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CITOKININEK HATÁSA *ACTINIDIA CHINENSIS* VAR. *DELICIOSA* 'JENNY' *IN VITRO* ÉS AKKLIMATIZÁLT ÁLLOMÁNYAIBAN

THE EFFECT OF CYTOKININS IN *ACTINIDIA CHINENSIS* VAR. *DELICIOSA* 'JENNY' *IN VITRO* AND ACCLIMATIZED STOCKS

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Összefoglalás

Az Actinidia chinensis var. deliciosa 'Jenny' in vitro szaporítása során a sarjképződést 1 és 2 mg/l MT, 2 mg/l BAR serkentette leginkább, míg a sarj- és levélhosszra, levél pigmenttartalomra az MT mellett a KIN bizonyult megfelelőnek, de csak alacsonyabb (0.25 és 0.5 mg/l) dózisban. A KIN ezen kívül a gyökeresedésre is optimálisan hatott, míg MT használatakor gyökerek nem, csak kallusz képződött. Az akklimatizált növényeken az in vitro szakaszban előzőleg alkalmazott citokininek utóhatásaként főként a BA több sarjat, hosszabb sarjakat és leveleket, az utóbbiakban pedig magasabb pigment-átlagokat eredményezett, szintén elsősorban az alacsonyabb koncentrációk esetén.

Abstract

During in vitro propagation of Actinidia chinensis var. deliciosa 'Jenny', shoot proliferation was stimulated mainly by 1 and 2 mg/l MT, 2 mg/l BAR, while KIN (but in lower, 0.25 and 0.5 mg/l doses only) was the best for development of longer shoots and leaves, and leaves with higher pigment contents. Additionally, KIN was also optimal in root production, on the other hand, MT resulted only callus formation but not rooting. In the acclimatized plants, citokinins (which were only used previously during the in vitro phase) after-effected more and longer shoots, longer leaves with higher pigment contents especially in the case of BA (optimally in low concentrations as well).

1. Introduction

Kiwifruits (*Actinidia* spp.) are one of the most important fruit crops in China, widely called "the king of fruits" due to their high vitamin C content and balanced nutritional profile [13]. It also represents a key export commodity for New Zealand, valued around NZD 4 billion annually, which has motivated and encourage intensive breeding programs [9]. Although these plants cultivated commercially for less than a hundred years, kiwifruit has become a significant horticultural product in Chile, Greece, Italy, New Zealand and in several Chinese provinces, while still accounting for less than 0.25% of global fresh fruit production [14]. The interesting-looking, peculiar-shaped fruits are

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rich in vitamins, organic acids and polyphenols, making the kiwifruits both medicinal and decorative plants [5].

Kiwifruits belong to the family *Actinidiaceae* and the genus *Actinidia* that includes about 60–66 species, most of them originate from China [12]. Most *Actinidia* species are dioecious, therefore both sexes must be planted together to ensure fertilization [3]. *A. chinensis* var. *deliciosa* is the only species cultivated worldwide, largely due to its fruit size, productivity and long shelf-life [11].

Conventional propagations through sowing, grafting or cutting are limited by genetic variability and late sex determination, while vegetative methods are labour-intensive [11]. In contrast, *in vitro* propagation ways allow rapid multiplication, uniform planting material and long-term preservation of germplasm. The most widely used MS [8] medium has been adapted with various cytokinins such as 2iP, BA or meta-topolin to enhance shoot proliferation [6, 10, 5]. High regeneration rates, up to almost 85 % have been achieved [11], and studies confirmed that cytokinin treatments also influence water retention and survival of plants during acclimatization [7].

Overall, kiwifruits combine commercial importance with significant botanical diversity, and modern micropropagation techniques provide effective solutions for breeding, conservation and large-scale production.

2. Material and method

For the trial, sufficient number of *Actinidia chinensis* var. *deliciosa* ‘Jenny’ *in vitro* plant individuals with an average of 2.5-3 cm sized, rootless shoot with at least two leaves was needed previously. In order to collect shoots, as source, we used a stock that had been subcultured for years in the laboratory of the Department of Floriculture and Dendrology. During the handling, clumps (looked like a miniature shrub) were carefully divided into separate shoots, and selected ones were placed in Erlenmeyer flasks (size: 250 ml) filled with hormone-free “S”-medium containing 20 g/l sucrose, 5 g/l agar and Jámborné-Márta [4] macro- and Heller [2] microelements. 6-8 weeks later, chosen shoots were planted on experimental media supplemented with the same nutrients and sugar, solidifying accessories plus different cytokinins: 6-benzylaminopurine (BA), 6-benzylaminopurine riboside (BAR), kinetin (KIN) and meta-topolin (MT) at 0.25, 0.5, 1, and 2 mg/l concentrations. The control medium did not contain cytokinin.

