Acta Biologica Marisiensis sciendo

STIMULATION OF PHYSIOLOGICAL PROCESSES IN ST. JOHN'S WORT (*HYPERICUM PERFORATUM* L.) SEEDLINGS BY TREATMENTS WITH TRIACONTANOL AND BENZYLADENINE

Laszlo FODORPATAKI[1*], Reka BERKECZI[2], Tekla Amalia LUNKA[1]

[1]Department of Horticulture, Faculty of Technical and Human Sciences, Sapientia University, Târgu Mureș, Romania

[2] Faculty of Biology and Geology, Babeș-Bolyai University, Cluj-Napoca, Romania

*Correspondence:
Laszlo FODORPATAKI
lfodorp@gmail.com

Received: 26 August 2022; **Accepted:** 23 October 2022; **Published:** 30 December 2022

Abstract: Treatment of St. John's wort plantlets with 1 µM triacontanol and 2 µM benzyladenine stimulates growth and metabolic processes, being an environmental-friendly approach for optimizing the cultivation of these valuable medicinal plants under controlled conditions. When the two growth regulators (a bioactive cuticular wax constituent and a cytokinin) are applied simultaneously, they act synergistically, enhancing each other's effect on the biomass accumulation and on certain parameters of the photosynthetic light use efficiency, such as the effective quantum yield of photosystem II and the overall vitality index of the photosynthetic apparatus which performs the conversion of light energy into usable forms for carbon dioxide assimilation. The results concerning the interactions between the two externally applied growth regulators during the early development of St. John's wort plants may lead to a more efficient cultivation of this herbal medicinal product, including the possibility to modulate the production of pharmacologically active metabolites.

Keywords: biomass production, carotenoids, chlorophyll fluorescence, growth regulators, photosynthetic quantum yield

1. Introduction

Cultivation of medicinal plants ensures better predictability and control of the production of pharmacologically active compounds, because plant growth and metabolism can be modulated by the culture conditions and by selected treatments (Tsukagoshi and Yamori, 2020). At present, herbal medicines suitable for complementary and alternative medicine are used at an increasing scale around the world, as ethnopharmacological traditions become more and more supported by scientific evidence regarding the biochemical composition of herbs and the action mechanisms of the different plant metabolites. In most of the European countries, plants used for registered herbal medicinal products are grown under controlled agricultural conditions (cultivated), excluding any contamination with pesticides and use of genetically modified plants (Edwards et al., 2015). Under these circumstances, the production of medicinal plants can be optimized in an environmental-friendly and cost-effective man-

ner by priming or hardening the plant with exogenous application of very small concentrations of bioactive substances. These compounds are able to induce metabolic changes that stimulate growth and tolerance to adverse environmental conditions, especially during the most sensitive periods of the plant's life cycle, such as germination and early vegetative development. The use of such growth regulators may significantly increase biomass production of medicinal plant, also leading to a more intense biosynthesis of the pharmacologically active metabolites (Garcia-Garcia et al., 2020; Godoy et al., 2021). From among the many bioactive compounds that may stimulate plant growth and metabolism both under normal and extreme (stressful) conditions, triacontanol is a natural constituent of the epicuticular waxes present on the surface of aboveground plant organs with primary structure, while benzyladenine is a synthetic cytokinin, being the cheapest among the representatives of this group of plant hormones (Naeem et al., 2012; Oshchepkov et al., 2020).

The growth regulating properties of triacontanol (Tria) were recently discovered as compared to most of the plant bioregulators, and its action mechanisms are still largely unknown. Recent studies have demonstrated that when this natural wax constituent of the plant cuticle comes in contact with a certain receptor in the plasma membrane, it induces the formation of secondary messengers (such as the 9-β-L-adenosine generated from AMP) which trigger signal transduction pathways that lead to transcriptional activation of certain genes, to regulation of specific enzyme activities (e.g. of Rubisco in the photosynthetic Calvin cycle and of nitrate reductase in the nitrogen assimilation) and membrane transporters. All these lead to stimulation of growth and developmental processes, both under normal and stress conditions. Applied as a foliar spray or added to the aqueous nutrient solution in concentrations as low as 10^{-8}-10^{-6} M, Tria could efficiently counteract the deleterious effects of high salinity, drought, extreme temperatures, hypoxia, high light intensity, heavy metal pollution, UV-B radiation and other environmental stressors on several physiological processes in various crop plants (Islam and Mohammad, 2020; Zaid et al., 2020; Tompa et al., 2022). In different medicinal and aromatic plants, external application of micromolar amounts of Tria led to an increased production of alkaloids and essential oil-constituting monoterpenes (Naeem et al., 2012).

