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ARTICLE

IBA uptake and metabolism of different type of plum rootstocks hardwood cuttings

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ABSTRACT The 50% alcoholic soluted 2000 ppm and 4000 ppm IBA (indole-3- butyric acid) uptake and the decomposition of IBA in the examined plum rootstock hardwood cuttings was determined by HPLC. 'INRA Marianna'GF 8-1', 'Myrabalan B', 'MY-KL-A', 'INRA Saint Julien GF 655/2', 'Fehér besztercei' plum rootstocks were used as plant material. The cuttings were collected on the 26 of October 2006, the basal parts IBA content was measured regularly by HPLC during the cold storage and rooting period. We found that all the cuttings was able to take up IBA and the 4000 ppm treatment cases double exogenous auxin level in the cuttings basal part parallel to the 2000 ppm. The uptake and decomposition of IBA by the myrobalan type 'MY-KL-A', 'GF 8/1' and 'Myrobalan B' rootstocks was the fastest, by others was slower, especially the 'GF 655/2'. In the half-life of IBA in the basal parts of the hardwood cuttings we found large differences. The half decomposition took only two weeks at the myrobalana type rootstocks while in case of 'Fehér besztercei' it was more than 60 days.
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KEY WORDS

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In the autovegetative propagation of plum rootstocks a type of synthetic auxin the IBA is widely used to get the highest rooting percentage. Most of the experiments made on the field of propagation by hardwood cuttings aimed to get the answer on the optimal concentration of the IBA to the root stimulation. Most of them concentrated on the outer environment of the cuttings during the rooting, because temperature, soil and the effects of IBA treatments on rooting percentage are parameters easy to investigate. The inner conditions of cuttings can be even so important for us, they can indicate the real processes in the cuttings. We wanted to get more information about the IBA uptake and metabolism, because it plays a main role in understanding the hormonal bases of the exogenously stimulated adventitious root formation.

The treatment with 2000 ppm indole-butyric acid gives the highest rooting percentage is accepted and used in the experiments in a wide range (Nahlawi and Howard 1972; Nahlawi and Howard 1973; Nicotra and. Damino 1975; Lemus 1987; Rana and Cadha 1992). But there is no recored in the optimal concentration. Swedan et al. 1993 found that the 1000ppm is the best for the Marianna rootstock. Kracikova 1996 reported that the 2500 ppm is the more effective; while in other resarches the 3000 ppm has been found to be the best (Kapetanovic et al. 1972; Fontanazza and Ruigini 1980; Rathore 1983; Sharma and Aier 1989). It is widely accepted that the treatment with IBA is usually necessary. It is also known that the too high concentration decreases the survival numbers of rootstocks. Szecskó et al. (2003) reported that generally the IBA treatment has no affect on the rooting of 'Marianna GF 8-1' plum. The IBA has also no effect on rooting reported Tofanelli et al. in 2001. The differences between the optimal treatments depend on a lot of things like the climate, the varieties, the weather and on other conditions may be on the uptake of the IBA treatment and metabolism. We have found no data about the IBA uptake in case of plum rootstocks, and about the decomposition of the IBA in the plum hardwood cuttings. The hormonal basis of adventitious root formation is not clear at all, many paradoxes can be found in the literature. That is the reason why more experimental data is needed in order to reveal this issue.

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Materials and Methods

Propagation conditions

Hard wood cuttings of 'INRA Marianna'GF 8-1', 'Myrabalan B', 'MY-KL-A', 'INRA Saint Julien GF 655/2', 'Fehér besztercei' plum rootstocks were used as plant material. The mother plants were grown in the Research and Experimental Farm of the Faculty in Soroksár. The length of the prepared cuttings was 15 cm, their diameter was between 7-12mm. On the basal part straight cuts were made about 0,5 cm below the bud, on the apical end a slanting cut were made about 1 cm above the last bud. After two hours, their basal parts were taken about 1 cm deep into the 2000 ppm, 4000 ppm IBA containing alcoholic solution. The cuttings were collected at 26 of October 2006. The hormone concentration of the basal