Each cultures (25 individuals per medium) were replicated twice and *in vitro* plants maintained under 16-hour illumination with cool and warm white fluorescent lamps (Polylux XLR FT8/30W/830 & 860, USA) at 20-25 °C. After two months, we examined the number and length of shoots, the length of the largest leaf, the ratio of rooted individuals, and the number and length of roots. The rooted specimens were acclimatized in plastic pots (diameter: 10 cm) containing a substrate mixture combined 2/3 parts *Sphagnum* peat and 1/3 part perlite. Before planting, we cleaned the root system gently and left only the strongest shoot (one piece per each individual). In the first two weeks of the acclimatization in the department’s greenhouse, we kept the plants under white vlies, additionally, moderate humidification and irrigation were done once a week. During the next half month, we removed this covering gradually, and water supply was performed more frequent (2-3 times per week). The plants did not receive any nutrient supplementation or cytokinin treatment (thus, the after-effects of these hormones were examined only). At the end of the second month, we collected data about the shoot number and length, and the length of the largest leaf. Roots were not examined because we did not want to harm the plants (most of them were used for private gardens later).

To determine the chlorophyll (a+b) and carotenoid content of the leaves of *in vitro* and acclimatized plants, 4 x 100 mg leaves were collected from each group. As leaf-preparation, samples were grounded in a mortar using a dash of quartz sand and were supplemented to 10 ml final volume with 80% acetone, and then, samples were stored in refrigerator for 24 hours (temperature: 4 °C). The absorbance of the clear extract was analyzed with a Genesys 10vis (Thermo Fisher Scientific Inc., USA) spectrophotometer at wavelengths of 480, 644 and 663 nm. The pigment contents of the leaf were calculated using formulas (1) and (2):

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

$$\text{Carotenoid content } (\mu g/g) = 5.01 \text{ x } A480 \text{ x } V/w \quad (2)$$

V = volume of tissue extract (10 ml), w = fresh weight of tissue (0,1 g), A = absorbance [1]

All data were evaluated using SPSS 23.0 software (IBM Corp., USA), and significance differences between groups was determined by one-way analysis of variance (ANOVA). In the presence of significances, means were compared using Tukey-Kramer test at probability levels of $p \leq 0.05$, $p \leq 0.1$.

3. Results

3.1. *In vitro* plants

Shoot number, shoot and leaf length

Compared to the control with low value (averagely only 1.2 shoots), all cytokinins at every dose resulted significantly more shoots in most cases, excepting KIN. This growth regulator usually allowed the formation of no more than 2 shoots. The highest multiplication (6.52 shoots) was produced by the plants grown on media with 1.0 mg/l MT, followed by 2.0 mg/l MT and BAR (equally 5.52 shoots). With the exception of KIN (where 0.5 mg/l concentration proved to be the best), the cytokinins generally effected the greatest number of shoots at higher doses (1.0 or 2.0 mg/l, especially BA and BAR), and comparing with the shoot values obtained at lower concentrations, the differences were significant even within the same cytokinin type.

Shoots also grew longer in most citokinin-groups compared to the control (which resulted shoots barely longer than 25 mm), however, difference was significant only in the case of certain cytokinins (all KIN doses and MT at concentrations of 0.25 and 0.5 mg/l). On the other hand, enhancement the concentration of every cytokinins led to increasingly shorter shoots (in the presence of BAR and MT, the difference between the effect of the lowest/highest dose was significant as well). Average data lower than the control were obtained in the case of 1.0 and 2.0 mg/l BAR (24.6 and 23.48 mm) and 2.0 mg/l MT (21.56 mm), while we achieved the highest values on medium containing 0.25 and 0.5 mg/l KIN (50.76 and 53.2 mm) and 0.25 mg/l MT (54.52 mm).