Cytokinins are plant hormones with well-known developmental and metabolic effects, being mostly responsible for stimulation of cell divisions in meristematic regions (root tip, shoot apex, embryo of the seed), for the delay of senescence due to maintenance of a metabolically active state of plant organs, for the development of thylakoid membrane system in the chloroplasts, as well as for acclimation mechanisms to drought, high salinity, chilling and heat stress (Haisel et al., 2006; El-Ghamery and Mousa, 2017; Li et al., 2020). Because it is less expensive than the pure form of the natural cytokinins, the synthetic cytokinin benzyladenine (BA, also known as 6-benzyl-aminopurine) is the most widely used one in the *in vitro* plant tissue and organ cultures, mainly to induce callus formation and generation of new shoots, in co-action with different auxins. Upon the perception of cytokinins, hystidine kinases located in the plasma membrane or in the membrane of the endoplasmic reticulum initiate intracellular signaling pathways which will regulate the activity of several genes, resulting in coordinated developmental changes (Hwang et al., 2012; Rademacher, 2015). Although many effects of cytokinins in plants were demonstrated under various growth conditions, their application in horticulture, in

ex vitro cultivation of medicinal plants and in human therapy is still very limited.

St. John's wort (*Hypericum perforatum* L.) is one of the most popular medicinal plants, being an excellent example for how modern phytochemical research can be effective in the development of traditional remedies (Nahrstedt and Butterweck, 2010). It is mainly used to treat mild and moderate forms of depression, having similar effectiveness but fewer side effects than standard synthetic antidepressants. This effect is related to the fact that its certain active compounds act on noradrenergic, dopaminergic and serotonergic systems in the human brain. The main active secondary metabolites responsible for these effects are the hypericins (naphthodianthrones, which include hypericin, isohypericin, protohypericin and pseudohypericin) and the hyperforins (prenylated phloroglucinols represented by hyperforin, adhyperforin and their derivatives). These components are also used to treat anxiety and certain types of insomnia. Other, more common active metabolites are phenolic compounds such as the flavonoids quercetin (with high antioxidant capacity), biapigenin and kaempferol, as well as chlorogenic acid and caffeic acid (Kasper et al., 2012; Russo et al., 2014; Shrivastava and Dwivedi, 2015). Oil-based extracts of the herb are used externally to treat wounds, burns, swellings and skin bruises. It was demonstrated that hyperforins strengthen the skin barrier function by reducing harmful radical formation, and they are also responsible for the activation of cytochrome P450-metabolizing enzymes, while hypericins inhibit the enzyme protein kinase C and the release of arachidonic acid, thus having an anti-inflammatory effect. The skin healing effect is also related to the fact that extracts of St. John's wort stimulate collagen synthesis and fibroblast migration (Edwards et al., 2015). Recently it was established that the plant's extracts possess antibacterial effects, including against mycobacteria, and they are also efficient against certain types of viruses, such as the influenza virus and retroviruses (Avato et al., 2004; Mortensen et al., 2012). Furthermore, hypericins and hyperforins were effective in the *in vitro* inhibition of tumor cell divisions and in the induction of apoptotic cell death in lymphomas (Shrivastava and Dwivedi, 2015).

