MS media containing heterocyclic compounds derivatives of pyridine, pyrimidine and pyrazole. The current study confirms perspective of using of low molecular weight five and six-membered nitrogen-containing heterocyclic compounds at the concentration 10⁻⁸ M/l of the MS medium as new effective substitutes of traditional growth regulators auxin NAA and cytokinin BAP for regeneration of *Linum usitatissimum* L. cultivar heavenly *in vitro*.

Keywords: Plant Growth Regulators, MS Medium, NAA, BAP, Nitrogen-Containing Heterocyclic Compounds, Pyridine, Pyrimidine, Pyrazole, Isoflavones, *Linum usitatissimum* L., Organogenesis *in Vitro*.

Introduction

An actual problem for the successful development of plant biotechnology is elaboration of new effective plant growth regulators to improve technology of introduction to *in vitro* culture of different plant species with low morphogenetic potential, to produce virus-free and pathogen-free lines of plants, to improve techniques of microclonal breeding of plants, to increase synthesis of biologically active compounds and secondary metabolites in the isolated tissue cultures of agricultural and medicinal plants [1-4]. Today, the traditionally known synthetic and natural plant growth regulators with auxin and cytokinin activity such as IAA,

NAA, IBA, 2,4-D, 2,4,5-T, Zeatin, Kinetin, 2iP, BA, BAP, BPA [5-11] and new plant growth regulators such as BSAA (3-(benzo[b]selenienyl)acetic acid), 5,6-Cl₂-IAA-Me (5,6-dichloroindole-3-acetic acid methylester) [12, 13], TDS (thidiazuron) [13-18], as well as natural bioregulators [19-26] are widely used in the biotechnological practice for cultivation of isolated plant tissues and organs and microclonal propagation *in vitro* of medicinal and agricultural plants. Therefore, the elaboration of new plant growth regulators of synthetic or natural origin as effective substitutes of traditional regulators for practical using in the plant biotechnology is very important and urgent task.

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At the Institute of Bioorganic Chemistry and Petrochemistry of NAS of Ukraine together with the National Enterprise Interdepartmental Science and Technology Center "Agrobiotech" of NAS and MES of Ukraine the several new ecologically safe plant growth regulators of synthetic origin: Ivin and natural origin: Emistim, Poteitin, Zeastimulin, Charkor, Biolan, Biogen, Radostim, Radostim-super, Regoplant and Stimpo that accelerate plant growth, increase yield crop of vegetables, grains, legumes and fruit crops, as well increase plant immune protection against pathogenic and parasitic organisms were created [27-31]. Our previous studies have shown the possibility of using of new synthetic and natural compounds in the isolated tissue cultures of agricultural and medicinal plants to induce morphogenetic processes and increase the synthesis of secondary metabolites [32-34].

Over the last years the another classes of chemical compounds - functionally substituted of low molecular weight five and six-membered nitrogen-containing heterocyclic compounds were also synthesized at the Institute of Bioorganic Chemistry and Petrochemistry of NAS of Ukraine. Most of them showed anti-radical, antioxidant, antitumor, antidepressant, antimicrobial and antiviral activity and could potentially be used as medical drugs for treatment of cancer, immune, cardiovascular, nervous, viral, infectious and other diseases [35-42]. The another strategic focus of our researches is study the influence of low molecular weight heterocyclic compounds derivatives of classes pyridine, pyrimidine, pyrazole and isoflavones on the plant growth and development with the prospect of their using as effective substitutes of traditional plant growth regulators in the biotechnological practice. In favor of target area of our researches witness numerous studies conducted by another authors [43-53].

