Determination of auxine content of soft wood cutted 'Marianna GF8/1' (*Prunus cerasifera* x *P. munsoniana*) by High Performance Liquid Chromatography during rooting period

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Summary: The content of different auxins of soft-wood cutted plum rootstock 'Marianna GF8/1' (*Prunus cerasifera* x *P. munsoniana*) was determined during the rooting period. The level of auxin-concentration (exogenous and endogenous) of basic and internodal part of cuttings was determined by WATERS HPLC equipment every 7 days during rooting period. The lengths soft wood cuttings were app. 30 cm long. The basal part of shoots were treated with 2000 µg/g concentrated indole-butiryc acid in talcum powder. After treatment the cuttings were placed in propagation green-house under intermittent mist. The plant hormones were extracted by methanol the solution was cleaned by paper-filter, and further cleaned by centrifuge. The effluent was examined by reversed phase High Performance Liquid Chromatography, with WATERS 2487 dual detector at 220 nm on Symmetry C18 4,6×150 column. Recovery and reproducibility assessment indicates good accuracy and acceptable relative standard deviation (RSD) 5%. Linear responses (r20.997) for calibration curve was obtained with IAA, IPA and IBA standard in range, with a limit of quantification of 0.15 $g \cdot ml^{-1}$. The concentration of IAA, IPA and IBA in the basal part of cuttings were measured, during the rooting period. We proved the external IBA was taken up by the plants. In the plants were found the IBA, and the IAA concentration of IBA treated cuttings was higher, than the untreated one.

Key words: HPLC, plum rootstock, auxin, 'GF 8/1', rooting

Introduction

The propagation technique of rootstocks has important role in the fruit growing, because it is common to use grafted plants. The rootstock could be propagated by seeds, or one kind of vegetative propagation and the science will be budded or grafted on it. Nowadays, in the modern fruit growing, the vegetative propagation of the rootstock is spreading.

The part of vegetative propagation, as well the autovegetative propagation is used to produce true-to-type plant material. In the fruit nursery the new roots are generated on the stems.

The plant hormones have significant influence on plant vigour, growth and development of new organs, as like the roots formation too. The plant hormones are formed in different places. The cytocinins are in the top of the roots, and the auxins in the top of the shoots, and in the young leaves. In the horticultural praxis, in the case of vegetative propagation the most important group is the auxins, because the content of it could inducate the root formations too.

In the nursery practice the hard-wood cutting propagation method is a cheap and fast method to get true to type plum rootstocks (*Szecskó* et al., 2003). The plum rootstocks, like 'GF 8/1' can be propagated by hard-wood cutting and soft-wood cuttings too. The problem of plum rootstock was examined by many researchers (*Howard* and *Ridout*, 1994, *Reighard* et al., 1990), the 'Marianna GF 8/1' proved to be promising one in Hungary (*Hrotkó* et al., 2002).

To induce adventitious roots before using of synthetic root-promoting growth regulators in rooting stem cuttings, many chemicals were tried with limited success (*Kefford*, 1973). The discovery of natural auxins, such as indoleacetic acid (IAA), and synthetic one indolebutyric acid (IBA), could stimulate the promotion of adventitious roots on different part of plants. Hence auxins is not always the limiting chemical component in rooting (*Hartmann* et al. 1990).

The analysis of IAA, as plant growth factors mostly is difficult, because the low account of hormons, easy oxidation and the photodecomposition (*Archbold* and *Dennis*, 1984). The HPLC method is the highest sensitive possibility to determine the quality and quantity of plant hormones (*Rodriguez* and *Tames*, 1984). *Guinn* et al. (1986) used HPLC-method to determine abscisic acid and indole acetic acid concentration of young fruit cotton (*Gossypium hirsutum*) and leaves of grapefruit (*Citrus paradisi*), mulberry (*Morus alba*) and ash (*Fraxinus uhdei*). *Blazkova* et al. (1997) examined how can the IBA treatments exercise influence on root produce in young and mature clones of *Sequoia sempervirens*.

Materials and methods

1. Plant material:

The 'Marianna' plum originated in Texas, as an open-pollinated seedling of the myrobalan plum and supposedly, *P. munsoniana*. It can be propagated true to type by hardwood cuttings and softwood cuttings too. This variety could be rootstock of different kind of stone fruits. 'GF 8/1' mostly used as rootstock for plum, apricot, and partly peach. Some plum has grown well on it; others have not (*Hartmann* et al., 1990). The studied variety 'Marianna GF 8/1' was selected in France.