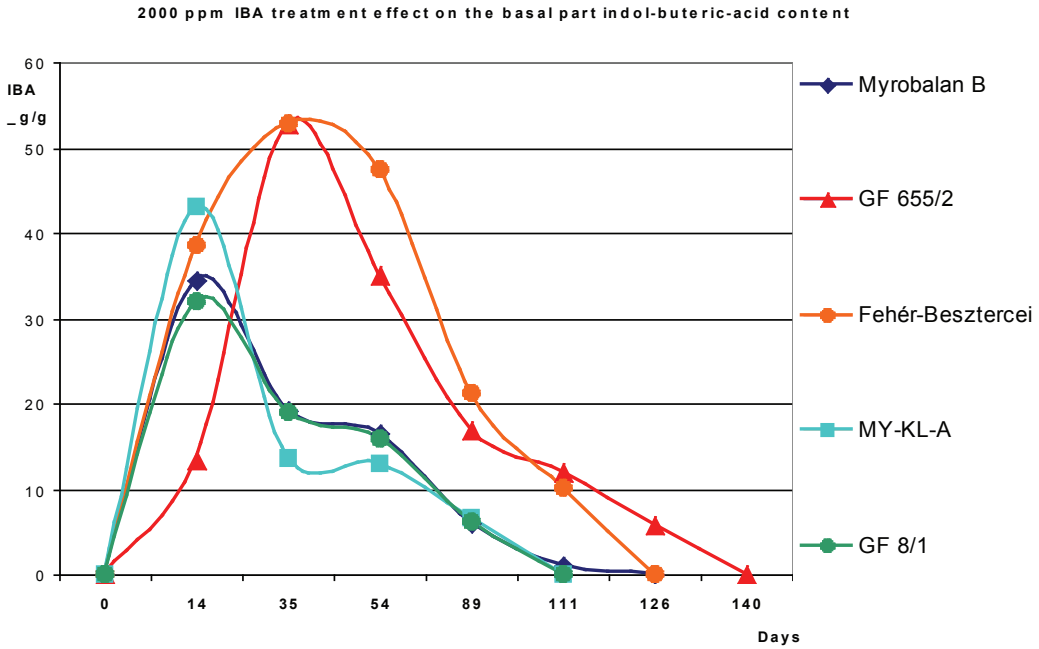

Figure 1. The uptake and decomposition time of indole-3-butyric acid in the basal parts of the different plum rootstocks varieties cuttings using 2000ppm IBA treatment.

parts of the cuttings was measured during the dormancy period every month, otherwise in every two weeks. The exact date's of samples preparing in the 2006, 2007 years was: Okt. 26., Nov. 08., Nov. 29., Dec. 18., Jan. 22. Febr. 13., Febr. 28, March 14. During the hard winter period the hormonal changes are not intensive because of the intensive dormancy, from our point of view, so there was not so much point in examining it too intensively. The cuttings was stored at 4°C in perlite in a coldroom. All the cuttings were planted out in the middle of March in Soroksár, in one long row using 5-8 cm distance.

IBA quantity in the basal parts of cuttings (cca. 1-1,5 cm), and in the 1-1,5 cm long part of the middle and upper part was determined till the beginning of May. After 14 of March we found no IBA in the cuttings.

Analytical conditions

Chemicals

Analytical grade IBA (indole-3-butyric acid) [133-32-4], methanol [67-56-1] and acetic-acid [64-19-7] (HPLC-grade) were purchased from Sigma Aldrich Chemical Co. The double distilled water was further cleaned by 0,45 µm Millipore-filter. IAA was used in a methanol stock solution (0.01 g/50 ml) and a 50X dilution of it was used as working standard in HPLC.

Sample preparation

The plants were collected, and approximately 1-1,5 cm long, basal parts were cut, and the extraction of them was analysed monthly during the winter dormancy otherwise in every two weeks during storage and every two weeks during the rooting period. Every sample was made from three cuttings, after they were cleaned from the perlite by washing all of them was destructed by hammer. The plant hormones and the exogenously added IBA was extracted in cool (4°C), dark place to avoid the IAA degradation with 5 ml 80% methanol in one day, added BHT (butylated-hydroxi-toluene) (Wyndaele et al 1985). To remove the chlorophyll content 500 µl solutions was transferred to Eppendorf-tubes and given 300 µl 4M NaOH. For the HPLC analysis we need to moderate the pH level under 7 so 100 µl acetic acid was also added and centrifuged for 5 minutes on 15.000 rpm. The well centrifuged extraction was filtered on 0,45 µm MILLEX-HN Syringe Driven Filter Unit and injected into the HPLC.

HPLC conditions

A WATERS High Performance Liquid Chromatograph equipped 2487 Dual Detector, and 1525 Binary HPLC Pump, controlled with EMPOWER™2 software were purchased from Waters Corporation (34 Maple street Milford MA 01757 USA). A SYMMETRY C18 5µm 4.6 x 150 mm column was installed. Mobile phase methanol: water 60:40 v/v% containing 0.5% glacial acetic acid. The flow rate 1 $cm^3 \cdot min^{-1}$, the pressure on the column was 2300±15 psi at ambient room temperature (Végvári and László 2004). Each injected volume was 20 µl, and the runing time take 6 minutes.