The aim of this work is to enhance growth and photosynthetic performance of young St. John's wort plants with the foliar application of micromolar concentrations of triacontanol and benzyladenine, as well as to study the possible interactions between the effects of the above-mentioned bioactive compounds when they are applied simultaneously. The starting prediction is that if Tria and BA induce different signal transduction pathways in triggering certain metabolic and developmental processes, their effects may be synergistic or additive, resulting in a more pronounced growth stimulation than in the case of their separate application. Our results may contribute to optimization of biomass production of this medicinal plant during cultivation, this being a prerequisite for an enhanced yield of herbal medicinal product.

2. Materials and methods

Plant material and experimental conditions

Seeds of St. John's wort (*Hypericum perforatum* L.) were purchased from Agrosel S.R.L., soaked for 24 h in dechlorinated tap water and sown individually in round pots (9.0 cm diameter and 11.8 cm height) filled with wet perlite as an inert soil substitute. The pots were randomly arranged in a growth chamber, where the temperature was kept constantly at 22 °C, the relative air humidity was set to 60%, and the photosynthetically active photon flux density, provided by blue and red LEDs, was 355 µM m^{-2} s^{-1} for a daily 14 h photoperiod.

After germination, the plantlets were watered every three days with Hoaglans's mineral nutrient solution, which provided in an optimal molar ratio every essential macro-, micro- and ultramicronutrient, having the pH value set to 5.6 (Hoagland and Arnon, 1950). Treatments with bioactive compounds started when plantlets were six days old and lasted for 15 days, being repeated once in three days, during the morning hours. Thus, each plant received a total number of five treatments during the experiment. For every experimental variant seven similar plantlets were selected, representing a total of 28 plants in separate pots. Control plants were not treated with any biostimulant, one set of plantlets received once in three days, as a foliar spray, 1 µM triacontanol (Tria, purchased from Nutri-Tech Solutions, Yandina, Australia), another group of plants was treated with the same periodicity with 2 µM benzyladenine (BA, 6-benzyl-aminopurine, provided by Sigma-Aldrich, Darmstadt, Germany). Another set of seven plantlets was provided simultaneously, as foliar spray, with 1 µM Tria and 2 µM BA. The solvent of the Tria and BA solutions was distilled water. The concentrations were chosen based on previous sets of experiments and on literature (El-Ghamery and Mousa, 2017; Tompa and Fodorpataki, 2021). Plants were rerandomized after each treatment event to avoid any positional influence.

Growth and yield measurements

During germination of seeds, parameters such as germination percentage, rate of germination, mean germination time and germination speed were recorded or computed. After germination, plant shoot height (from the collet to the stem apex) was measured on a daily basis, for seven plants for each experimental variant. On the fifteenth day after the initiation of treatments (i.e. 21 days after the beginning of germination), after performing the *in vivo* and *in situ* measurment of induced chlorophyll fluorescence parameters, the fresh biomass of the aboveground shoot and the fresh weight of leaves (including the young ones emerging from the apical bud and possessing their own petiole) of all plantlets were determined separately, using an analytical scale.

Determination of induced chlorophyll fluorescence parameters

Pulse amplification modulated and conventional parameters of chlorophyll fluorescence were determined with an FMS-2 type fluorometer (Hansatech, Norfolk, UK) on the adaxial side of the third fully developed leaf blade from the base of the stem. Conventional parameters, such as ground fluorescence level (Fo), maximal fluorescence (Fm), fluorescence variation (Fv = Fm – Fo) and potential quantum yield of photosystem II (Fv/Fm) were determined in leaves that were dark-adapted for 15 min before measurements, while light pulse amplification modulated parameters (the Fo' modulated ground fluorescence, the Fm' modulated maximal fluorescence, the Fs steady-state fluorescence value and Φ, the effective quantum yield of photosystem II, calculated with the relation (Fm' – Fs) / Fm') were established in leaves exposed to ambient illumination (Haisel et al., 2006; Tompa et al., 2022). The vitality index of the photosynthetic apparatus of thylakoid membranes involved in the light reactions of photosynthesis, expressed as the chlorophyll fluorescence decrease ratio [Rfd = (Fm – Fs)/Fs], was determined according to Lichtenthaler et al. (2005), applying very strong white light (with the intensity of 3000 µmole photons m^{-2} s^{-1}) for five minutes.