The change of leaf length showed the same trend to shoot length, so, plants with shorter shoots usually had shorter leaves, and longer leaves were found on more elongated shoots. Thus, BA, BAR resulted lower values, while KIN and (primarily 0.25 and 0.5 mg/l) MT effected longer leaves, inversely proportional to the increase in dose. Compared to the control, significant difference was observed under the effect of 2.0 mg/l BA and 0.25 mg/l MT, which resulted the formation of the shortest (18.88 mm) and longest (39.16 mm) leaves. Numerical data of the above parameters are shown on Figure 1 (vertical lines represent the standard deviations, and differing letters indicate significant differences between groups, as in the cases of all the next graphs).

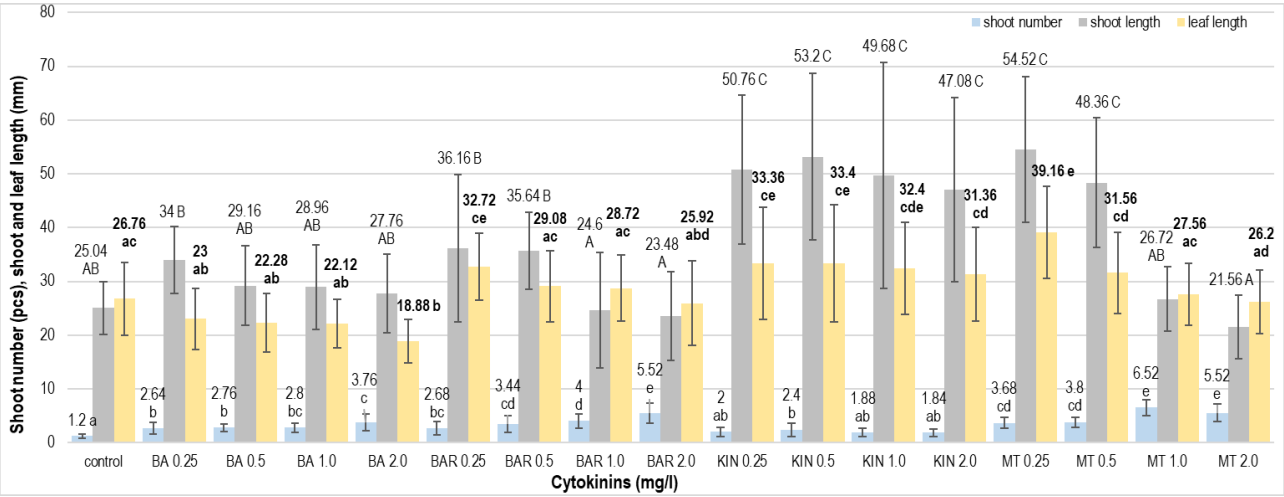

Figure 1: The effect of cytokinins on shoot number, shoot and leaf length of in vitro Actinidia chinensis var. deliciosa 'Jenny' plants

Figure 2 illustrates the different effects of cytokinins on shoot and leaf formation at equal concentrations (2 mg/l).

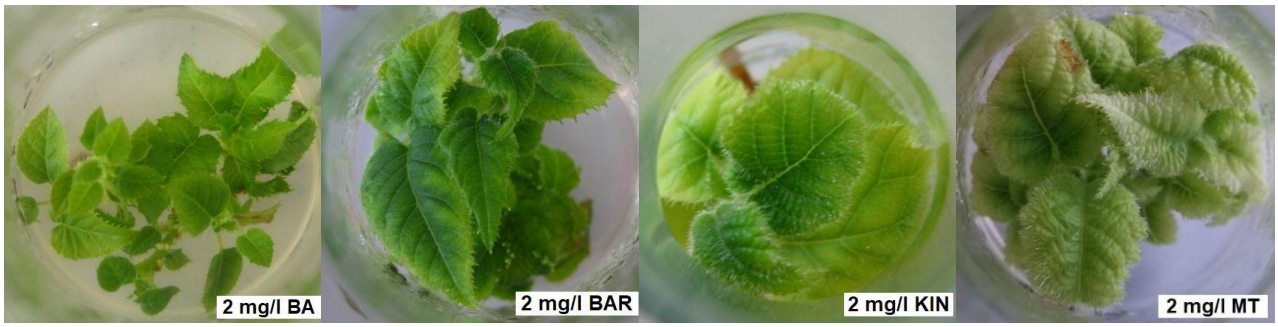

Figure 2: Shoot development on media with different cytokinins in the same (2 mg/l) concentration