Linum usitatissimum L. (flax or linseed) is one of the oldest economically important crops that are widely used in biotechnology [54-60]. *Linum usitatissimum* L. belongs to *Linaceae* family which consists of more than 300 species mainly known as ornamental plants, but only one species *Linum usitatissimum* L. has a practical importance [55]. Flax fiber and seed have industrial significance as a source of cellulosic fiber for textile and paper industry and seed oil for pharmaceutical, cosmetic and food industry [56, 57]. Flax seed contains various bioactive compounds, the major components include: 41% fat, 20-31% protein, 28% total dietary fiber, 7.7% moisture, and 3.4% ash, the minor components include: cyanogenic glycosides, phytic acid, phenolics, trypsin inhibitor, linatine, lignans (phytoestrogens), minerals, vitamins, cadmium, selenium and cyclolinopeptides (CLs) [57, 58]. The essential amino acids such as arginine, aspartic acid, and glutamic acid are the major protein components of the flax seed [57, 58]. The flax seed oil is an important source of omega-3 fatty acid - alpha-linolenic acid (ALA) which accounts for more than 50% of fatty acid content of oil [56-59]. The major components of flax seed oil are triacylglycerides which include a mixture of the fatty acids: linolenic (52%), linoleic (17%), oleic (20%), palmitic (6%), and stearic (4%) acids, the minor lipids and lipid soluble compounds include: monoacylglycerides, diacylglycerides, tocopherols, sterols, sterol-esters, phospholipids, waxes, CLs, free fatty acids (FFAs), carotenoids, chlorophyll, and other compounds [57]. Today linseed oil is commonly used in clinical practice for prevention and treatment of cardiovascular disease because it has antioxidant effect and decreases blood total plasma cholesterol, triglyceride (TG), and low-density lipoprotein (LDL) cholesterol without a significant decrease in high-density lipoprotein (HDL) cholesterol

[56-58]. Moreover, another beneficial effect of flax seed oil is due to the presence ALA component which takes part in the biosynthesis of hormone-like eicosanoids that regulate inflammation and immune function in higher animals [57]. The widespread use of dietary food supplemented with flax seed oil omega-3 fatty acid and flax seed lignan secoisolariciresinol diglucoside (SDG) prevents different diseases such as cancer, arthritis, atherosclerosis, diabetes, inflammatory diseases, depression, heart disease, hypertension, memory problems, weight gain, some allergies, kidney disorders and lupus nephritis [56, 60].

Because of using *Linum usitatissimum* L. in the biotechnology as an important source for bioactive compounds, numerous studies devoted to elaboration of effective techniques for *in vitro* regeneration of this plant have been conducted for many years [54, 55, 61-66]. Nevertheless, the elaboration of new efficient methods for *in vitro* cultivation and regeneration of this plant is very actual problem today. Based on the mentioned, the considerable theoretical and practical interest is study the possibility of using of new low molecular weight five and six-membered nitrogen-containing heterocyclic compounds synthesized in the Institute of Bioorganic Chemistry and Petrochemistry of NAS of Ukraine for stimulation of organogenesis of *Linum usitatissimum* L. *in vitro* conditions.

The objective of this work is study of impact of low molecular-weight synthetic five and six-membered nitrogen-containing heterocyclic compounds derivatives of pyridine, pyrimidine, pyrazole and isoflavones on shoot organogenesis of *Linum usitatissimum* L. cultivar heavenly *in vitro*.

Materials and Methods

Chemicals

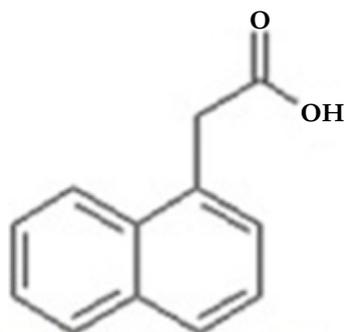
In our experiments the biological activity of synthetic low molecular weight five and six-membered nitrogen-containing heterocyclic compounds derivatives of pyridine ((1H-pyrrolo[2,3-c]pyridin-3-yl)-acetic acid), pyrimidine(6-Methanesulfonyl-imidazo[1,2-d]pyrimidine-5-ylamine), pyrazole (5-hydrazino-1-phenyl-1H-pyrazole-4-carbohydrazide and 3-Ethyl-7-methyl-3, 7-dihydropyrazole[3,4-d][1,2,3]triazin-4-one) and isoflavones (5-hydroxy-7-methoxy-8-(methoxymethyl)-3-(4-methoxyphenyl)-4H-chromen-4-one) was studied. These compounds were synthesized at the Department for Chemistry of Bioactive Nitrogen-Containing Heterocyclic Compounds of Institute of Bioorganic Chemistry and Petrochemistry of NAS of Ukraine. The methods of synthesis of these heterocyclic compounds are described in the works [67-71].

