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Original Article

Mixture of Growing Medium with IAA, Biochemical and Chemical Properties in *Euphorbia Pulcherrima* Cultivation

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Abstract

In the experiment there were used rooting of cuttings of poinsettia *Euphorbia pulcherrima* 'Prestige Early Red'. This cultivation of plants was carried out in cell trays with the use of commercial peat growing medium 'Klasmann Steck Medium', containing four concentrations of indole-3-acetic acid (IAA), (natural concentration and 200, 300 and 400 µg kg^{-1} additionally). Data indicates that the mixture of substrate with IAA had an impact on chemical compounds and enzymes activity before and after rooting of cuttings of poinsettia. These investigations revealed that higher activity of xanthine oxidase and peroxidase were observed before cultivation of poinsettia. However, different results related to phenol oxidase, urease and nitrate reductase activity were noted.

Keywords: poinsettia cultivation, peat substrate, biochemical and chemical properties, IAA.

1. Introduction

There are many areas in agriculture, horticulture, pomiculture, moriculture, etc., where phytohormones can be used in successful cultivation in order to obtain greater yield.

The availability of free indole-3-acetic acid (IAA), the biologically active form of auxin, plays an important role in the development of a plant throughout its life cycle.

Auxin regulates several fundamental cellular processes including division, elongation, and differentiation of cells, represents one of the most important classes of signaling molecules described in plants.

Indole-3-acetic acid, the principal form of auxin in higher plants, is first synthesized within young apical tissues, then conveyed to its basal target tissues by a specialized delivery system termed polar auxin transport. Strict regulation of the endogenous IAA level is of great importance and its homeostasis results from the balance between its biosynthesis and metabolism, including conjugation, deconjugation, and catabolism [7, 9].

The production of greenhouse crops involves a number of cultural inputs. Among these, probably the most important is the type of growing medium used. Due to the relatively shallow depth and limited volume of a container, growing media must be amended to provide the appropriate physical and chemical properties necessary for plant growth. Growing demand for highly specialised and standardised substrates allowed for fast development of substrate industry, as well as research focusing on solving problems arising during production of high quality substrates [16].

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Growth medium typically consists of an organic component, fertilizer, and sand or sandy soil. The organic component is often peat excavated from natural bogs and fens. Peat is plant debris that in its natural setting degrades very slowly and thus peat can be considered bound biogenic carbon [2]. Substrates designed for vegetables cultivation have also high total porosity, excepting substrate produced with higher content of black peat, which had essentially lower values. The addition of perlite to those substrates mixes of black and white peat influenced profitably on the porosity increase. The horticultural growers more and more frequently use ready-made substrates produced on industrial scale with the use of homogeneous components that are of tightly controlled quality [10].

Soil organic matter affects biochemical, chemical, biological and physical soil properties that control soil microbial activity. Enzyme activity reflects an essential part of the functional diversity in soils, which is driven by the genetic diversity of soil microorganisms, plants and soil animals in close relation to environmental effects and ecological interactions [15].

The aim of our investigations was to estimate biochemical and chemical properties of growing medium with different concentrations of IAA in poinsettia cultivation.

2. Material and Methods

In the experiment there were used rooting of cuttings of poinsettia *Euphorbia pulcherrima* 'Prestige Early Red'. From April to August 2011 rooting of cuttings was carried out in cell trays with the use of commercial peat growing medium of 'Klasmann Steck Medium', containing four concentrations of IAA (natural concentration and 200, 300 and 400 μg kg^{-1} additionally). The experiments were conducted in accordance with EPPO norms (European and Mediterranean Plant Protection Organization - Guideline for the efficiency evaluation of plant growth regulators, Rooting of cuttings, PP 1/186(2).