Leaf chlorophyl a+b and carotenoid content

1.0 mg/l BA, 1.0 mg/l or 0.5 mg/l BAR, 0.25 and 0.5 mg/l KIN or MT resulted higher pigment values than the control, but these values did not differ significantly. The highest pigment content (3118.1 µg/g chlorophyll a+b, 709.54 µg/g carotenoid) were obtained when 0.25 mg/l MT was used, and the lowest was achieved in the presence of 2.0 mg/l BA (1039.8 µg/g chlorophyll a+b, 251.69 µg/g carotenoid). In the case of every cytokinin, a decrease was observed at the highest dose, and (except for BAR) the lowest values obtained in such cases were also significantly lower than the pigment content measured in the leaves of plants grown mainly on medium containing 0.25, 0.5 mg/l KIN or MT (Figure 3).

Root formation, number and length of roots

KIN proved to be optimal for root formation (Figure 4/A), especially at lower doses (0.25 and 0.5 mg/l) – in both cases the rate was over 90% (96 and 92%). Application of BA and BAR, these concentrations also effected higher values (the former resulted 64% and the latter, 84%), but non-wanted callus was also developed on some plants (Figure 4/B). None of the shoots produced roots under the influence of MT (unfortunately, all individuals had large, massive callus clump – Figure 4/C and 4/D), while the rooting rate was 56-60% on the control and 1.0 or 2.0 mg/l BA-containing media. Therefore, even on hormone-free medium, plants could develop roots spontaneously. The number of roots was uniform (no significant difference was observed between any of the groups); however, the highest doses of all cytokinin reduced the roots' number. The length of the roots also followed quite similar trend; values (mostly above 50 mm) exceeded the control only in the presence of 0.5 or 1.0 mg/l BA, 0.5 BAR, and 0.25 or 0.5 mg/l KIN, however, with no significant differences. Such significance was only demonstrated between the highest value (63.08 mm) achieved on the medium containing 0.25 mg/l KIN and the shortest roots (mostly not or barely more than 40 mm) resulting from the 0.25 or 2.0 mg/l BA and BAR, 1.0 or 2.0 mg/l KIN supplements (Figure 5).

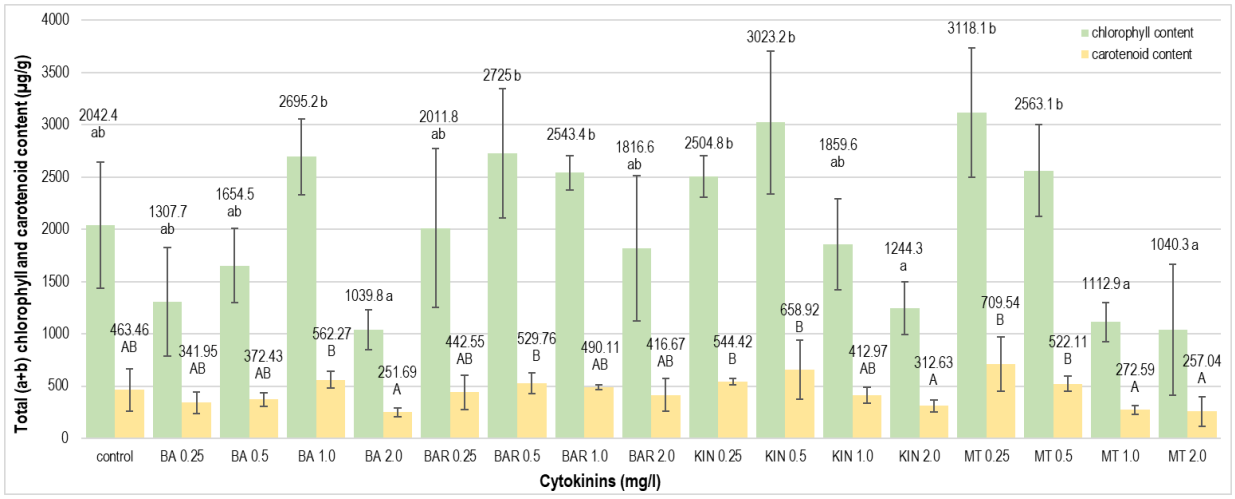

Figure 3: The effect of cytokinins on leaf pigment content of in vitro Actinidia chinensis var. deliciosa 'Jenny' plants