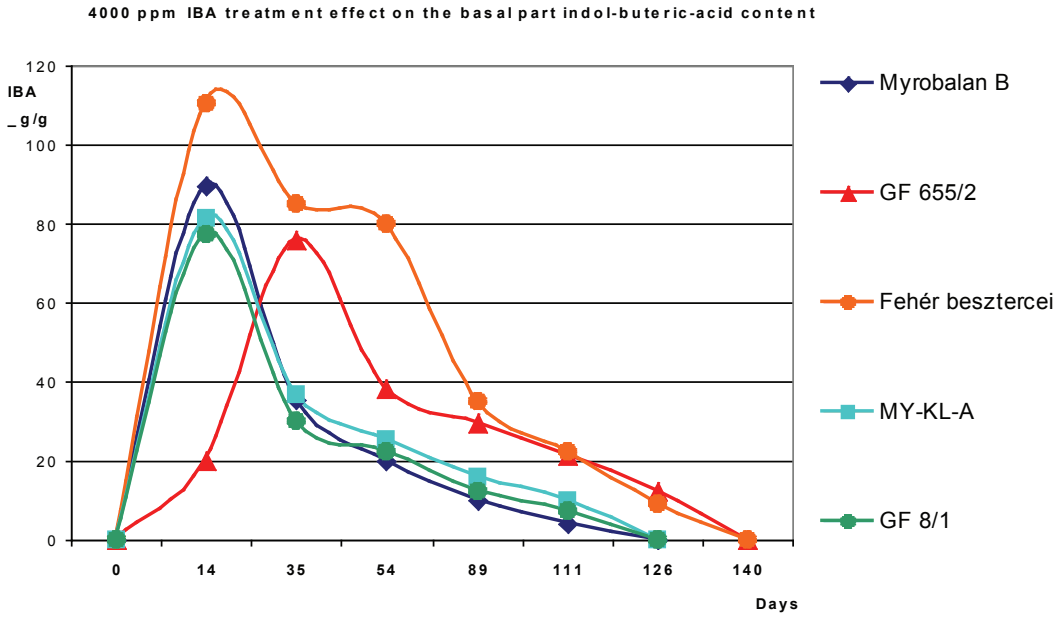

Figure 2. The uptake and decomposition time of indole-3-butyric acid in the basal parts of the different plum rootstocks varieties cuttings using 4000ppm IBA treatment.

The IBA was monitored at a wavelength of 280 nm. The retention time of IBA in standard solution was 4.4 min. The concentration was calculated in $\mu g \cdot g^{-1}$ in the fresh plant material.

Results

We have found that the *Prunus cerasifera* cv. myrabolana and its hybrids are able to take up IBA quickly; the maximum uptake was measured on the second week in case of 'Myrobalan B', 'MY-KL-A', 'GF 8/1'. The *Prunus insititia* Jusl. 'GF 655/2' has had a one-month long uptake. On the second week we could find only about the one-third of the maximum concentration of IBA, in our point of view the other part of it was only in the outside of the basal part of the cutting so it was removed by the washing. The maximum of IBA content in the cuttings was obtained two more weeks later, only after a monthof treatment.

The *Prunus domestica* L. cv. Fehér besztercei is an intermediate type, the 4000 ppm IBA treatment cased maximum IBA level in the basal part of the cuttings was measured on the second week, but the 2000 ppm was noticed only on the fourth week.

The shortest decomposition time the 89 days was noticed at the 2000 ppm IBA treated 'GF 8/1' Fig. 1. The longest was 126 days in case of GF 655/2 2000 and 4000ppm and 'Fehér besztercei' in the 4000 ppm treatments. Generally, the lower 2000 ppm IBA treatment had a shorter decomposition time.

The half life time of IBA content in the basal part of the cuttings was at the myrabolan type plum rootstocks the shortest it was only two weeks. In case of 'Fehér besztercei' this period was the longest it is more then 60 days, the 'GF 655/2' was about 30 days.

Discussions

We found that all the cuttings were able to take up the alcoholic solution of IBA. In case the myrabolan type rootstocks quick uptake and decomposition was noticed. The Fehér besztercei and even more the 'GF 655/2' hormonal transport and metabolism were slower. The more common differences between the types in aspect of root stimulation and adventitious root formation is the half-life time of the IBA in the cuttings. We found here very large differences. The retention time of myrabolan was about two weeks, whereas in case of the 'Fehér besztercei' it was more than 60 days.

The 4000 ppm treatment causes double uptake value compared to the 2000 ppm. We have found in case of the examined rootstocks no block in the IBA uptake so differences in the adventitious root stimulation and rooting percentage can really depend on the concentration of the treatments.

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