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THE EFFECT OF CYTOKININS AND AUXINS IN ROSA HYBRIDA 'SOPHIA LOREN' L. IN VITRO CULTURES

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Abstract
During in vitro propagation of 'Sophia Loren' rose variety, the effect of different cytokinins and auxins were examined, in order to find optimal type and concentration. BAR resulted the best shoot production, and KIN was optimal for shoot elongation and leaf length enhancement. Primarily, auxins stimulated root development, and better results (higher rooting percentage, more and longer roots) achieved when IBA was applied, this auxin was more effective than NAA.

1. Introduction

Commercially, roses usually propagated by cutting, however, budding and grafting can be also done, but these procedures are more difficult [5], and sometimes, environmental conditions (precipitation, soil etc.) are not optimal or the multiplication rate was not acceptable during conventional propagation [12]. In vitro propagation techniques are more expensive but significantly more pathogen-free, genetically uniform plantlets can be produced in shorter time and round the year. That is why, micropropagation is an alternative way of rose multiplication which is becoming more and more popular [9].

Usually, researchers wanted to find the optimal type and/or concentration of certain accessories of the culture media. In order to prevent *Rosa hybrida* 'Baby Love' *in vitro* plants' abnormal leaf abscission, silver nanoparticles in 2 mg/l was the best [4]. Silver nitrate at 100 mg/l dose resulted the longest shoots and greatest leaf number of *R. canina* [15]. The addition of silver nitrate also efficiently improved the ratio of shoot regeneration in three *R. hybrida* genotypes, and the highest bud development and shoot elongation were obtained in the case of 1.0-2.0 mg/l KIN, but BA was not optimal [6]. The latter growth regulator in 1.0 mg/l concentration resulted the maximum number, length and weight of shoots of *R. centifolia* and *R*. 'Gruss an Teplitz' [3]. 30-70 mg/l silver nitrate proved to be effective (with a combination of 1 mg/l BA and 0.5 mg/l IBA) for elimination of *R. indica* leaf yellowing [13]. For *R. hybrida* 'Al-Taif Rose' plants' shoot production, 1.0 mg/l BA + 1.0 mg/l KIN was optimal [2], and for *R. indica*, 1 mg/l BA + 0.5 mg/l KIN [16]. The same PGR combination but with tripled BA, doubled KIN (3.0 mg/l BA + 1.0 mg/l KIN) was efficient for 'Red Masterpiece', furthermore, this variety developed in vitro flowers on medium containing 2 mg/l BA [7]. In the case of *R. damascena*, 4.0 mg/l BA + 0.25 mg/l IAA + 0.2 mg/l gibberellic acid resulted more and longer shoots [11].

For rooting (the next step after *in vitro* shoot multiplication and elongation), mainly growth regulator-free, usually Murashige and Skoog [10] MS-medium was the best for different *R. hybrida* varieties' rooting [6, 7, 13]. The use of cobalt nanoparticles (in 4.65 µg/l) increased root number and root length of *R. hybrida* 'Baby Love' [4]. Other roses, such as *R. centifolia*, R. 'Gruss an Teplitz' developed the most and longest roots in the highest percentage on medium with 0.5 mg/l IBA [3], and *R. hybrida* 'Al-Taif Rose' when 2.0 mg/l IBA was added [2]. Other auxins such as NAA (0.1 mg/l)

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given the best rooting of *R. hybrida* 'Landora' [14]. Both IBA and NAA successfully stimulate rooting of *R. persica*, but there was not significant difference among the results according to the type and dose of auxins [8].

In this trial, during in vitro shoot multiplication and root development of *R. hybrida* 'Sophia Loren', the aim was to find the best type and concentration of cytokinins and auxins.