The biological activity of chemical compounds was compared with activity of growth regulators: auxin NAA (Naphthalen-1-yl-acetic acid) and cytokinin BAP (N-(Phenylmethyl)-1H-purin-6-amine or 6-Benzylaminopurine). Chemical structures and relative molecular mass of growth regulators NAA, BAP and heterocyclic compounds used for bioassays are shown on the Figure 1.

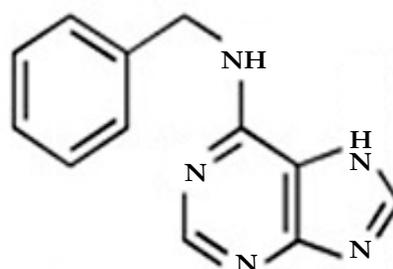
Plant Growing Conditions

To study impact of synthetic heterocyclic compounds on shoot organogenesis *in vitro* seeds of *Linum usitatissimum* L. cultivar

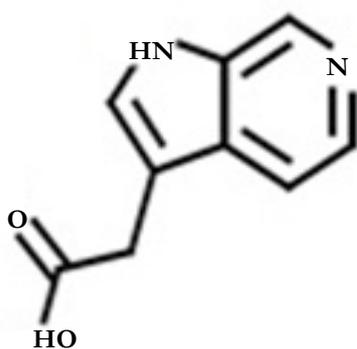
Figure 1. Chemical structures of synthetic compounds used for bioassays.



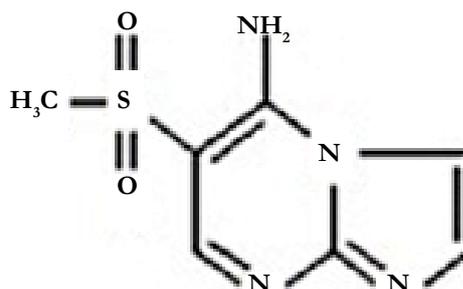
Naphthalen-1-yl-acetic acid;
Molar mass=186.21 g/mol; (10^{-8} M/
l=0,0018621 mg/l)



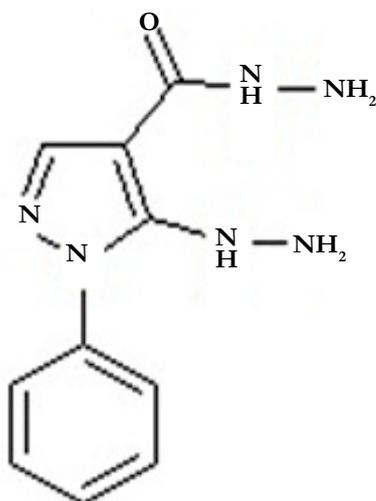
N-(Phenylmethyl)-1H-purin-6-amine;
Molar mass=225.249 g/mol; (10^{-8} M/
l=0,00225249 mg/l)



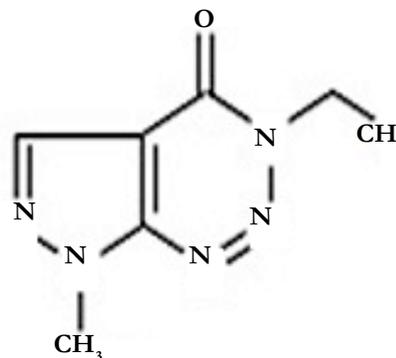
H-pyrrolo[2,3-c]pyridin-3-yl-acetic acid; Molar
mass=176.175 g/mol;
(10^{-8} M/l=0,00176175 mg/l)