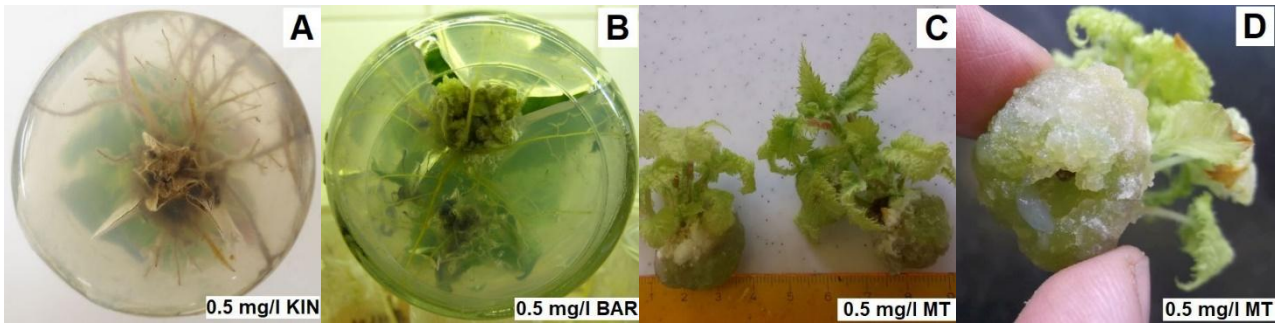

Figure 4: Different in vitro Actinidia chinensis var. deliciosa 'Jenny' root and callus production on media with the same (0.5 mg/l) concentration of KIN (A), BAR (B) and MT (C, D)

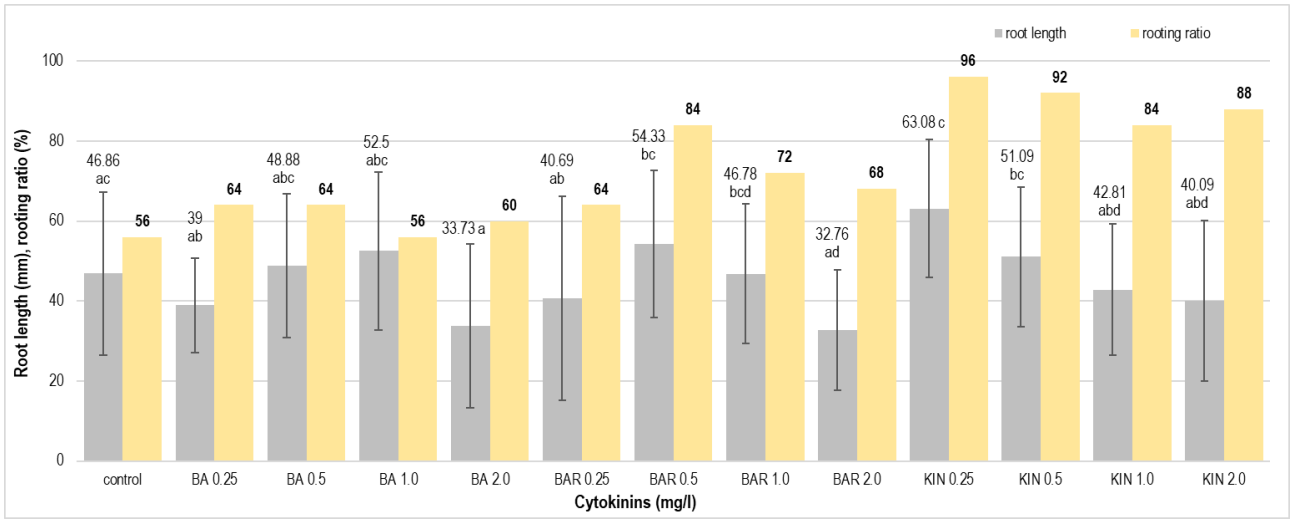

Figure 5: The effect of cytokinins on rooting parameters of in vitro Actinidia chinensis var. deliciosa 'Jenny' plants

3.2. Acclimatized plants

Only rooted plants were acclimatized, so the plants grown on media with MT did not participate in this process.