2. Material and methods

In order to collect averagely 12-14 mm sized shoots for the trial, an *in vitro* culture of tea hybrid *Rosa hybrida* 'Sophia Loren' (originated from an old mother plant aged more than 15 years, located in a private garden) maintained in the laboratory of the Department of Floriculture and Dendrology, Hungarian University of Agriculture and Life Sciences. As in vitro multiplication and rooting, shrubby plant clumps were divided into separate shoots and transferred them onto Murashige and Skoog [10] basic media with 20 g/l sucrose and 6.5 g/l agar (one shoot per 250 ml sized Erlenmeyer flask). I separated three main groups according to the cytokinins, auxins and a control with no plant growth regulators (PGRs). As cytokinins, 6-benzylaminopurin (BA), 6-benzylaminopurin riboside (BAR), kinetin (KIN), meta-topolin (MT) in four (0.25-0.5-1.0-2.0 mg/l) doses, and as auxins, indole-3-butyric acid (IBA), 1- naphthaleneacetic acid (NAA) was also applied in four (0.1-0.25-0.5-1.0 mg/l) concentrations. Each culturing (in vitro multiplication and rooting) was replicated twice.

The plants (30 specimens per every media) were grown under 16-hour illumination with cold and warm white fluorescent lamps (Polylux XL_R FT8/30W/830 and 860, USA), at 20-25 °C. After 2 months, shoot number and length, leaf length, rooting ratio, root number and length examined. Additionally, for determination of leaf total (a+b) chlorophyll content, I collected 3 x 100 mg leaves from each culture group. After leaf homogenization (in a mortar with the use of quartz sand and 80% acetone) and 24 hour refrigeration on +4 °C, absorbance of the suspension was analysed by Genesys 10vis (Thermo Fisher Scientific Inc., USA) spectrophotometer at 644 and 663 nm wavelength. In order to calculate leaf pigment level, I used the following formula (1):

$$\text{Total (a+b) chlorophyll content (µg/g)} = (20.2 \times A644 + 8.02 \times A663) \times V/w \quad (1)$$

V= volume of tissue extract (10 ml), w= fresh weight of tissue (0.1 g), A= absorbance [1]

All data evaluated by the application of SPSS 23.0 (IBM Corp., USA) software and analysis of variance (ANOVA) was conducted to determine the statistical significance between the groups. In cases of significant differences, the means separated by Tukey's test at $p \leq 0.05$.

3. Results

3.1. The effects of cytokinins

Shoot number, shoot and leaf length

Among the various cytokinins, higher (1.0 and 2.0 mg/l) BA concentration resulted the most (4.13 and 5.13) shoots, with a significant difference in the majority of cases compared to the other groups. Only KIN did not stimulate shoot formation (usually less than 1.2 pieces per plant), anyway, in the case of the other three cytokinins, increasing the dose enhanced shoot development.

The length of the shoots was opposite to their number, because 0.5 and 1.0 mg/l KIN resulted significantly the longest shoots with values 19.29 and 22.33 mm (only the latter exceeded the average of 19.92 mm on the control group), and the shortest shoots (12.2 and 9.46 mm) at 1.0 and 2.0 mg/l BAR. In the case of BA and KIN, it increased with higher concentration (up to 1.0 mg/l), while when MT or BAR was used, shoot length did not change or decrease.

Higher cytokinin doses usually shortened the leaves. An exception to this was only the KIN, which effected significantly longer (in all KIN concentrations, longer than 20 mm) leaves compared to the other cytokinins and the control, but here again only up to a dose of 1.0 mg/l. The highest BAR concentration (2.0 mg/l) resulted considerably the lowest value (8.83 mm), not only in terms of shoot length, but also in the case of leaf length (**Figure 1**).