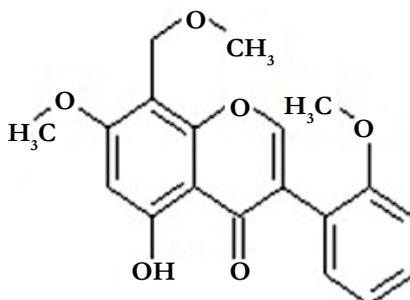
6-Methanesulfonyl-imidazo[1,2-a]pyrimidine-
5-ylamine; Molar mass=212.23 g/mol; (10^{-8} M/
l=0,0021223 mg/l)



5-hydrazino-1-phenyl-1H-pyrazole-4-carbohy-
drazide; Molar mass=232.25 g/mol; (10^{-8} M/
l=0,0023225 mg/l)



3-Ethyl-7-methyl-3,7-dihydropyrazole[3,4-d]
[1,2,3]triazin-4-one; Molar mass=179.18 g/mol;
(10^{-8} M/l=0,0017918 mg/l)



5-hydroxy-7-methoxy-8-(methoxymethyl)-3-(4-methoxyphenyl)-4H-chromen-4-one;
Molar mass=342.35171 g/mol (10^{-8} M/l=0.0034235171 mg/l)

heavenly were initially sterilized in the laminar box using magnetic stirrer with 70% ethanol solution for 1 min and in 5% sodium hypochlorite solution for 6 min at the temperature 20-22°C. The surface of sterilized seeds was washed three times with sterile distilled water for 10 min. Then, the seeds were placed in Petri dishes (9.0 cm in diameter) each containing 25 ml MS (Murashige and Skoog) basal medium [72] supplemented with half-strength MS macro- and micro salts, 20 g/l sucrose and 8 g/l agar, then the media were adjusted to pH 5,7-5,8 before autoclaving. Then Petri dishes were placed in the box for plant cultivation in which seedlings were grown for 5-6 days at the 16/8 h light/dark conditions, the temperature was 24-25°C, light intensity was 3000 lux and air humidity was 60-80 %. After 5-6 days of seed germination the seedlings with open cotyledons were formed.

With the aim to prepare explants for *in vitro* experiments the segments of hypocotyl of 7th days seedlings were aseptically cut into segments (2-3 mm length) and placed horizontally in the Petri dishes (each containing 25 pcs) on the surface of the nutrient MS medium. Three Petri dishes were used in the each variant. Culture media for explant cultivation were supplemented with macro- and micro salts contained in MS basal medium [72], 20 g/l sucrose, 8 g/l agar and each chemical low molecular weight heterocyclic compound at the concentration of 10^{-8} M/l of MS medium.

MS basal medium containing 1 mg/l BAP and 0.05 mg/l NAA was used as a control sample for shoot regeneration of flax explants. The efficiency of regeneration was determined according to: a) the number of explants with regeneration (in%) and b) the number of shoots longer than 1 cm (in%) obtained in the experimental samples relative to the control. The obtained shoots were rooted on hormone-free MS medium containing half-strength MS macro- and micro salts and vitamins, 10g/l sucrose and 7 g/l agar.

All the 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 \pm SD [73].

Results

The inducing effect of low molecular heterocyclic compounds derivatives of pyridine ((1H-pyrrolo[2,3-c]pyridin-3-yl)-acetic acid), pyrimidine (6-Methanesulfonyl-imidazo[1,2-a]pyrimidine-5-ylamine), pyrazole (5-hydrazino-1-phenyl-1H-pyrazole-4-carbohydrazide and 3-Ethyl-7-methyl-3,7-dihydropyrazole[3,4-d][1,2,3]triazin-4-one) and isoflavones (5-hydroxy-7-methoxy-8-(methoxymethyl)-3-(4-methoxyphenyl)-4H-chromen-4-one) as well as growth regulators NAA and BAP on shoot organogenesis in the isolated tissue cultures of *Linum usitatissimum* L. cultivar heavenly was studied. It was found that heterocyclic compounds at the concentration 10^{-8} M/l of MS medium revealed maximal stimulating effect on shoot regeneration as compared to lower effect of 1 mg/l BAP and 0.05 mg/l NAA supplemented into control MS medium (Figure 2 and Figure 3).

