

THE INFLUENCE OF GROWTH REGULATORS ON ROOT INDUCTION *IN VITRO* OF THE *MUSA* GENUS

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Abstract

The influence of growth regulators on the root induction of the *Musa* genus plants cultivated within *in vitro* conditions has been compared. The plant material used was the 'Cavendish' cultivar. Different concentrations of growth regulators (naphthaleneacetic acid, indole-3-acetic acid, 6-benzylaminopurine, 2,4-dichlorophenoxyacetic acid) has been used and as control ones MS and half concentrated MS media without addition of growth regulators. The induced roots were evaluated in conditions *in vitro* and *ex vitro*. The amount and length of the roots were evaluated, as well as the capacity of absorption of the roots by conductivity was determined. The experiments have proven that the most roots are created by using naphthaleneacetic acid (5.4 μM), but the longest roots provide the control variant (MS medium). After 7 weeks of the transfer to *ex vitro* conditions the plants that were growing on medium with addition indole-3-acetic acid have the best vitality and root absorption.

Key words: banana, Cavendish, conductivity, micropropagation, root evaluation.

INTRODUCTION

The majority of bananas planted for the fruit production is seedless, therefore they are reproducing mostly vegetatively using sprouts or chopped rootstocks with eyelets. However, these types do not allow to prepare sufficient amount of suitable vegetal material in case, when the new breed shall replace the old one or in case when it is necessary to propagate enough plants of the new resistant cultivars for the field testing purposes and its spreading. These causes have been an impulse for the banana micropropagation technologies development, that is effective way of propagating of large amount of genetically identical entities in aseptical *in vitro* conditions. The average rate of shoot formation produced by this technique are 4–5 shoot per monthly subculture (Priyono, 2001). Naturally, banana has high potency to produce large number of small corms. Thus the *in vitro* technique for inducing cormlet initiation followed by its development to form micro sucker is prospective method to improve the commercialised of banana *in vitro* technique (Priyono, 2001). Even though many authors have already been dedicated to the problematics of the *in vitro* banana growing technologies (Roels et al., 2005; Albany et al., 2005; Assani et al., 2003; Bajaj, 1995); Cronauer and Krikorian, 1984; Hwang et al., 1984) and to the optimisation of separate phases of micropropagation to speed up its proceeding, there is still space remaining for certain improvements and comparisons of different variants and solutions are being offered by individual authors. In the presented work the suitability of media

containing various concentrations of growth regulators (auxins and cytokinins) is being tested for its influence on root induction of the banana cultivated *in vitro* and, root quality that enables successful transfer to *ex vitro* conditions.

MATERIAL AND METHODS

Plant material

Triploid cultivar of the Cavendish group was used. Triploid of the type acuminata with AAA constitution, significantly spread species bringing huge yields of quality fruit bananas, is distinguished with increased resistance against colder climatic circumstances, is panama disease resistant, but sigatoca disease susceptible (Pospisil and Hrachova, 1990). The group AAA in comparison to planteins (group AAB) many well-developed suckers, in condition *in vitro*, the micropropagation rate is 3 to 5 fold every multiplication cycle (Roels et al., 2005).

Establishment phase

The explants were before the transfer to *in vitro* conditions surface-sterilized for 1 min in 70% ethanol, 23 min in 1% solution of NaClO with a few drops of Tween 20. They were rinsed then tree times with sterile destiled water and transferred to proliferation medium (Cronauer and Krikorian, 1984) into 50 ml flasks filled with 25 ml of medium.

Proliferation phase

After 28 days the developing shoots were subcultured

at least four times on the same medium before use as a starting material for the experiments.

METHODOLOGY

Media and culture condition for root induction

The experiment testing the suitability of the media for root induction of the banana was initiated with following variants: 1) MS (Murashige and Skoog, 1962) 2) ½ MS; 3) MS + 5.4 µM NAA (naphthaleneacetic acid) + 13.3 µM BA (6-benzylaminopurine); 4) MS + 2.3 µM 2,4-D (2,4-dichlorophenoxyacetic acid); 5) MS + 4.6 µM 2,4-D; 6) MS + 2.7 µM NAA; 7) MS + 5.4 µM NAA; 8) MS + 2.9 µM IAA (indole-3-acetic acid); 9) MS + 5.7 µM IAA; 10) MS + 8.7 µM IAA; 11) MS + 11.6 µM IAA; 12) MS + 14.5 µM IAA; 13) MS + 17.4 µM IAA.