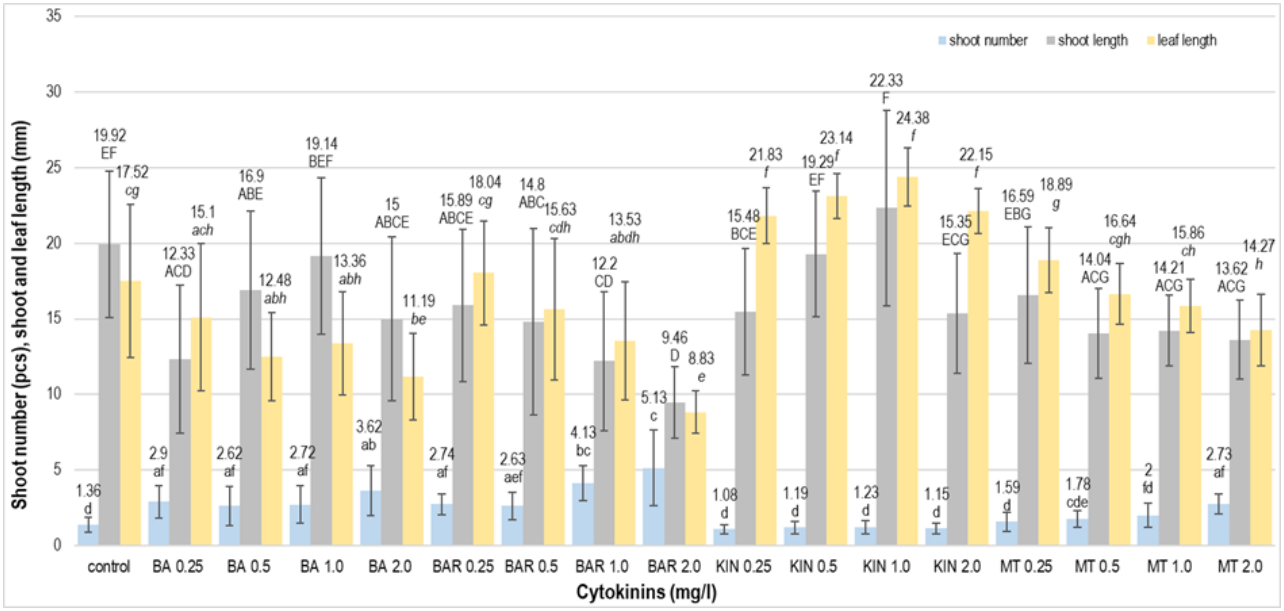

Figure 1: The effect of cytokinins on shoot number, shoot and leaf length of R. hybrida 'Sophia Loren'

Figure 2 illustrates the different effect of various cytokinins on shoot formation at the same concentration (2.0 mg/l).

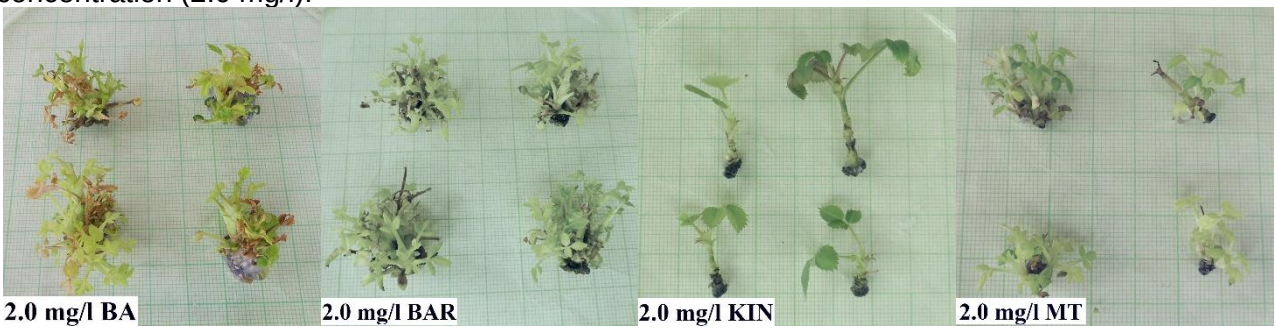

Figure 2: Shoot development on media with different cytokinins in the same (2 mg/l) concentration

Leaf chlorophyll content

None of the cytokinins increased the leaves' chlorophyll content compared to the control (which resulted an average of over 3000 µg/l), the pigment values of all treated groups were significantly lower. Nevertheless, KIN significantly enhanced chlorophyll levels (in all concentrations: above 2000 µg/l) and especially the highest (1.0 and/or 2.0 mg/l) doses of BA, BAR, MT decreased the amount of this leaf physiological characteristic (**Figure 3**).