The maximal index of explants with regeneration (in %) that exceeded almost twice the control index was obtained on the MS medium containing heterocyclic compound derivative of isoflavones (5-hydroxy-7-methoxy-8-(methoxymethyl)-3-(4-methoxyphenyl)-4H-chromen-4-one). At the same time the activity of this compound according to index of shoots elongated more than 1 cm

(in %) was lower as compared with activity of heterocyclic compounds derivative of pyridine ((1H-pyrrolo[2,3-c]pyridin-3-yl)-acetic acid) and pyrimidine (6-Methanesulfonyl-imidazo[1,2-a]pyrimidine-5-ylamine) and was similar to the activity of pyrazole (5-hydrazino-1-phenyl-1H-pyrazole-4-carbohydrazide) (Figure 4).

According to index of explants with regeneration (in %) the chemical compounds derivative of pyrimidine (6-Methanesulfonyl-imidazo[1,2-a]pyrimidine-5-ylamine), pyrazole (5-hydrazino-1-phenyl-1H-pyrazole-4-carbohydrazide) and pyridine ((1H-pyrrolo[2,3-c]pyridin-3-yl)-acetic acid) showed activity that exceeded one and a half times activity of growth regulators NAA and BAP containing in the control MS medium (Figure 4).

It was observed that according to index of shoots elongated more than 1 cm (in %) the activity of heterocyclic compounds derivative of pyrimidine (6-Methanesulfonyl-imidazo[1,2-a]pyrimidine-5-ylamine), pyrazole (5-hydrazino-1-phenyl-1H-pyrazole-4-carbohydrazide) and pyridine ((1H-pyrrolo[2,3-c]pyridin-3-yl)-acetic acid) exceeded almost twice activity of growth regulators NAA and BAP supplemented into control MS medium (Figure 4).

At the same the activity of heterocyclic compound derivative of pyrazole (3-Ethyl-7-methyl-3,7-dihydropyrazole[3,4-d][1,2,3]triazin-4-one) according to index of explants with regeneration (in %) and index of shoots elongated more than 1 cm (in %) was less as compared with activity above mentioned heterocyclic compounds but somewhat exceeded activity of growth regulators auxin NAA and cytokinin BAP (Figure 4).

The obtained shoots were rooted on the hormone-free MS medium elaborated in our previous work [74] (Figure 5).

Thus, the results of this work confirm that synthetic low molecular weight five and six-membered nitrogen-containing heterocyclic compounds derivatives of pyridine, pyrimidine, pyrazole and isoflavones showed high stimulating activity on direct shoot organogenesis in the isolated tissue culture of *Linum usitatissimum* L. cultivar heavenly *in vitro* which exceeds the activity of growth regulators auxin NAA and cytokinin BAP.

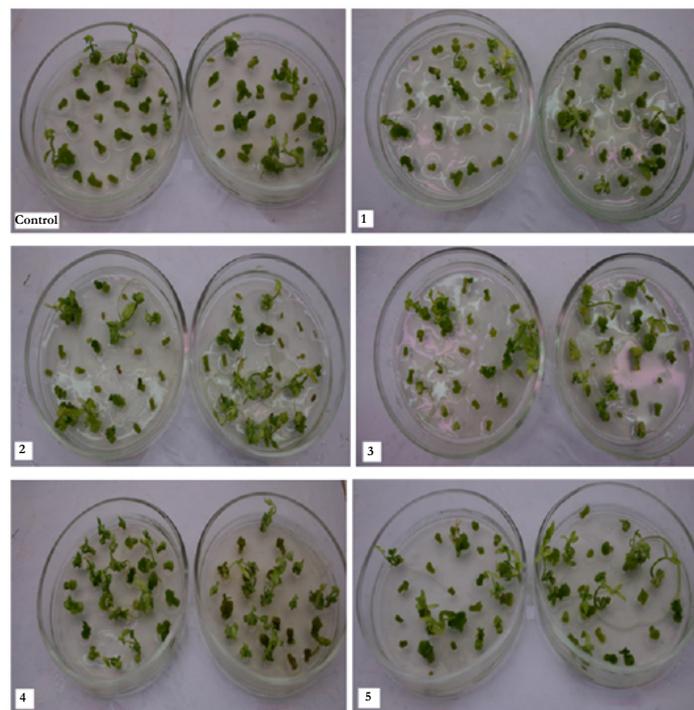
Discussion

Data of our previous researches and numerous data of other authors witness that regeneration ability of flax (*Linum usitatissimum* L.) is under the influence of various types of growth regulators and their different concentrations in the culture media as well as origin of the plant explants [55, 62-66, 75-78].