2. Propagation conditions:

The experiment was carried out in June, in the Research field of the Department of Fruit Science in Soroksár, when the mother plants shoot- length were 40–50 cm long. The 35–40 cm long, 3–4 mm thick shoots were collected in the morning, and the soft top of those were cutted, and the leaves on the basal part of shoots were removed too. The basal parts of the shoots were treated with 2000 µg/g indole-butiryc acid on talcum-powder.

The rooting media was composed by mixture of 30% sphagnum moss and 70% perlite. During the rooting period the cuttings were in a plastic-foil covered specialized propagation greenhouse equipped with automatic fog-generator. During the rooting period, the relative humidity was kept at 90%. The temperature of the house was 20–27 °C. The rooting period was taken 4 weeks.

The HPLC analysis was carried out every 3 days during the rooting period. The IAA and IPA content in the new collected, and in the rooting plants the IBA, IAA and IPA until the developing of well functioning roots we determined by HPLC. The different auxins content of cuttings was determined in the basic parts of cuttings (cca. 1 cm).

2. Analytical conditions:

Chemicals:

Analytical grade 3-Indoleacetic acid: IAA [87-51-4], Indole-3-propionic acid: IPA [830-96-6] and Indole-3-butyric acid: IBA [133-32-4], 2,6-Di-tert-butyl-p-cresol: BHT [128-37-0], ethanol, methanol and acetic-acid (HPLC-grade) were purchased from Sigma Aldrich Chemical Co. The double distilled water was further cleaned by Millipore-filter until the HPLC-grade. The standards of IAA, IPA and IBA were used in a methanolic stock solution (0.01 g/50 ml) and a 50X dilution of those were used as working standard in HPLC.

Sample preparation

The plants were collected, and cca. 1 cm long bottom and internodal parts were cutted from them. The weights were 2,7–4,5 g. The IAA, IPA, and IBA was extracted from the fresh plants with 10 ml ethanol in one day, added BHT in cool (4 °C), dark place to avoid the auxine degradation. The chlorophyll content was removed from the solution with petroleum-ether, three times. The solution of extraction was filtered on filter-paper, and the solvent was moved in HEIDOLPH rota-dest on 20 °C. The residue was redissolved onto 1 ml methanol. The solutions were taken onto the Eppendorf-tube, and centrifuged for 10 minutes on 15 000 rpm. The well centrifuged extraction was injected onto the HPLC.

HPLC conditions:

A WATERS High Performance Liquid Chromatograph equipped 2487 Dual Detector, and 1525 Binary HPLC Pump, controlled with BREEZE software. A SYMMETRY C18 5 µm 4.6 x 150 mm column was installed. Mobile phase methanol: water 60:40 v/v% containing 0.05% acetic acid. The flow rate 1 $cm^3 \cdot min^{-1}$, the pressure on the column was 1800±15 psi. The each injected volume was 20 µl.

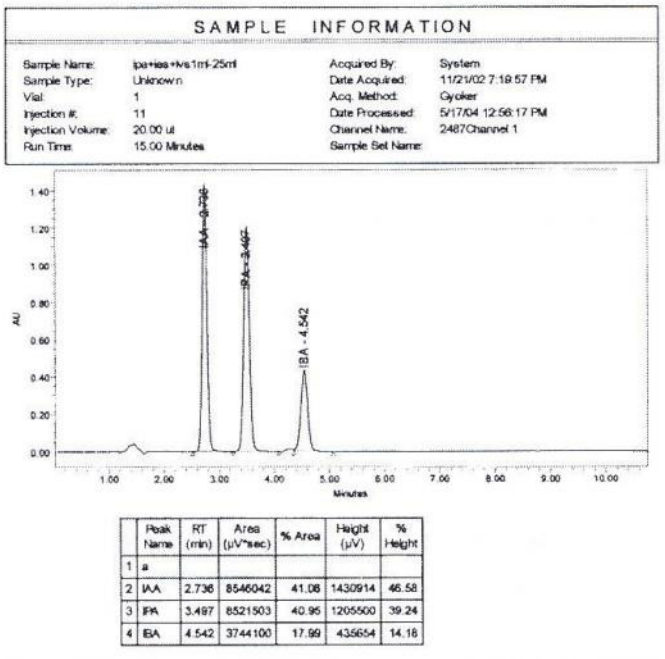
The plant hormones were monitored at a wavelength of 280 nm. The retention time of IAA in standard solution was 2.736, the IPA 3.497 and IBA 4.542 min.