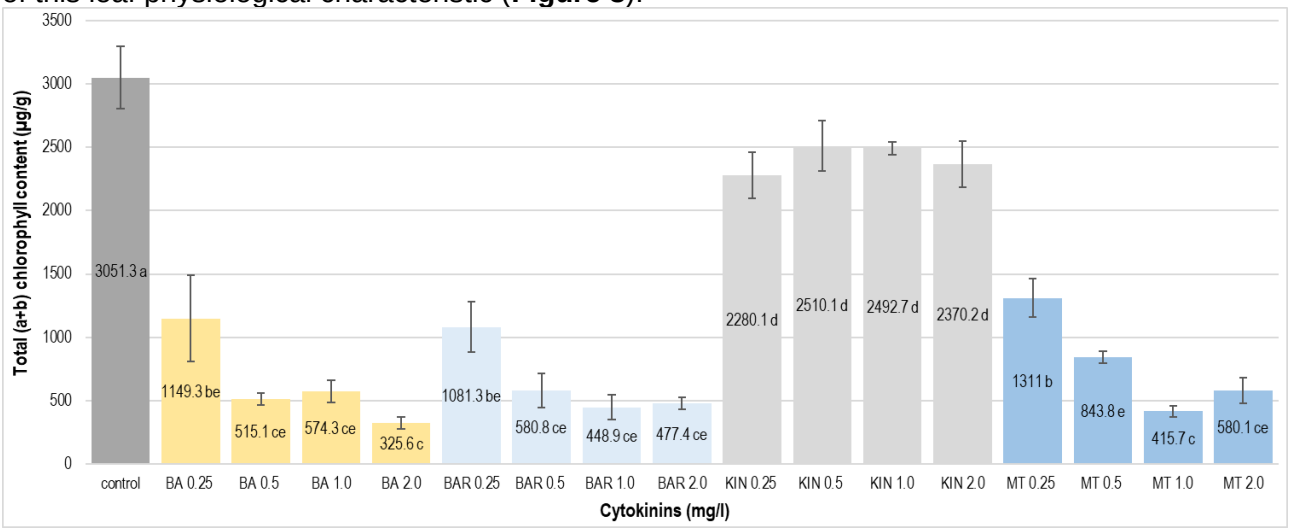

Figure 3: The effect of cytokinins on leaf chlorophyll content of R. hybrida 'Sophia Loren'

3.2. The effects of auxins

Shoot number, shoot and leaf length

None of the auxins had significant effect on shoot formation, especially in the case of NAA, when the number of shoots in did not exceed 1.2 pcs per plant. The most (1.66) roots were developed on medium supplemented with 0.25 mg/l IBA, and this value was significantly higher than which was obtained in the presence of almost all NAA concentrations and 0.5 or 1.0 mg/l IBA.

The shoot length exceeded the control only in the case of 0.1 and 0.25 mg/l IBA (both doses resulted elongated shoots longer than 20 mm), this auxin led to the development of significantly longer shoots than NAA in all doses. However, increasing the concentration of both auxins (0.5 and 1.0 mg/l) decreased shoot length.

The length of the leaves also changed similarly with the increase of the auxins dose, and IBA effectively lengthened the leaves than most NAA concentrations. Nevertheless, leaf length averages longer than 19 mm resulted by 0.1 and 0.25 mg/l IBA were the only ones that exceeded the control (**Figure 4**).