For example in our previous work [65] we studied morphogenetic responses of 12 fibre flax genotypes (*Linum usitatissimum* L.) on two hormone combinations in different culture media: MS-BN containing 1 mg/l BAP and 0.05 mg/l NAA and MS-DZ containing 0.4 mg/l 2,4-D and 1.6 mg/l Zeatin. It was shown that light green compact calli and shoot proliferation were obtained from hypocotyl explants on medium MS-BN containing 1 mg/l BAP and 0.05 mg/l NAA.

Similar studies were conducted in the works [55, 66, 74-78]. The inducing effect of various concentrations of growth regulators NAA, BAP, TDZ and 2iP added into basal MS medium on the formation of callus, shoot and root from hypocotyl explants of

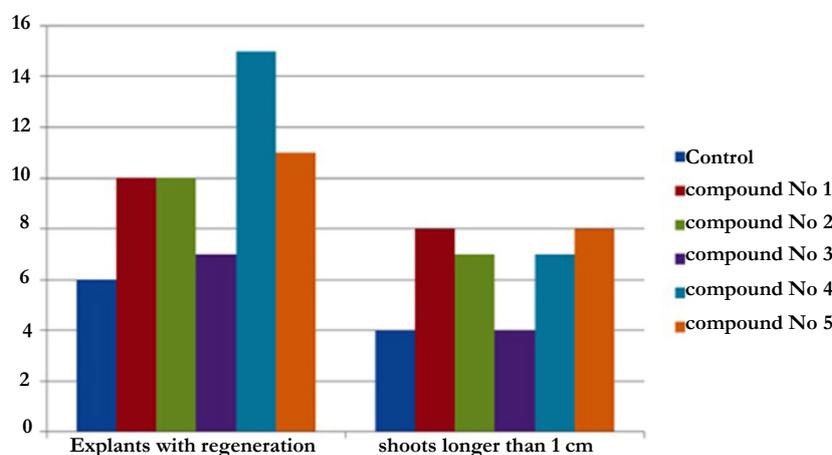
Figure 2. Impact of growth regulators NAA and BAP (control) and synthetic five and six-membered nitrogen-containing heterocyclic compounds (experiment) on shoot organogenesis of *Linum usitatissimum* L. cultivar heavenly *in vitro*.



Control - NAA+BAP,

1. 6-Methanesulfonyl-imidazo[1,2-*a*]pyrimidine-5-ylamine, 2. 5-hydrazino-1-phenyl-1H-pyrazole-4-carbohydrazide,
3. 3-Ethyl-7-methyl-3,7-dihydropyrazole[3,4-*d*][1,2,3]triazin-4-one, 4. 5-hydroxy-7-methoxy-8-(methoxymethyl)-3-(4-methoxyphenyl)-4H-chromen-4-one,
5. (1H-pyrrolo[2,3-*c*]pyridin-3-yl)-acetic acid

Figure 3. Average number of explants with regeneration and number of shoot longer than 1 cm obtained per one Petri dish.



Control - NAA+BAP,

1. 6-Methanesulfonyl-imidazo[1,2-*a*]pyrimidine-5-ylamine, 2. 5-hydrazino-1-phenyl-1H-pyrazole-4-carbohydrazide,
3. 3-Ethyl-7-methyl-3,7-dihydropyrazole[3,4-*d*][1,2,3]triazin-4-one, 4. 5-hydroxy-7-methoxy-8-(methoxymethyl)-3-(4-methoxyphenyl)-4H-chromen-4-one,
5. (1H-pyrrolo[2,3-*c*]pyridin-3-yl)-acetic acid