Vegetative explantates were cultivated at $23 \pm 2^\circ\text{C}$ under cool white fluorescent tubes (NARVA LT 36 W/010) providing a photosynthetic active radiation of $30 \mu\text{mol. m}^{-2} \cdot \text{s}^{-1}$ with a photoperiod of 16 hours.

Plant transfer to ex vitro condition

After 30 days of cultivation in conditions *in vitro* the plants were transferred to semi-sterile substrate (mixture of soil and perlite in ratio 3:1). The temperature during the cultivation in *ex vitro* conditions (greenhouse conditions) was reaching on average 21.5°C during the day and 18.0°C during the night. At the beginning the plantlets were covered with PP film (polypropylene film) to maintain high RH (relative humidity).

Root evaluation, statistics

The formation of roots by individual variants has been observed for 28 days in regular intervals of 7 days. The amount and length of the roots have been registered. To complete the experiment, the formation of shoots has also been observed. The growth of the roots (that is the number and length of roots) by different plants has been processed by statistic program STATISTICA 6.0 CZ (statistica base cz) with level of significance of $\alpha = 0.05$. The average values of partial parameters in the single variants were entered into the Table 1. Any contaminated cultures were deleted from the experiment. The success of transfer to *ex vitro* conditions was evaluated in percentage.

Measurement of absorption strength of the root by conductivity

The root adsorption strength was tested using the conductivity. After 4 weeks of cultivation of the banana in the substrate the plants were taken out again, the roots rinsed with water and compared with the status in the beginning of *ex vitro* transfer. The experiments were held during 10 days. The principle of the testing, which has to compare the different adsorption capacity of the root, is in the decrease of ions dissolved in the ½ MS solution (without sugar and agar) during cultivation

of single banana plants in this solution. To compare the adsorption power of the roots, created on the control medium MS, medium MS with addition of IAA at the concentration of 5.7 µM and medium MS with 5.4 µM of NAA, a trial based on conductivity of solution has been proposed. The three tested variants were chosen as representative according to the growth regulator used and also its amount (i.e. 1 mg. l^{-1}). Specific conductivity was measured on conductometer in Lab, Cond Level 1 under constant temperature of 21°C . Conductometer measures the resistance of given solution and gives the final value in $\mu\text{S.cm}^{-1}$ to mS.m^{-1} .

RESULTS

From the results obtained in the statistical analysis emerges that the largest number of roots has been found with the variant 5.4 µM NAA (14.79 roots per plant). The variants 2.3 µM and 4.6 µM 2,4-D did not create any roots at all (Table 1). Evidential differences were not found among variants with addition of IAA at concentrations of 5.7 µM, 11.6 µM and 14.5 µM and also among variants 2.9 µM IAA and ½ MS. The biggest average length of the roots has been measured with variant MS (36.54 mm), Table 1. The differences in length of the roots have not been evidential among variants 8.7 µM IAA, 14.5 µM IAA and 5.4 µM NAA. The Figure 1 shows visibly the indirect proportion between average length and number of the roots created within the framework of variants. This trend is mostly visible among variants ½ MS, MS and medium containing NAA. The roots created at the control media MS and ½ MS were in comparison with other variants longer (avg. length 35.81 mm), but their number was lower (avg. 8.77 roots; Figure 2a). Roots created on the media with addition of IAA were similar to those on MS medium (Figure 2b). On the other hand the roots created on the media supplemented with NAA were rather short (avg. length 23.31 mm), but their number was in comparison with other variants high (avg. amount of 14.00 roots; Figure 2c).

The comparison of variants NAA (5.4 µM) + BA (13.3 µM) with variant NAA (5.4 µM) is also worth mentioning. Whilst the average length of the root with variant 5.4 µM NAA was 21.99 mm and the amount was 14.79 roots, the variant NAA + BA created on average only 2.20 roots with average length of 8.49 mm. Using the same concentration of NAA the variant NAA + BA created only 14.87% of the roots of variant NAA and the roots reached 38.61% of the length of 5.4 µM NAA variant. The addition of BA at the concentration of 13.3 µM in the medium with 5.4 µM NAA has suppressed the creation of adventive roots of 85.13% and average length of the root of 61.39%. Nevertheless the creation of sprouts has been increased by 70.02%.