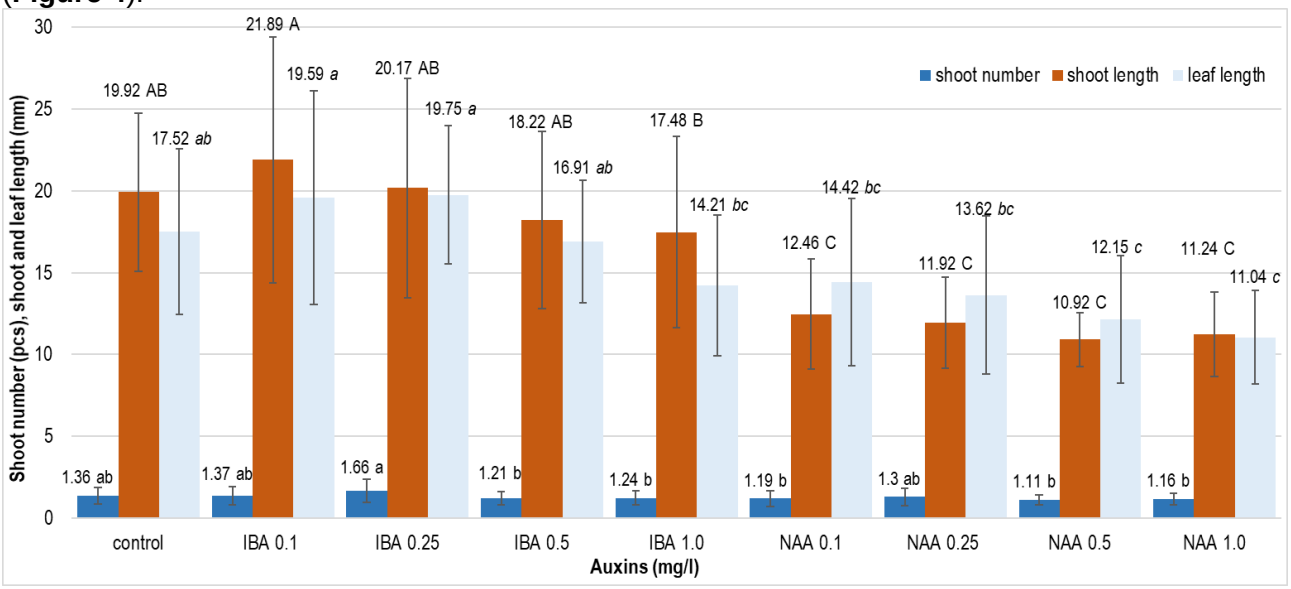

Figure 4: The effect of auxins on shoot number, shoot and leaf length of R. hybrida 'Sophia Loren'

Leaf chlorophyll content

The application of auxins also did not result higher chlorophyll values than the control, and concentrations of 0.25 mg/l or above significantly decreased this pigment's levels (especially in the case of NAA, a definite lowering was shown with the increasing of the dose). However, comparing the two auxins, IBA had a more favorable effect: even with the same concentrations, leaves had higher chlorophyll contents (**Figure 5**).

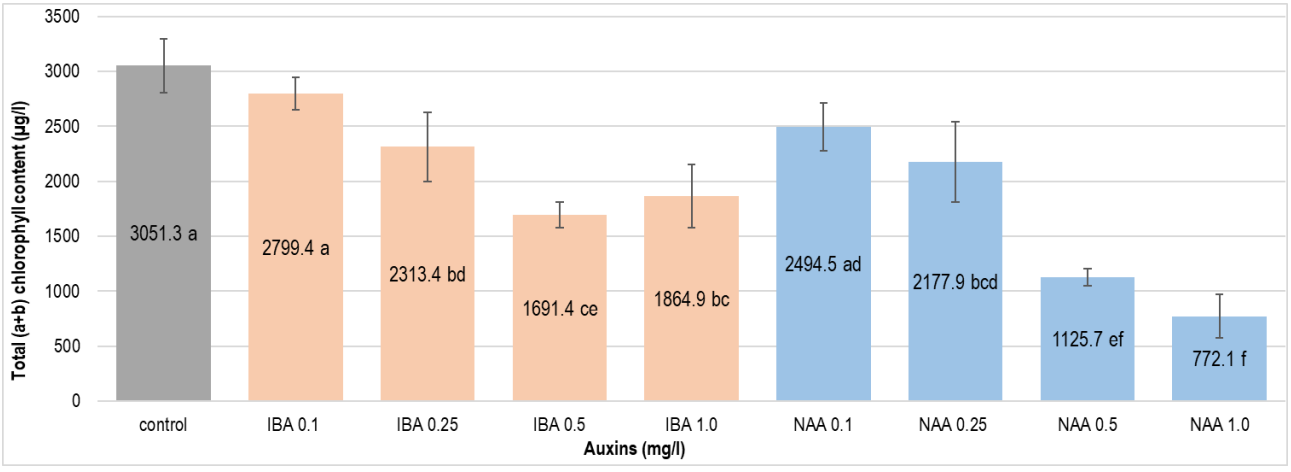

Figure 5: The effect of different auxins on leaf chlorophyll content of Rosa cv. Sophia Loren