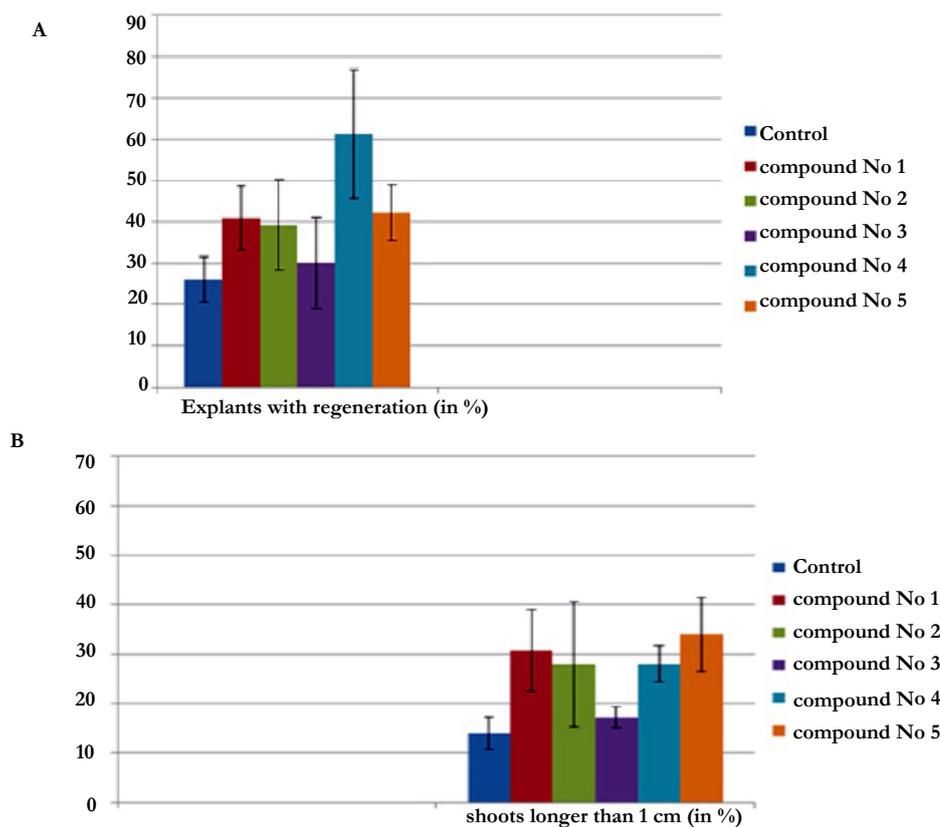
Linum usitatissimum L. was studied [55]. It was found that highest regeneration effectiveness was observed on the MS media supplemented with 1 mg/l BAP.

The effect of different concentrations and combinations of growth regulators BAP, NAA and TDZ on shoot regeneration from hypocotyl explants of three flax cultivars (*Linum usitatissimum* L.) was investigated [66]. The best results of shoot regeneration (up to 70.0-100 %) were obtained on the MS medium containing 1 mg/l BAP and 0.02 mg/l NAA, at the same time increase of

TDZ concentrations above 1 mg/l significantly reduced the shoot regeneration frequency due toxic effect of TDZ on explant (resulting in tissue necrosis and explant death).

In the work [75] the most effective shoot multiplication (that was about twice higher in the light-grown cultures than those in the darkness) from hypocotyl explants of oil flax (*Linum usitatissimum* L., cv. 'Szafrir') was obtained on MS medium containing 0.05 mg/l 2,4-D and 1 mg/l BA.

Figure 4. Efficiency of regeneration: A) the number of explants with regeneration (in %) and B) the number of shoot longer than 1 cm (in %) obtained in the experimental samples relative to the control.



Control - NAA+BAP,

1. 6-Methanesulfonyl-imidazo[1,2-*a*]pyrimidine-5-ylamine,
2. 5-hydrazino-1-phenyl-1H-pyrazole-4-carbohydrazide,
3. 3-Ethyl-7-methyl-3,7-dihydropyrazole[3,4-*d*][1,2,3]triazin-4-one,
4. 5-hydroxy-7-methoxy-8-(methoxymethyl)-3-(4-methoxyphenyl)-4H-chromen-4-one,
5. (1H-pyrrolo[2,3-*c*]pyridin-3-yl)-acetic acid

Figure 5. Rooting of shoots on the hormone-free MS medium.