Media with NAA + BA addition, which has created a great number of shoots and small number of roots; were

more difficult to induce root and 37% did not survive the transfer into *ex vitro* conditions. While this variant was highly successful in conditions *in vitro*, the most of the plants perished in conditions *ex vitro* out of all the compared variants that created roots.

Variants with the addition of IAA had the highest survival percentage (90%), followed by variants with NAA (78%). All the transferred plants of variant 2.4-D perished.

The results of evaluation of roots by the means of conductivity

Variants labelled "I" were tested on conductivity immediately after the end of 4-week cultivation *in vitro* conditions labelled "II" were kept another 4 weeks in substrate (*ex vitro*). When the plants were taken out of substrate, their rootage, created already *in vitro* conditions, seemed to be still operational.

Plants tested immediately after the transfer from *in vitro* conditions had generally lower sucking power (expressed by the average fusion volume shortage – 30 ml) in comparison with the plants cultivated in *ex vitro* conditions (63 ml). The difference was more than double (Table 2).

Roots created on the medium with addition of IAA kept approximately the same sucking power (measured by the fusion volume decrease), done either out of *in vitro* conditions (decrease by 29 ml) or after 4 week cultivation in substrate (decrease by 35 ml). While in the first case roots showed the average performance in comparison with the other variants, in the second case suction capacity was below standard. After 7 weeks of cultivation in the soil new functional roots started to appear and they replaced the older ones created in conditions *in vitro*, which allowed a fluid nutrition and the plants of this variant at the end of the experiment had the best growth.

Roots created on the medium with addition of NAA had really small absorbing power after the transition into *in vitro* conditions (decrease of fusion by 23 ml). These roots though adapted on soil conditions really fast and their suction capacity became the highest out of all the tested variants (decrease by 83 ml). After next 3 weeks of cultivation all the roots die out and the plants desisted in growth.

MS medium gave rise to roots with good suction capacity, which increased proportionally with the age of the plant and the transition into the *ex vitro* conditions (MS I recorded the decrease by 39 ml, MS II 71 ml). After 7 weeks in conditions *ex vitro* new roots began to replace the old ones like in variant IAA.

Plants tested immediately after the transition from *in vitro* conditions had on average lower sucking power of the roots - expressed by the conductivity of the solution – out of original 2.77 mS.cm⁻¹ to 2.39 mS.cm⁻¹ in comparison with the plants cultivated consequently in *ex vitro* conditions (out of original 2.77 mS.cm⁻¹ to 1.84 mS.cm⁻¹), Table 2.

DISCUSSION

The experiments showed that medium with the supplement of synthetic auxin 2.4-D is absolutely unsuitable for the optimization of the rootage. Even though Prochazka and Sebanek (1997) mention that its low concentrations stimulate the production of roots and very often induce the production of the callus, neither of these presumptions was not confirmed. Not even the medium with the supplement of auxin and cytokinin, as recommended by Prochazka and Sebanek (1997) for the production of adventitious root does not appear suitable for the rootage of the banana. The above mentioned medium would be more suitable for the proliferation phase of micropropagation, where the main goal is to get as high number of reproduction propagules. Control media MS (without growing regulators) and ½ MS, as recommended by Pocasangre (1993) reveals to be efficient. Another tested medium with the supplement of NAA was published by Cronaur and Krikorian (1984), Ganapathi et al. (1995), pointed out on the ability of NAA to induce the root formation. Banana plants created on the medium with NAA had different rootage in comparison with the others regulators – there were more roots and were shorter. Roots were growing in several waves. Using media with the supplement of IAA and NAA and MS medium without addition of growth regulators roots really started creating after 5–7 days. On medium with the supplement of NAA and BA the roots started appearing the 12th day.

In accessible publications there is no data that informs on the functionality of the roots induced *in vitro*. Our results demonstrated that the induced roots maintain their functionality after the transfer to *ex vitro* conditions. After 4 week cultivation of the bananas in conditions *ex vitro* the greatest absorption has the roots induced on the medium with the addition of NAA. After next 3 weeks these roots die out. The best variant is therefore medium with the addition of IAA where the older roots are gradually replaced with the new ones.