The pH was measured in 1N KCl in air-dried of peat substrates using a 1:5 v/v soil solution suspension. The total organic carbon was analyzed on Total Organic Carbon Analyzer (TOC 5050A) with Solid Sample Module (SSM-5000A) produced by Shimadzu (Japan). Hot water extractable carbon (C_{HWE}) was evaluated by Smolander and Kitunen method [22] on TOC 5050A equipment produced by Shimadzu (Japan). Total nitrogen was determined by the Kjeldahl method. Ammonium and nitrate ions were measured by chromatographic method [27].

Indole-3-acetic acid (IAA) concentrations were assayed in the resulting of soil extraction fluorimetrically in the bottom layer at $\lambda_{excitation}$=290 nm and $\lambda_{emission}$=368 nm. The IAA concentration was calculated from the analytical curve, the IAA content ranged from 50-300 ng mL^{-1}, prepared similar to investigated soils samples [27, 29, 30].

Xanthine oxidase activity was determined by Krawczyński method [11, 27, 29]. Xanthine is used as a substrate for measurement of activity of the xanthine oxidase. The absorbance of the solution was measured colorimetrically at λ_{max}=290 nm using a UV-VIS spectrophotometer Beckman DU®-68 USA.

Nitrate reductase activity was determined using potassium nitrate as a substrate and 2, 4-dinitrophenol as inhibitor of nitrite reductase according to Kandeler [8, 25, 26, 29]. The field-moist soil samples were incubated under waterlogged conditions for 24h at 25ºC. Nitrite released as a result of incubation was extracted with potassium chloride solution and determined colorimetrically at λ_{max}=520 nm.

Urease activity in soils was determined by Hoffmann and Teicher method [27, 29]. This method involves determination of the ammonium released by urease activity when soil is incubated with buffered (pH 6.7) urea solution and toluene at 37°C for 3 h. The absorbance of the solution was measured colorimetrically at λ_{max}=630 nm using a UV-VIS spectrophotometer Beckman DU®-68 USA.

Phenol oxidase was determined by Perucci method [17, 27, 29, 31]. Catechol is used as a substrate for measurements of phenol oxidase activity in soil samples. The absorbance of the solution was measured colorimerically at λ_{max}=525 nm using a UV-VIS spectrophotometer Beckman DU®-68 USA.

Peroxidase activity in soils was determined by Bartha and Bordeleau method [27, 29]. In the presence of peroxidase, phenolic substrates are oxidized to metoxyl and carboxyl groups through demethoxylation or decarboxylation reactions. Peroxidase activity was determined by following the H_2O_2 mediated oxidation of o-dianisidine. The absorbance of the solution was measured colorimetrically at λ_{max}=460 nm using a UV-VIS spectrophotometer Beckman DU®-68 USA. Enzymes activity in peat substrates was calculated from the early-prepared analytical curve according to the Lambert-Beer light absorption law by means of the least squares formulas.

3. Results and Discussions

Substrates should have physical and chemical properties conducting for plant growth and be uniform, consistent, light weight, affordable and absent of weed seeds and harmful pathogens. When

plants are producted in container their roots are restricted to a small volume. Consequently, the demands made on the substrate for water, air, nutrients, and support are more intense than those made by plants grown in a field production situation where unrestricted root growth can occur [5].

The pH seems to be the most important physicochemical parameter among many soil properties affecting plant growth in peat substrates. Higher pH was confirmed after rooting of cuttings of poinsettia (from 5.01 to 5.41) than before cultivation (from 4.58 to 4.72). Similar pH has been demonstrated among IAA additions (natural concentration and 200, 300 and 400 μg kg^{-1}) (table 1).