Determination of photosynthetic pigment content of leaves

For the measurement of photosynthetic pigment content (chlorophyll *a*, chlorophyll *b* and total carotenoids, i. e. carotenes and xanthophylls) the same leaves were used as the ones on which the *in vivo* determination of induced chlorophyll fluorescence was performed previously (from seven plants for each experimental variant). The leaf blades were used for extraction of chlorophylls and carotenoids after the fresh weight of shoots and leaves was measured. 0.2 g of leaves were ground in a prechilled mortar with 5 mL 80% (v/v) acetone, in the presence of a small amount of magnesium carbonate, to avoid transformation of chlorophylls into phaeophytin. The acetonic extracts were centrifuged for 10 min at 4000 g and 4 °C. The absorbance of 4 mL of supernatant was measured with a spectrophotometer (V-750 UV-Vis Jasco, Midrand, South Africa) at 470 nm, 646 nm and 663 nm, using as reference the 80% acetone (Wellburn, 1994). The chlorophyll and carotenoid pigment contents were expressed as mg per gramm fresh weight of leaves, using the formulae: chlorophyll *a* content = (12.21 x A663 – 2.81 x A646) x V x D / 1000 x d x W, chlorophyll *b* content = (20.13 x A646 – 5.03 x A663) x V x D / 1000 x d x W, carotenoids content = (A470 – 3.27 x chlorophyll *a* content – 104 x chlorophyll *b* content) x V x d / W x d, where A is the absorbance of pigment extract at the given wavelength, V is the volume of the acetonic leaf extract (in mL), D is the coefficient of dilution, d is the width of the measuring cuvette through which the light beam crosses the pigment solution (in cm), and W is the fresh weight of leaf blade used for the extraction (in g).

Statistical analysis of experimental data

Data analysis was performed using the R statistical package (R Core Team, software environment version 4.1.0). Normality of experimental data distribution was tested with the Shapiro-Wilk test, while Bartlett's test was applied for establishing the homogeneity of variances. Significance of differences between experimental variants was determined with one-way ANOVA, followed by the Tukey HSD multiple means comparison test. Every experimental variant had 7 replicates, and every measurement was performed with two technical repetitions (i. e. every measurement was repeated twice). The data were represented as mean ± standard error, and differences were interpreted as significant at $p < 0.05$.

3. Results and discussions

Influences on vegetative growth and development

21 days after germination, the shoot elongation of young plantlets was stimulated in the same degree by triacontanol and by benzyladenine, but the combination of the two bioactive compounds did not result in further enhancement of internode elongation (**Fig. 1**). This reflects that there is no interaction (neither synergism, nor antagonism) between triacontanol and benzyladenine in the stimulation of stem length. With all the three types of treatments, an increment of about 50% could be recorded in the shoot height as compared to the untreated control, which represents a significant growth stimulation and enables the plantlets to develop higher stems in shorter times, which may facilitate a better tolerance to adverse conditions, thus increasing the survival expectations of the vulnerable young plantlets. Similar results were reported for onion plantlets, where BA treatment stimulated root

and shoot length (El-Ghamery and Mousa, 2017), and for spinach, where even smaller concentrations of Tria (25 nM) significantly increased shoot height (Tompa and Fodorpataki, 2021). The combined treatment did not result in any significant difference from the separate treatments with Tria and BA, respectively. While no synergism could be demonstrated between Tria and BA in the stimulation of stem elongation, the fresh biomass of the vegetative shoots (stems with leaves) was significantly higher when the two bioactive substances were provided together, as compared with the control and with the separate treatments with Tria and with BA, respectively (**Fig. 2**).