Rooting ratio, root number and length

IBA also had a more favorable effect on the rooting ratio than NAA, as more than 70% of the shoots developed roots at concentrations of 0.1-0.25-0.5 mg/l IBA, while NAA resulted in a maximum of 50% rooting at 0.25 mg /l dose. Higher concentrations (0.5 and 1.0 mg/l) of both auxins reduced the rooting rate, especially when NAA was used.

Compared to the control, only 0.25, 0.5 and 1.0 mg/l IBA resulted more roots. The number of roots increased in direct proportion with the enhancement of the IBA dose, and the values of 4.17 and 4.63 roots resulted by 0.5 and 1.0 mg/l IBA were also significantly higher than the lowest average (1.84 pcs) that was obtained on medium contained 0.25 mg/l NAA. The latter auxin did not prove to be effective, a maximum of 2.77 roots formed under the influence of this auxin at a dose of 0.5 mg/l.

The length of the roots did not exceed the control under any of the auxin treatments, but IBA was better effect in this respect as well, because longer roots developed on the media supplemented this auxin, especially when 0.1 and 0.25 mg/l concentrations was applied (24 and 24.58 mm). These two latter values differed significantly compared to the lowest average (8.89 mm) obtained under the influence of 0.25 mg/l NAA. Increasing the concentrations of both auxins usually reduced the length of the roots (**Figure 6**).

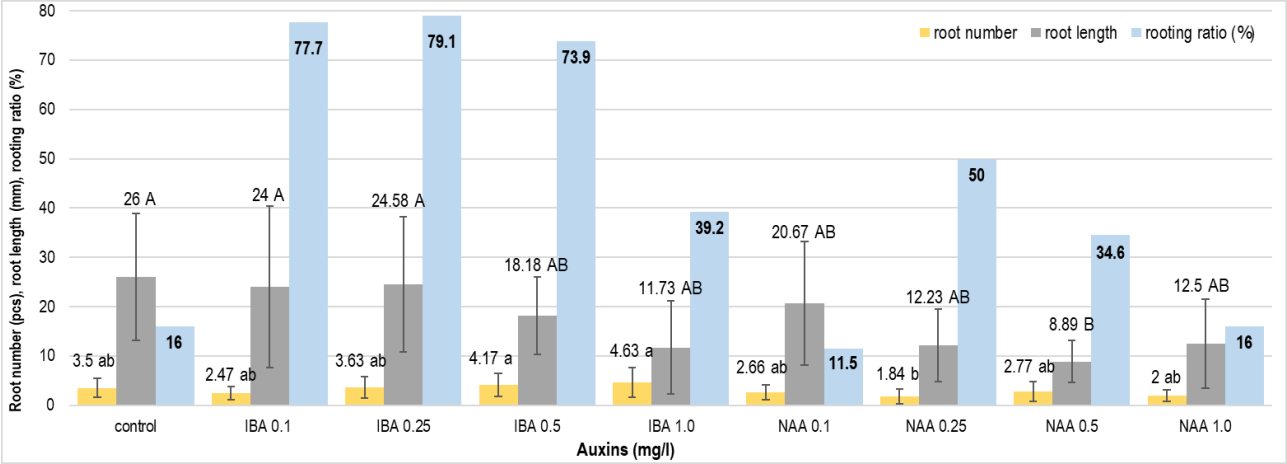

Figure 6: The effect of auxins on shoot number, shoot and leaf length of Rosa cv. Sophia Loren

Figure 7 shows the differences between the effects of the two auxins on rooting, based on type and concentration.