Shoot number, shoot and leaf length

The acclimatized plants that had previously grown on medium with 0.5 mg/l BA produced the most shoots (2.25); and the same concentration also resulted a relatively high shoot number (averagely more than 2) as an aftereffect of both BAR and KIN cytokinins. 0.25 mg/l BAR effected the fewest shoots (only 1.25), and equally 1.33 shoots by 2 mg/l BA, and 0.25 or 2.0 mg/l KIN. The highest doses (in all three cytokinins) decreased shoot production, but no significant difference was observed between any of the groups.

Shoot length was the highest in the group that grown previously on media containing 0.25 mg/l BAR (231.5 mm), this value was significantly higher than the lowest data (135.67 and 124.75 mm) after effected by 2.0 mg/l BA and the same amount of BAR; the latter also considerably differed from the control. Shoot length of the acclimatized plants (similar to shoot number) decreased when the highest doses of any cytokinins were used during their previous (*in vitro*) stage.

Leaf length followed a similar trend as shoot length. Notably, almost the same cytokinin types and concentrations led as an aftereffect to the smallest/largest average values in the acclimatized stocks, with the only difference being that the longest (168.75 mm) leaves were developed in the 0.5 mg/l BA group. Furthermore, this average was significantly different from the smallest results (BAR: 112 mm, BA: 124 mm, KIN: 124.33 mm) generated by all three cytokinins at their maximum dose 2.0 mg/l (Figure 6).

Leaf chlorophyll a+b and carotenoid content

Compared to the pigment contents obtained in the control stock (chlorophyll a+b: 987.91 µg/g, carotenoid: 278.59 µg/g), the values were higher in all acclimatized groups; the difference - based on the chlorophyll content - was significant in the case of 0.5 and 1.0 mg/l BA, which resulted the highest levels (1759.6 and 1622.8 µg/g). The former BA dose led to the highest carotenoid content (449.18 µg/g), which significantly exceeded both the average (278.84 µg/g) achieved in the control and 0.25 mg/l BAR groups (Figure 7).

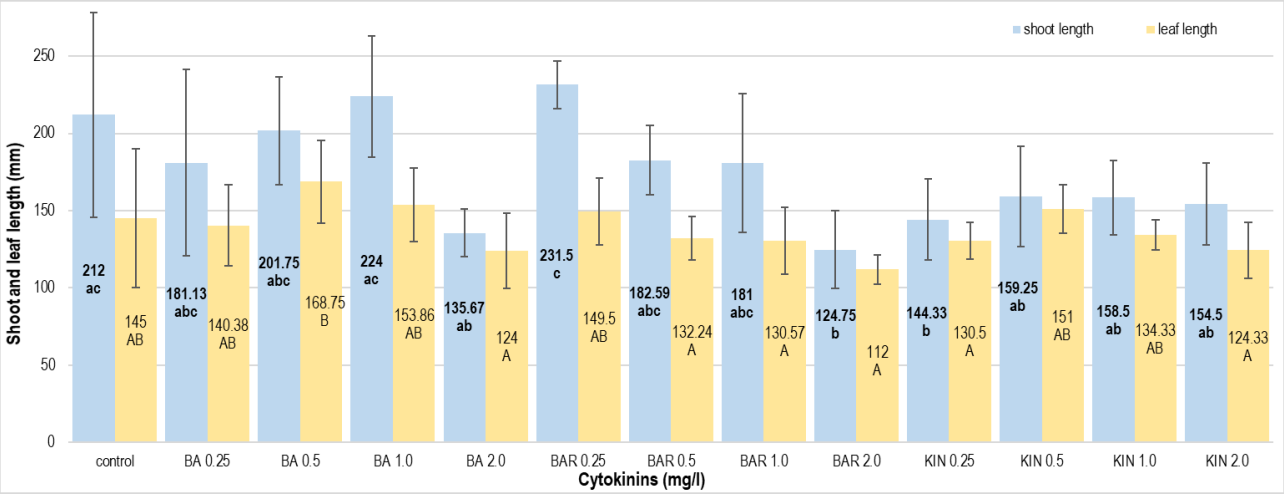

Figure 6: The effect of auxins on, shoot and leaf length of acclimatized Actinidia chinensis var. deliciosa 'Jenny' plants