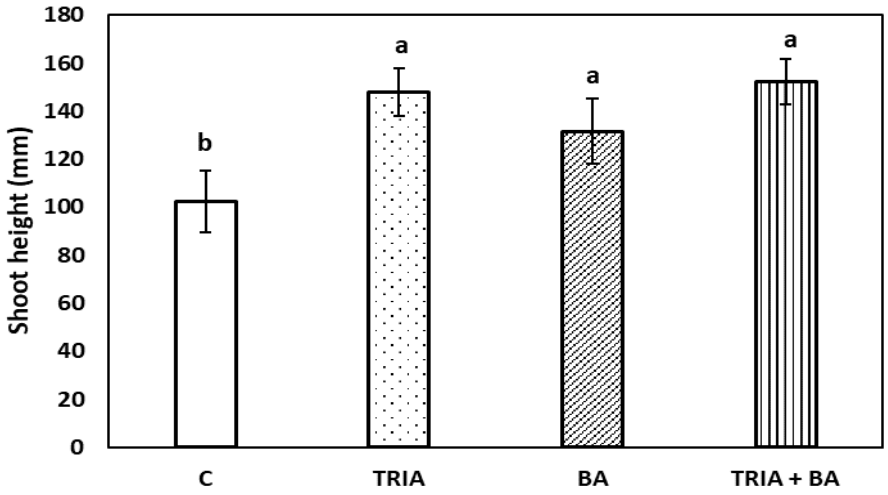

Fig. 1. Stimulation of shoot elongation by exogenously applied 1 µM triacontanol (TRIA), 2 µM benzyladenine (BA) and their combination (TRIA + BA) in 21 days old St. John's wort plantlets. C – control group. Vertical bars show the ± standard errors from means (n = 7), and the different letters represent significant differences at $p < 0.05$

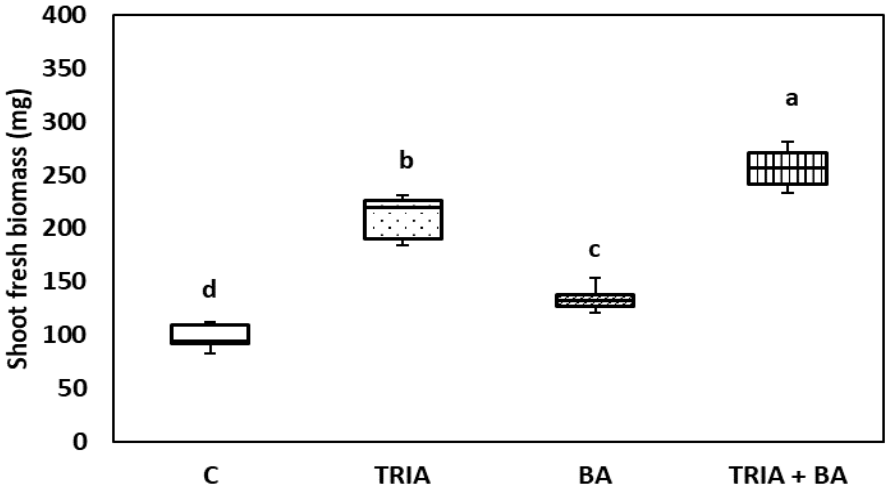

Fig. 2. Influence of 1 µM triacontanol (TRIA), 2 µM benzyladenine (BA) and their combination (TRIA + BA) on shoot fresh biomass accumulation of 21 days old St. John's wort plantlets. C – control group. Vertical bars show the ± standard errors from means (n = 7), and the different letters represent significant differences at $p < 0.05$

This reveals a positive interaction between Tria and BA in stimulating fresh weight accumulation of young St. John's wort plantlets, because none of the two growth regulators could induce such an increment by its own, at least in the applied concentrations and for the 15 days period of treatments. Applied individually, Tria and AB still enhanced in a statistically significant degree the net biomass production of plants, the stimulating effect of triacontanol being more pronounced than the one exerted by benzyl-adenine.

Increased aboveground vegetative biomass growth induced with Tria was also reported for spinach plants, the same degree of stimulation being achieved with 25 nM and 1 µM concentrations (Tompa et al., 2022). For BA, the highest shoot number was obtained when 2 mg L^{-1} BA was added to the nutrient medium of *in vitro* shoot cultures of St. John's wort, while the highest hypericin percentage in the fresh biomass was achieved when 1 mg L^{-1} BA was supplied exogenously (Karakas et al., 2009). It is worth mentioning that the plantlets produce their own quantity of cytokinins, and the added benzyladenine is an extra amount which increases the overall cytokinin content, also modifying the molar ratio between the different growth regulators, thus leading to further changes in growth and development (Li et al., 2020).