Many soils contain compounds which exhibit strong auxin-like activity and differ in their indole-3-acetic acid (IAA) synthesizing capacity depending on the fertility status and organic matter content [21]. Our results indicated that the contents of IAA were the lowest before rooting of cuttings (from 128.29 to 142.54 μg kg^{-1}), and increased after cultivation of plants (from 158.37 to 190.06 μg kg^{-1}) and reaching a maximum with 200 μg kg^{-1} of IAA additions (table 1). Many compounds are released by plant roots, including inorganic ions and organic substances: amino acids, amides, sugars, aliphatic acids, aromatic acids, vitamins, peptides, proteins, enzymes, ketones, urea, phytoalexins and plant hormones like IAA [4]. Earlier investigations by Szajdak and Maryganova [30] suggested that the soil flora also produces appreciable amounts of auxin under natural condition, particularly when organic material is present to support microbial growth. IAA is biosynthesis, apart from the higher plants; however, it is also produced by a number of soil microorganisms, and in particular, plant growth-promoting rhizobacteria. IAA seems to fulfill an important function in nature as a result of its influence in the regulation and development of plant growth.

The degradation of organic matter used in substrates may be due to physical processes such as the mechanical action of roots breaking the matter's structure, to chemical effects such as changes in the pH, or to biological effects such as the activity of microorganisms. Substrate decay has an effect on crop growth. It may cause nitrogen fixation, phytotoxicity due to the appearance of new organic compounds, changes in cationic exchange capacity, or a salinity increase in the crop growing medium. The loss of organic matter and structure in a substrate may also cause a decrease in its aeration capacity, leading to a lack of oxygen and root death [3]. Our investigations have shown higher concentrations of TOC and C_{HWE} after rooting of cuttings of poinsettia in this substrate. This increase was on average 8% for TOC and 28% for C_{HWE} (table 1).

Table 1. The contents of chemical compounds in commercial growing medium before and after rooting of cuttings of *Euphorbia pulcherrima*

Commercial growing medium	pH (KCl)	IAA [μg kg^{-1}]	TOC [g kg^{-1}]	C_{HWE} [g kg^{-1}]	NH_4^+ [mg kg^{-1}]	NO_3^- [mg kg^{-1}]	N_{total} [g kg^{-1}]	C/N
Before rooting of cuttings of *Euphorbia pulcherrima* '								
Control (natural concentration of IAA)	4.58	134.47	391.30	10.47	25.59	54.20	8.96	44
Substrate+IAA (200 μg kg^{-1})	4.65	142.54	393.70	10.96	43.56	43.37	9.52	41
Substrate+IAA (300 μg kg^{-1})	4.72	128.29	388.10	10.94	40.84	42.35	9.56	41
Substrate+IAA (400 μg kg^{-1})	4.72	134.47	395.20	7.33	27.01	37.77	9.62	42
After rooting of cuttings of *Euphorbia pulcherrima*								
Control (natural concentration of IAA)	5.01	158.37	425.20	14.52	7.89	3.94	10.08	42
Substrate+IAA (200 μg kg^{-1})	5.16	190.06	421.70	12.81	4.40	4.93	10.36	41
Substrate+IAA (300 μg kg^{-1})	5.27	182.12	426.35	14.47	2.43	2.19	10.92	39
Substrate+IAA (400 μg kg^{-1})	5.41	170.25	433.70	13.81	3.24	1.11	11.20	39

The availability of nitrogen has a regulatory effect on plant litter decomposition and the formation and stabilization of soil organic matter. Increasing nitrogen availability influences the decomposition rates of plant litter and organic matter; the direction of impact depends on the stage of decomposition. In fresh litter, high external and internal concentrations of nitrogen stimulate degradation, while in later