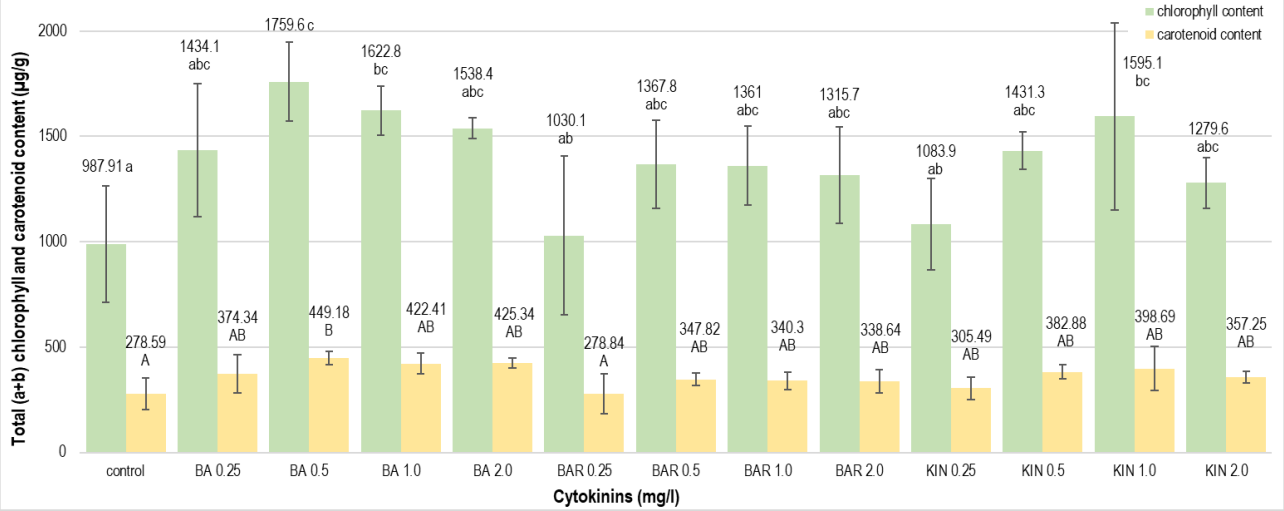

Figure 7: The effect of cytokinins on leaf pigment content of acclimatized Actinidia chinensis var. deliciosa 'Jenny' plants

4. Conclusions

Plants that developed roots under *in vitro* conditions used for acclimatization only, so the stocks grown on MT media were no longer included in this last experimental stage. There were no statistically significant differences in the number of shoots (mostly less than 2 shoots were found in the majority of groups), however, in the case of all cytokinins, the highest dose decreased shoot number, shoot and leaf length. Interestingly, mainly KIN resulted the longest shoots in the *in vitro* stocks, while in the acclimatized plants – as an after-effect – usually BA, BAR resulted more shoot elongation, but only at lower doses. Certainly, *in vitro* plants were tiny with short leaves/stems due to the limited space of 250 ml sized Erlenmeyer flask, but their size has multiplied during acclimatization. In this trial, plants had spectacular progression: while *in vitro* specimens that had

only 40-50 mm shoots and 25-35 mm leaves, they reached a length of 230 mm of the shoots and nearly 170 mm sized leaves by the end of the acclimatization, depending on the after-effect of the given cytokinin.

In contrast, the pigment content of the leaves of acclimatized plants decreased; sometimes by about half in the case of chlorophyll a+b (especially in the control and 0.25-1.0 mg/l BAR, 0.25-0.5 mg/l KIN groups), compared to their *in vitro* state. The increase in plant size could have been accompanied by a decrease in chlorophyll and carotenoid content of the leaves. On the other hand, the artificial illumination of the *in vitro* cultures for 16 hours and the addition of Fe-Na-EDTA (25 mg/l) and/or $MgSO_4$ (200 mg/l) as basic components of the medium had a presumable positive effect (nutrient supplementation was not used during acclimatization). In any case, similarly to the other morphological parameters, the pigment content of the leaves also decreased in the case of stocks originating from the medium with the highest (2.0 mg/l) cytokinin concentration.