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Tab. 1: Phenotype of plants after 30 days of cultivation

| Variation | Roots average per plant | Roots length average (mm) | Shoots quantity average per plant | Roots quantity average per shoot | Leaves average per plant |
|---------------------------------------|-------------------------|---------------------------|-----------------------------------|----------------------------------|--------------------------|
| 1/2 MS | 8.43±1.36 | 35.07±8.81 | 1.58±0.64 | 1.11±0.39 | 3.88±0.82 |
| MS | 9.10±1.21 | 36.54±9.11 | 2.07±0.59 | 1.26±0.45 | 4.47±0.93 |
| NAA (5.4 μ M) + BA (13.3 μ M) | 2.20±0.87 | 8.49±2.65 | 4.57±1.01 | 0.27±0.52 | 3.33±0.51 |
| 2.4-D (2.3 μ M) | 0.00 | 0.00 | 0.00 | 0.00 | 1.73±0.36 |
| 2.4-D (4.6 μ M) | 0.00 | 0.00 | 0.00 | 0.00 | 2.00±0.23 |
| NAA (2.7 μ M) | 13.20±1.32 | 24.62±5.04 | 3.73±0.88 | 1.10±0.54 | 3.72±0.77 |
| NAA (5.4 μ M) | 14.79±2.36 | 21.99±6.29 | 1.37±0.47 | 2.78±0.82 | 4.00±0.64 |
| IAA (2.9 μ M) | 8.97±0.98 | 28.53±7.45 | 1.67±0.87 | 1.52±0.78 | 4.60±0.87 |
| IAA (5.7 μ M) | 9.73±1.35 | 24.16±7.66 | 1.50±0.61 | 0.88±0.79 | 4.13±0.76 |
| IAA (8.7 μ M) | 11.05±2.02 | 22.71±6.84 | 1.85±0.79 | 1.59±0.85 | 3.25±0.84 |
| IAA (11.6 μ M) | 10.80±1.83 | 24.55±5.21 | 1.55±0.58 | 2.45±0.96 | 3.10±0.36 |
| IAA (14.5 μ M) | 10.85±1.33 | 22.33±6.33 | 1.15±0.21 | 2.74±1.15 | 3.32±0.57 |
| IAA (17.4 μ M) | 9.80±1.12 | 24.34±7.96 | 1.00±0.12 | 3.20±0.74 | 2.95±0.33 |

Tab. 2: Volume rate and conductivity value in selected variables

| Evaluation | date/days | IAA I | IAA II | NAA I | NAAIL | MS I | MS II |
|--------------------------------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| Volume (ml) | 1. day | 145 | 145 | 145 | 145 | 145 | 145 |
| | 7. day | 122±5.7 | 114±7.2 | 126±9.1 | 88±5.3 | 114±7.9 | 90±6.4 |
| | 10. day | 116±6.9 | 110±9.3 | 122±11.6 | 62±8.5 | 106±10.5 | 74±8.7 |
| Conductivity (mS. cm ⁻¹) | 1. day | 2.77 | 2.77 | 2.77 | 2.77 | 2.77 | 2.77 |
| | 7. day | 2.78±0.01 | 2.81±0.01 | 3.01±0.02 | 2.96±0.01 | 2.91±0.01 | 2.93±0.02 |
| | 10. day | 2.82±0.02 | 2.90±0.01 | 3.18±0.02 | 3.47±0.02 | 3.08±0.02 | 3.27±0.02 |
| | 10. day* | 2.29±0.02 | 2.20±0.02 | 2.64±0.03 | 1.57±0.02 | 2.25±0.02 | 1.75±0.03 |

Note: 10. day* = conductivity values of the measured solution the tenth day after adding the missing volume at the original of 145 ml
 The solution temperature during the conductivity measurement was 21°C.
 The solution evaporation (except for the leaves) during the experiment was stipulated in 10%.
 Maximum loss of rots during the test 5%.