Control - Shoots previously obtained on the MS medium supplemented with NAA+BAP;

1. Shoots previously obtained on the MS medium supplemented with 6-Methanesulfonyl-imidazo[1,2-*a*]pyrimidine-5-ylamine;
2. Shoots previously obtained on the MS medium supplemented with 5-hydrazino-1-phenyl-1H-pyrazole-4-carbohydrazide;
3. Shoots previously obtained on the MS medium supplemented with 3-Ethyl-7-methyl-3,7-dihydropyrazole[3,4-*d*][1,2,3]triazin-4-one;
4. Shoots previously obtained on the MS medium supplemented with 5-hydroxy-7-methoxy-8-(methoxymethyl)-3-(4-methoxyphenyl)-4H-chromen-4-one;
5. Shoots previously obtained on the MS medium supplemented with (1H-pyrrolo[2,3-*c*]pyridin-3-yl)-acetic acid

The effect of various concentrations and combinations of growth regulators BAP, GA3, Kin, IBA on shoot regeneration from shoot tip and nodal segment of linseed (*Linum usitatissimum* L., cv. neela) was studied [76]. It was shown that multiple shoot induction was achieved on the MS medium supplemented with 2.0 mg/l BAP, at the same time shoots were rooted most effectively in MS medium supplemented with 0.5 mg/l NAA.

In the work [77] an optimal composition of different culture media: Sh2 medium supplemented with 1.0 mg/l BAP and 0.05 mg/l NAA, Sh3 medium supplemented with 0.1 mg/l BAP, 0.5 mg/l NAA and 0.3 mg/l IAA as well as Sh50 medium supplemented with 0.02 mg/l BAP and 0.001 mg/l NAA were elaborated for the efficient production of flax plants regenerated from somatic explants from hypocotyls, cotyledons, leaves, stems and roots as well as plants regenerated from anther and microspore cells. It was found that medium supplemented with two carbon sources (2.5% sucrose and 2.5% glucose) was most effective for callus induction, whereas medium supplemented with sucrose at lower concentration (2%) was effective for shoots. Root formation was most efficient on the medium supplemented with 1% sucrose and reduced (50%) mineral concentration.

The effect of growth regulator combinations (1.0 mg/l kinetin and 0.1 mg/l IAA, 1.0 mg/l BAP and 0.05 mg/l NAA, 2.0 mg/l 2iP or 1.0 mg/l kinetin) on adventitious shoot organogenesis from hypocotyl-derived and stem segment-derived callus of different linseed cultivars was studied [78]. Obtained results showed that maximum shoot regeneration frequency was obtained from hypocotyl-derived four week-old callus in all cultivars tested on combined MSB5 media, supplemented with 2.0 mg/l 2iP.

Data obtained in this work testify that the low molecular weight five and six-membered nitrogen-containing heterocyclic compounds revealed high stimulating activity on direct shoot organogenesis of *Linum usitatissimum* L. cultivar heavenly *in vitro*. These results confirm possibility of using of low molecular weight heterocyclic compounds as new effective substitutes of growth regulators auxin NAA and cytokinin BAP for micropropagation of *Linum usitatissimum* L. cultivar heavenly *in vitro*.

Conclusion

Impact of low molecular weight five and six-membered nitrogen-containing heterocyclic compounds derivatives of pyridine, pyrimidine, pyrazole and isoflavones on shoot organogenesis of flax (*Linum usitatissimum* L.) cultivar heavenly *in vitro* was studied. According to indexes of shoot organogenesis it was found that heterocyclic compounds at the concentration 10^{-8} M/l showed similar to auxin and cytokinin activity which exceeds almost one and a half - twice the activity of growth regulators auxin NAA and BAP. Obtained data confirm possibility of using of low molecular weight five and six-membered nitrogen-containing heterocyclic compounds as new efficient plant growth regulators in the biotechnological practice.

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