References

[1] Arnon, D.I. (1949): Copper enzymes in isolated chloroplast. Polyphenoloxidase in *Beta vulgaris*. Plant Physiology 24(1): 1-15. doi: 10.1104/pp.24.1.1

[2] Heller, R. (1953): Recherches sur la nutrition minerale des tissues vegetaux cultives *in vitro.* Annales de Sciences Naturelles, de Botanique et de Biologie Vegetale 14: 1-21.

[3] Ivanova, R., Calugaru-Spataru, T., Dascaliuc, A. (2013): In vitro multiplication and cultivation of Actinidia arguta in the Republic os Moldova. Biologija 55(3): 301-308.

[4] Jámborné B. E., MÁRTA K. (1990): *In vitro* propagation of *Philodendron tuxtlanum* Bunting with BA. Acta Agronomica 39: 34l-348.

[5] Levchyk, N., Skrypchenko, N., Dziuba, O., Gajdosova, A., Liubinska, A., Zaimenko, N. (2022): Features of morphogenesis of *Actinidia arguta* leaf tissues at microclonal propagation. Journal of microbiology, biotechnology and food sciences, 12(1): e4667-e4667. https://doi.org/10.55251/jmbfs.4667

[6] Molkanova, O.I., Kozak, N.V., Konovalova, L.N., Malaeva, E.V. (2014): Biological peculiarities of far eastern species of the genus *Actinidia* Lindl. Bulletin of Udmurt University. Series Biology. Earth Sciences 1: 42-48.

[7] Moncaleán, P., Fal, M.A., Castañón, S., Fernández, B., Rodríguez, A. (2009): Relative water content in vitro proliferation and growth of Actinidia deliciosa plantlets are affected by benzyladenine. New Zealand Journal of Crop and Horticultural Science 37(4): 351-359. https://doi.org/10.1080/01140671.2009.9687590

[8] Murashige T., Skoog, F.: A revised medium for rapid growth and bio assays with tobacco tissue cultures, Plant Physiology, 1962, 15: 473-497. https://doi.org/10.1111/j.1399- 3054.1962.tb08052.x

[9] Nadarajan, J., Esfandiari, A., Mathew, L., Divinagracia, J., Wiedow, C., Morgan, E. (2023): Development, management and utilization of a kiwifruit (*Actinidia* spp.) in vitro collection: a New Zealand perspective. Plants 12(10): 2009. https://doi.org/10.3390/plants12102009

[10] Saeiahagh, H., Mousavi, M., Wiedow, C., Bassett, H. B., Pathirana, R. (2019): Effect of cytokinins and sucrose concentration on the efficiency of micropropagation of 'Zes006' *Actinidia chinensis* var. *chinensis*, a red-fleshed kiwifruit cultivar. Plant Cell, Tissue and Organ Culture 138(1): 1-10. https://doi.org/10.1007/s11240-019-01597-4

[11] Thakur, D., Sharma, P., Sharma, R., Kumari, C., Rana, V.S. (2022): Critical factors governing the efficient direct organogenesis in green-fleshed kiwifruit (*Actinidia deliciosa*) [A. Chev.] var. *deliciosa*. In Vitro Cellular & Developmental Biology – Plant 58: 1107-1116. https://doi.org/10.1007/s11627-022-10309-1

[12] Zhang, X.C., Bao. W.W., Znag, A.L., Pathirana, R-, Wanf, Q.C., Liu, Z.D. (2020): Cryopreservation of shoot tips, evaluations of vegetative growth, and assessments of genetic and epigenetic changes in cryo-derived plants of Actinidia spp. Cryobiology 94: 18-25. https://doi.org/10.1016/j.cryobiol.2020.05.004

[13] Zhong, W., Zhou, J., Tang, D., Huang, Y., Liu, F., Zhang, M., Wang, G., Wu, S., He, Y., Tang, J. (2021): Establishment of tissue culture system of *Actinidia deliciosa* cultivar "Guichang". Journal of Chemistry 2021(1): 9951949 https://doi.org/10.1155/2021/9951949

[14] Wu, J.H., Datson, P.M. (2015): Development of a method for in vitro germination of Actinidia seedlings to enable PSA bioassays and ploidy manipulation. Acta Horticulturae 1096: 383-388. https://doi.org/10.17660/ActaHortic.2015.1096.45