stages of decomposition and in lignified organic matter and humus, high nitrogen availability attenuates decomposition [1]. In this study higher concentrations of ammonium and nitrate ions were observed before rooting of cuttings of poinsettia. The ammonium ions ranged from 25.59 to 43.56 mg kg^{-1} before cultivation of plants and from 2.43 to 7.89 mg kg^{-1} after planting in growing medium. We observed slight decrease of the contents of ammonium ions between the mixture substrate with 200 and 400 μg kg^{-1} of IAA addition before rooting of cuttings of poinsettia to 38%. It was corresponded to decrease concentration of ammonium ions between substrate with natural concentration of IAA and mixture with 400 μg kg^{-1} of IAA after planting, which was 59%. Similar trend was shown for nitrate ions and were amounted from 37.77 to 54.20 mg kg^{-1} before cultivation of plants and from 1.11 to 4.93 mg kg^{-1} after planting (table 1). These results may indicate on absorb mineral forms of nitrogen by plants during cultivation. Additionally, Styła [23] confirmed the following amount of ammonium ions from 3.76 to 11.66 mg kg^{-1} and nitrate ions from 3.67 to 25.98 mg kg^{-1} in mineral soil under apple-tree cultivation. According to Potila and Sarjala [19], most of the plants are able to absorb and assimilate nitrate, ammonium, urea and amino acids as nitrogen sources, but the response to a particular form of nitrogen. Our studies have documented that the content of total nitrogen was lower before rooting of cuttings of poinsettia (from 8.96 to 9.62 g kg^{-1}) in comparison with plants after cultivation (from 10.08 to 11.20 g kg^{-1}) (table 1). Significant and sensitive indication of the degree of humification of the organic materials is C/N ratio.

The C/N ratio was similar before (from 41 to 44) and after (from 39 to 42) rooting of cuttings poinsettia and with combinations of different IAA additions in growing medium (table 1). Soil enzyme activities are indicator of soil degradation since they integrate information about microbial status, and also, from soil physico chemical conditions. They are used as sensors in studies on the influence of soil treatments on soil fertility [20]. The accumulation of organic compounds has been reported in the nutrient solutions of closed systems. Phenolic acids as organic acids are released by plant roots and microorganisms as metabolites or as biotransformation products in the rhizosphere and the nutrient solution [12].Xanthine oxidase and peroxidase plays an important role in redox processes of soils. These investigations have shown higher activity of xanthine oxidase and peroxidase before rooting of cuttings of poinsettia. Xanthine oxidase catalyzes the oxidation of hypoxanthine to xanthine and the latter to uric acid, although it has a low substrate specificity, oxidizing also several purines and aldehydes at a lower rate. This enzyme participates in the cycle of nitrogen in soils [14]. In all analyzed substrate samples xanthine oxidase activity ranged from 4.03 to 8.88 μmol h^{-1} g^{-1} (table 2). The investigations have shown a decrease of its activity to 24% when the IAA was added to substrate before rooting of cuttings of plants (from 200 μg kg^{-1} to 400 μg kg^{-1} IAA). Moreover, Szajdak et al. [29] measured xanthine oxidase activity in soil of Tagan peatland from Siberia. These authors determined higher activity in peat samples (from 22.93 to 54.73 μmol h^{-1} g^{-1}) in comparison with growing medium.

Table 2. Enzyme activity in commercial growing medium before and after rooting of cuttings of *Euphorbia pulcherrima*

Commercial growing medium	XOA [μmol h^{-1} g^{-1}]	PA [nmol h^{-1} g^{-1}]	POA [μmol h^{-1} g^{-1}]	NRA [μgN $24h^{-1}$ g^{-1}]	UA [μmol h^{-1} g^{-1}]
Before rooting of cuttings of *Euphorbia pulcherrima*					
Control (natural concentration of IAA)	6.13	1.34	8.06	0.29	9.36
Substrate+IAA (200 μg kg^{-1})	8.88	1.22	9.62	0.31	9.76
Substrate+IAA (300 μg kg^{-1})	7.23	2.59	9.20	0.40	9.12
Substrate+IAA (400 μg kg^{-1})	6.77	1.26	9.96	0.44	8.32
After rooting of cuttings of *Euphorbia pulcherrima*					
Control (natural concentration of IAA)	4.13	0.95	10.81	4.21	15.13
Substrate+IAA (200 μg kg^{-1})	5.85	1.01	12.99	5.04	16.65
Substrate+IAA (300 μg kg^{-1})	4.03	0.54	9.62	4.32	19.29
Substrate+IAA (400 μg kg^{-1})	5.41	0.59	13.11	5.51	16.81