Fig. 1: Comparison of average root length and quantity of roots in different variants

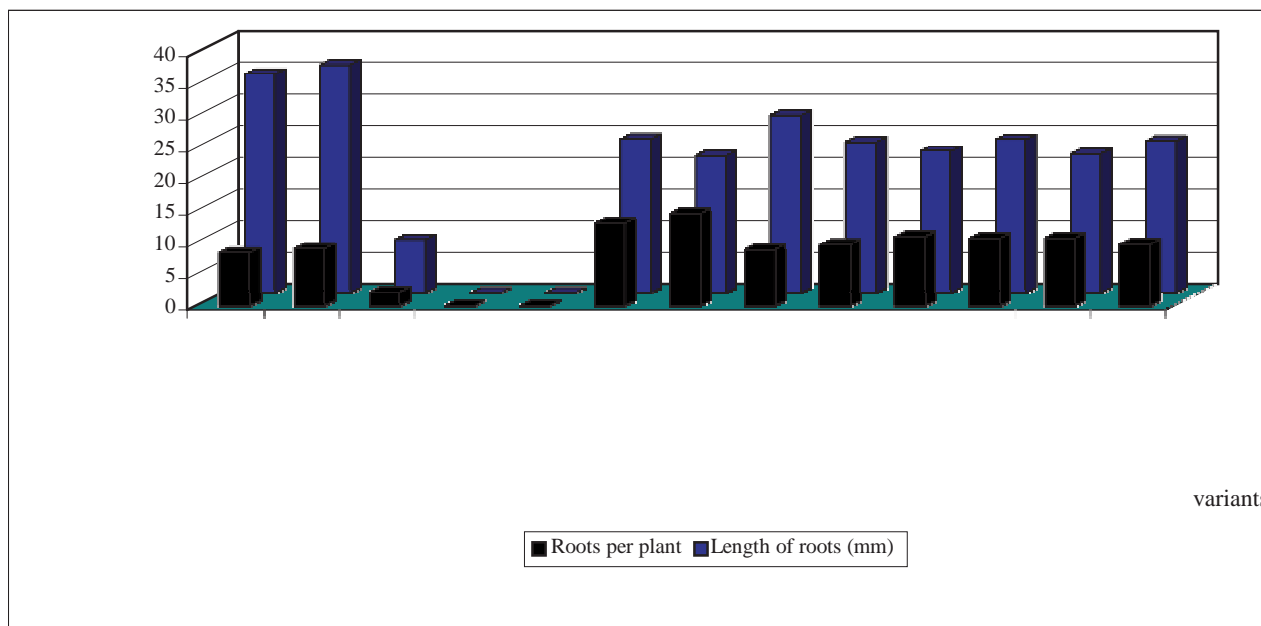
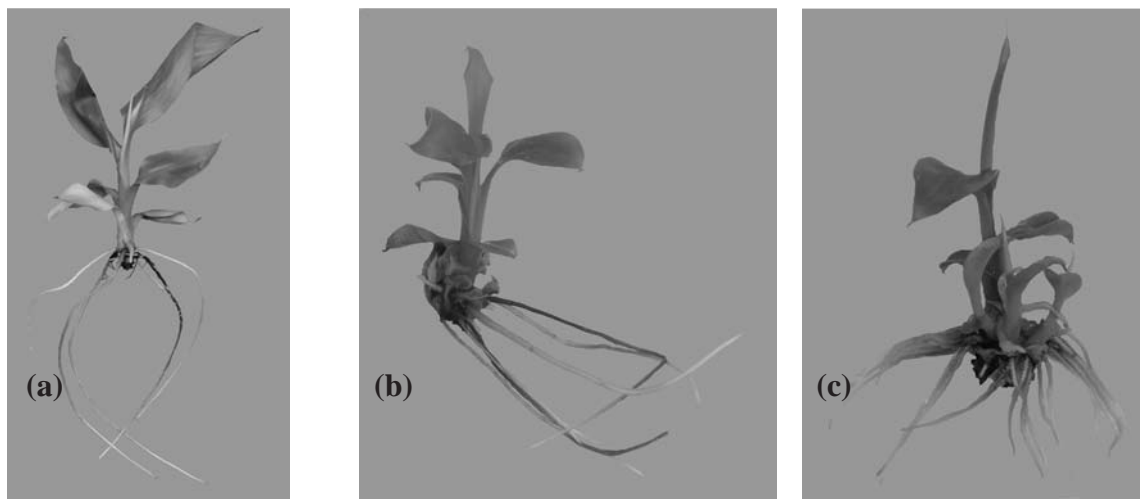


Fig. 2: Root formation on MS medium without growth regulators (control variant) – a) and with the addition of 8.7 μ M IAA – b) and 5.4 μ M NAA – c) after 4 weeks of cultivation in conditions *in vitro*. Significantly different roots are created on the medium with NAA, they grow up in several waves in radial direction and they are markedly shorter and thicker.



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