Results and discussion

The aim of this work was to consider to which extent softwood-cuttings 'Marianna GF8/1' (*Prunus cerasifera* x *P. munsoniana*) are able to take up IBA from the root-promoting substance.

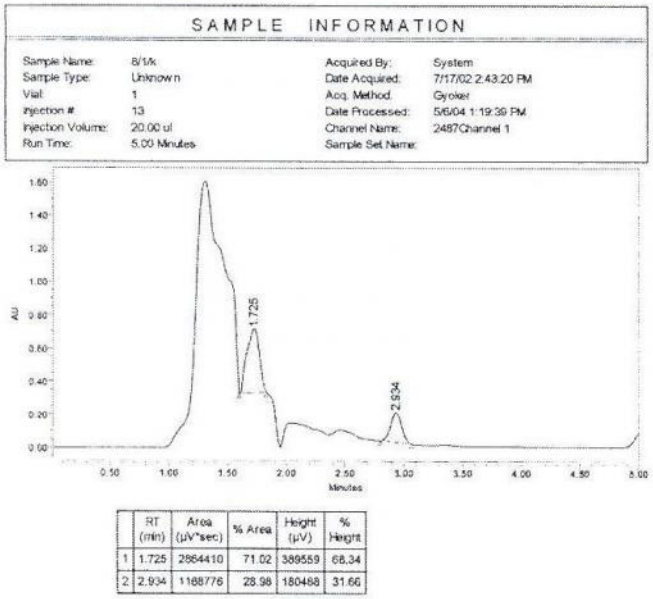
There were only few hints in relevant literature pertaining the auxin content of softwood-cutted plants.

Figure 1 shows the chromatogram of 20 µl injected mixture of IAA, IPA and IBA. The chromatogram is clear,



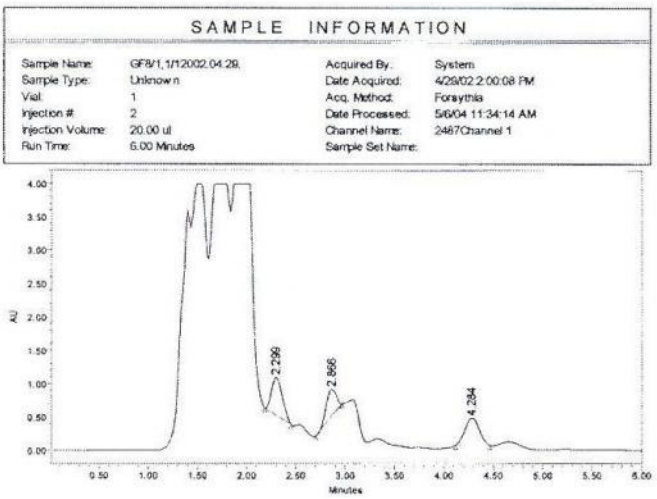
	Peak Name	RT (min)	Area (µV*sec)	% Area	Height (µV)	% Height
1	a					
2	IAA	2.736	8546042	41.06	1430914	46.58
3	IPA	3.497	8521503	40.95	1205500	39.24
4	IBA	4.542	3744100	17.99	435654	14.18

Figure 1 The characteristic HPLC chromatogram of IAA, IPA and IBA mixture standards



	RT (min)	Area (µV*sec)	% Area	Height (µV)	% Height
1	1.725	2864410	71.02	389559	68.34
2	2.934	1168776	28.98	180468	31.66

Figure 2 Characteristic chromatogram of untreated 'GF 8/1' cuttings



	RT (min)	Area (µV*sec)	% Area	Height (µV)	% Height
1	2.299	4354195	38.23	570938	41.09
2	2.866	2932214	25.75	408286	29.38
3	4.284	4101804	36.02	410277	29.53

Figure 3 Characteristic chromatogram of 2000 ppm IBA treated 'GF 8/1' cuttings

and the peaks show the retention time of different plant-hormones. On the *Figure 2* it can be see the chromatogram of untreated softwood-cuttings 'GF 8/1'. It shows the native IAA and IPA concentration in the plants. The *Figure 3* shows the chromatogram of 20 µl plant extract of IBA-treated cuttings. On the derived from 0,267 µg/g IBA content of plant. During the study of chromatograms, can be stated, the plants can take up IBA from the root-promoting substance. When we make a comparison between the chromatograms of treated and not treated cuttings, it can be see the IAA concentration of treated one is higher than the untreated one. The *Table 1* summarizes the different auxin (IAA, IPA and IBA) content measured on the 1st, 3rd, 6th ... etc., until 30th day. The RSD value of the determination was about 5% (n=5).