XOA - xanthine oxidase activity, PA - peroxidase activity, POA - phenol oxidase activity, NRA - nitrate reductase activity, UA - urease activity

Peroxidase is important in soil and plant litter, as various types of these enzymes are released by fungi during the decomposition of lignin in soil organic matter. They are a group of oxidoreductases that catalyze the reduction of peroxides, such as hydrogen peroxide and the oxidation of a variety of organic and inorganic compounds [6]. Peroxidase activity was amounted from 1.22 to 2.59 nmol h^{-1} g^{-1} before rooting of cuttings of poinsettia and from 0.54 to 1.01 nmol h^{-1} g^{-1} after planting. The highest peroxidase activity was found at 300 μg kg^{-1} of IAA addition before cultivation of plants (table 2). Our investigations [28, 32] have documented higher peroxidase activity in peat from Kusowo bog (from 5.41 to 8.22 nmol h^{-1} g^{-1}) and fen of Stążka Mire (from 4.22 to 22.34 nmol h^{-1} g^{-1}) than in growing medium (from 0.54 to 2.59 nmol h^{-1} g^{-1}). However, different results were obtained for phenol oxidase, urease and nitrate reductase activity. Generally, higher activities were observed in peat substrates after rooting of cuttings of poinsettia. Phenol oxidase catalyzes polyphenol oxidation in the presence of oxygen (O_2) by removing phenolic hydrogen or hydrogenes from radicals or quinines. According to Yang et al. [33] the stimulation of enzyme activity can help plant root exudates. These authors reported that the source of microbial enzymes is underground parts of plants and soil fauna. Phenol oxidase activity was amounted from 8.06 to 9.96 μmol h^{-1} g^{-1} before cultivation of poinsettia and from 9.62 to 13.11 μmol h^{-1} g^{-1} after planting. The investigation of the distribution of phenol oxidase activity has shown an increase of its activity from natural concentration to 400 μg kg^{-1} of IAA additions before rooting of cuttings of plants in peat substrates, which was 18%. Our investigations [29] have documented higher phenol oxidase activity from Tagan peatlands (from 6.18 to 46.01 μmol h^{-1} g^{-1}) than in growing medium (from 8.06 to 13.11 μmol h^{-1} g^{-1}). Major pathways for nitrogen removal in soils include mineralization of nitrogen in organic compounds, ammonia volatilization, assimilation into biomass, adsorption of ammonium onto the substrate. Denitrification is one of the important causes of nitrogen loss in soil. Under anaerobic conditions, NO_3^- is reduced to NO_2^- by nitrate reductase [13]. This enzyme activity was ranged from 0.29 to 0.44 μgN $24h^{-1}$ g^{-1} before rooting of cuttings of poinsettia and the highest from 4.21 to 5.51 μgN $24h^{-1}$ g^{-1} after planting (table 2). Furthermore, higher nitrate reductase activity has been recorded at 200 and 400 μg kg^{-1} of IAA additions after cultivation of plants than other. Szajdak et al. [28, 32] observed higher nitrate reductase activity in fen of Stążka Mire (from 1.02 to 16.83 μgN $24h^{-1}$ g^{-1}) than in peat from Kusowo bog (from 0.10 to 0.18 μgN $24h^{-1}$ g^{-1}).The specific groups of bacteria, collectively known as rhizobia, induce the formation of root or stem nodules of leguminous plants and establish a nitrogen-fixing symbiosis. Besides, nitrogen fixation, ammonia can be obtained from the other metabolic processes such as urease production and ammonification [18]. Our research pointed out the highest urease activity with 200 μg kg^{-1} of IAA addition before rooting of cuttings of poinsettia (9.76 μmol h^{-1} g^{-1}) and with 300 μg kg^{-1} of IAA addition after planting (19.29 μmol h^{-1} g^{-1}) (table 2). Correspondingly, the concentrations of ammonium and nitrate ions were higher before rooting of cuttings of plants. Styła and Sawicka [24] evaluated activity of this enzyme in mineral soil under apple-tree cultivation. According to these authors, urease activity ranged from 1.78 to 3.35 μmol h^{-1} g^{-1} in this object. The results of Yang et al. [33] indicated that enzymes in rhizosphere soil play essential roles in soil processes such as nutrient cycling and energy transformation by catalyzing numerous chemical, physical and biological reactions. They are mainly exuded by roots and microorganisms and their activities can also have significant effects contributing to the changes of nutrients.Different doses of IAA added to the commercial growing medium for rooting of cuttings of 'Klasmann Steck Medium' had a significant effect on the tested characteristics of rooted cuttings of poinsettia 'Prestige Early Red' (table 3).