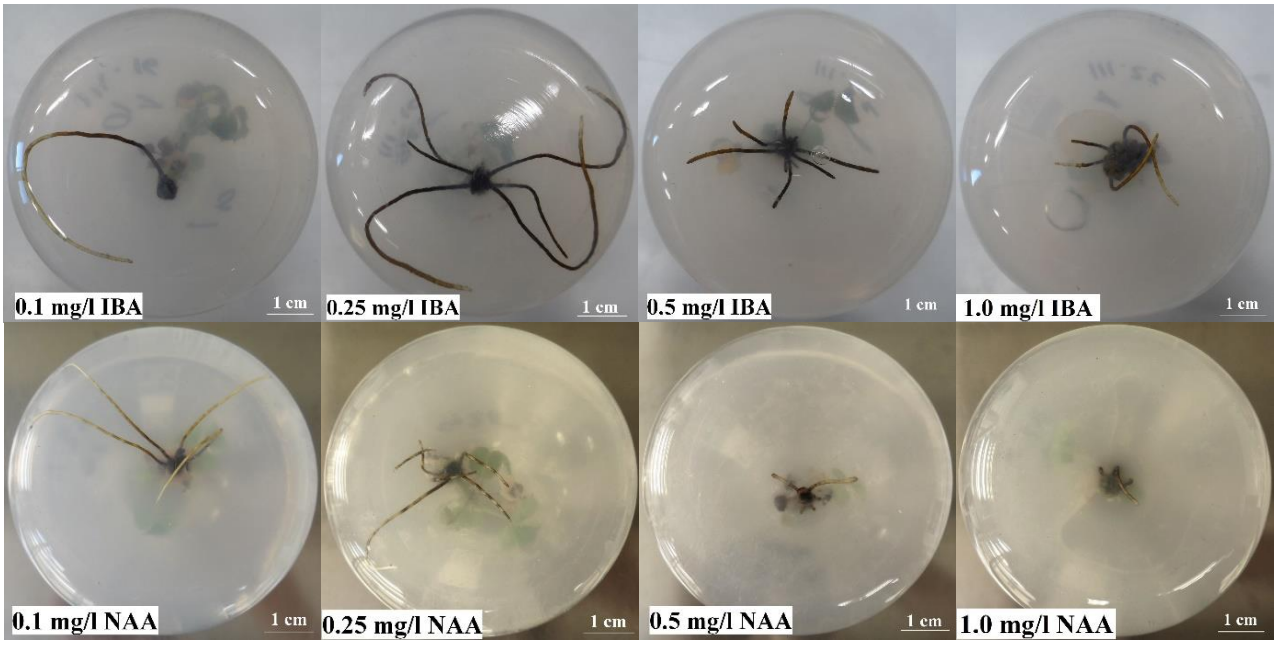

Figure 7: Root development on media with different auxin types and concentrations

4. Conclusions

For shoot stimulation, BA was the most effective cytokinin especially in higher, 1.0 and 2.0 mg/l concentrations, however, increasing the dose usually resulted shorter leaves with lower chlorophyll content. KIN was not optimal for shoot multiplying, but it can be used for elongation because longer shoots were developed in all medium supplemented with this cytokinin. Probably, combination of BA and KIN give better results, as other authors reported, although different rose varieties often required different optimal PGR concentrations [16, 7, 2]. During in vitro multiplication and rooting stages of 'Sophia Loren' rose variety, leaf chlorophyll contents of PGR-treated plants were lower than which obtained in the control group; especially BA, BAR, MT and the highest concentrations of IBA and NAA caused leaf yellowing. To solve this problem, silver nitrate may help, as [13] mentioned; furthermore, this component can be good for not only increasing the pigment level but for better shoot regeneration [6] or stimulating shoot elongation and leaf number enhancement [15]. IBA was more effective than NAA for 'Sophia Loren' in vitro rose plants' root development; and in the case of the control group, very low percentage of the shoots produced roots, despite of that mostly hormone-free [6, 7] or NAA-contained [14] medium proved to be the best for different roses' root induction. Thus, the efficiency of cytokinins and auxins usually based upon the rose taxon.

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