It can be stated, that the cuttings can take up the IBA. The IBA content until the 1st–6th day increases, following this on the 30th day significant decrease can be observed.

Composed the treated plants chromatogram and to the non treated one it can be ascertained that in the plants treated

Table 1 The tendency of plant hormone concentration during the rooting period

	Untreated (µg/g)			Treated (µg/g)		
Day	IAA	IPA	IBA	IAA	IPA	IBA
1	0,0345	0,031	–	0,0348	0,029	0
3	0,1002	0,044	–	0,101	0,046	0,133
6	0,1342	0,055	–	0,185	0,099	0,297
9	0,164	0,069	–	0,235	0,125	0,267
12	0,175	0,085	–	0,241	0,129	0,252
15	0,182	0,091	–	0,246	0,126	0,241
18	0,163	0,073	–	0,222	0,099	0,22
21	0,166	0,081	–	0,193	0,094	0,135
24	0,143	0,071	–	0,184	0,085	0,101
27	0,138	0,062	–	0,152	0,071	0,085
30	0,135	0,053	–	0,144	0,06	0,032

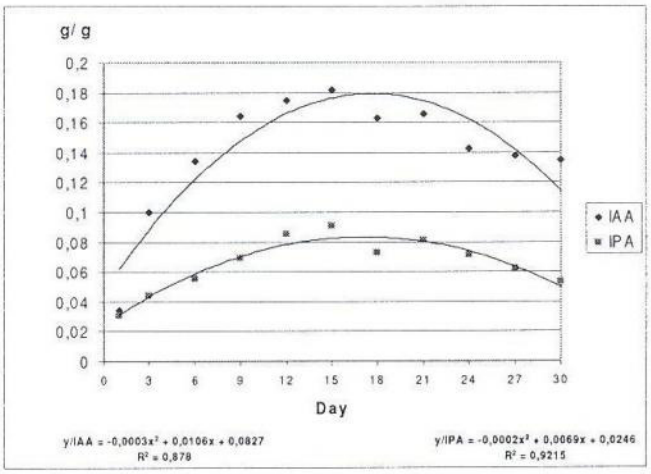


Figure 4 Changes of hormon content during the rooting-periode 'GF 8/1' cutting

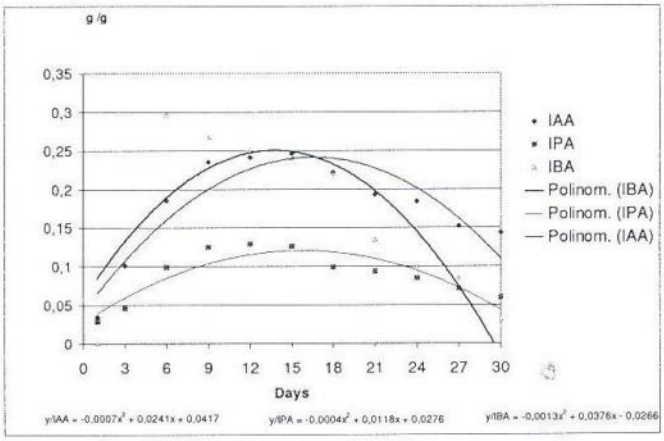


Figure 5 Changes of hormon content during he rooting-periode in IBA treated 'GF 8/1' cutting

with IBA the content of the native auxin is increased, so we could measure a higher concentration of IAA.

Our results lead us to believe that the synthetic auxin treated in exogenous way, helps to increase the concentration of the native auxin in the plant organism. In our opinion it can't be happened by a direct way, so the synthetic IBA surely not encourages the synthesis of the native auxins. Our measurements were accurate and it shows a higher level of the IAA content in the treated plants. From our experimental results, we have to conclude, that the explanation of the higher native auxin concentration can be the synthetic IBA, which links stronger to the auxin dissimilation enzymes, so the decomposition of the natural auxins slows down and this results a higher concentration and a higher activity. Another explanation for the higher IAA concentration in treated plants is also possibility: the level of the auxin decomposition enzymes does not grow dramatically, so the level of the native auxins is less reduced.

To understand a better for the problem, it's important to make carry out further experiments. This confirms the opinion of *Breen* and *Muraoka* (1974).

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