Table 3. The effect of commercial growing medium for rooting of cuttings of *Euphorbia pulcherrima*

Commercial growing medium	Assessment of root system [1-5 scale, according to EPPO]	Length of roots [cm]	Fresh matter of roots [g $plant^{-1}$]	Dry matter of roots [g $plant^{-1}$]	% of rooted cuttings
Control (natural concentration of IAA)	2.48 cd	4.70 abc	0.6179 abcd	0.0603 abc	97.5
Substrate + IAA (200 μg kg^{-1})	2.30 bc	5.30 bc	0.7530 abcde	0.0639 abcd	100.0
Substrate + IAA (300 μg kg^{-1})	2.05 ab	5.70 c	1.1904 ef	0.1198 bcde	100.0
Substrate + IAA (400 μg kg^{-1})	2.45 bc	7.90 d	1.3926 f	0.1269 cdef	92.5

Explanation: with columns, values followed by the same letter(s) are not significantly different at α = 0.05%

Most of the cuttings (100%) were rooted when the substrate was amended with IAA in the amounts of 200 and 300 µg kg^{-1}.

It also resulted preferably on the length of roots and their fresh and dry weight. However, the longest root and the highest fresh and dry weight of the roots were found when 400 µg kg^{-1} IAA was added to the substrate. Despite those significant differences, all poinsettia cuttings were characterized by good quality and were suitable for further cultivation independently on the content of IAA in the medium (fig. 1).

Figure 1. The effect of commercial substrate for rooting of cuttings of *Euphorbia pulcherrima* 'Prestige Early Red'. Explanations: **1/1** - natural IAA content, **1/2** - 200 µg kg^{-1} IAA, **1/3** - 300 µg kg^{-1} IAA, **1/4** - 400 µg kg^{-1} IAA with peat substrate

4. Conclusions

A distinct relationship among poinsettia and the index of soil chemicals represented by TOC, C_{HWE}, total nitrogen, nitrogen ions and IAA was observed. Our investigations have shown higher concentrations of TOC and C_{HWE}, total nitrogen and IAA after rooting of cuttings of poinsettia in substrate. It was corresponded to lower contents of ammonium and nitrate ions after cultivation of plants.

Data indicates that the mixture of substrate with IAA had an impact on chemical compounds and enzymes activity before and after rooting of cuttings of poinsettia. We observed slightly decrease content of ammonium ions between the mixture substrate with 200 and 400 µg kg^{-1} of IAA before planting. It was corresponded to the decrease in the concentration of ammonium ions between substrate with natural concentration and mixture with 400 µg kg^{-1} of IAA. Similar trend was shown for nitrate contents. Xanthine oxidase activity has shown a decrease of its activity when IAA was added to the substrate before rooting of cuttings of plants from 200 µg kg^{-1} to 400 µg kg^{-1} of IAA.

These investigations revealed higher activity of xanthine oxidase and peroxidase before rooting of cuttings of poinsettia. However, opposite phenomena related to phenol oxidase, urease and nitrate reductase activity occurred

The longest root and the highest fresh and dry weight of the roots were found when IAA was added to the substrate in an amount of 400 µg kg^{-1}.

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