Because the highest amounts of hypericins, hyperforins and other pharmacologically active secondary metabolites accumulate in leaves, especially in the large intercellular spaces that develop in the mesophyll (Shrivastava and Dwivedi, 2015), the influence of the two growth regulators on the total leaf biomass of each plant was also evaluated (**Fig. 3**). Tria did not cause a significant increase in the fresh biomass of leaves as compared to the control group, the exogenous application of 2 µM BA as foliar spray caused a moderate, but statistically significant decrement in the leaf biomass value per plant, and the only increment of this growth parameter was obtained when a combined treatment was applied with Tria and BA.

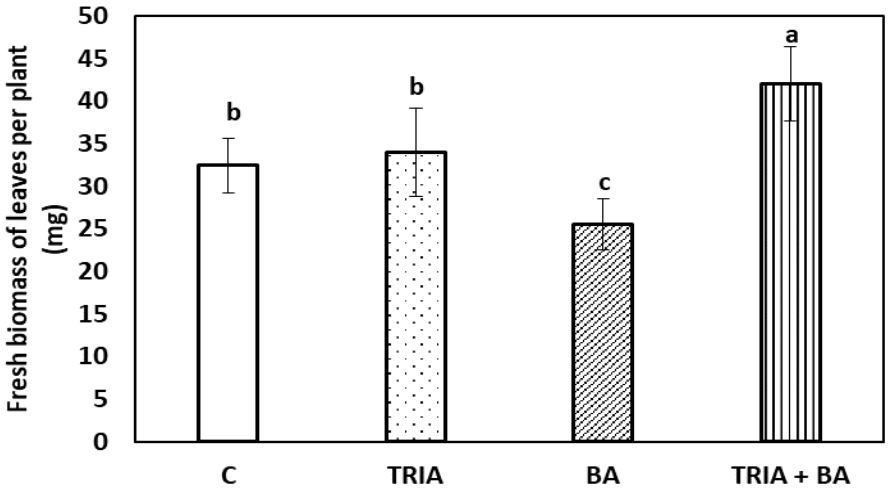

Fig. 3. Influence of 1 µM triacontanol (TRIA), 2 µM benzyladenine (BA) and their combination (TRIA + BA) on the fresh biomass of leaves of 21 days old St. John's wort plantlets. C – control group. Vertical bars show the ± standard errors from means (n = 7), and the different letters represent significant differences at $p < 0.05$

This means that there is such an interaction between Tria and BA in the plants that while none of the compounds increases the fresh weight of leaves when applied separately, their combination results in a significant stimulation of leaf growth. The internal mechanisms leading to this interaction still need to be elucidated with further investigations.

Influences on photosynthetic light use efficiency

The overall vitality of plants, their biomass production and the capacity to produce different amounts of metabolic products highly depend on their photosynthetic performace. This can be evaluated non-destructively by registering different parameters of the induced chlorophyll fluorescence in leaves. These parameters give an insight into the energetics of light reactions, rendering possible to evaluate the efficiency of photochemical conversion of the light energy absorbed by the photosynthetic pigments, in order to be stored and incorporated in the new organic compounds built-up by carbon dioxide assimilation (Lichtenthaler et al., 2005). A valuable parameter of the photosynthetic performance is the effective quantum yield of photosystem II (Φ), which correlates with the proportion of the absorbed light energy that under the given growth conditions can be used after conversion in the production of new metabolites. When St. John's wort plantlets were treated separately with Tria and BA, in both cases a moderate, but statistically significant increase of the effective quantum yield could be registered, while its highest value (approaching 0.8) was reached in plants that have received both growth regulators simultaneously (**Fig. 4**). This is one more evidence for the synergism between Tria and BA in stimulating metabolic processes, such as photosynthetic light conversion into chemical energy. The capacity of Tria to enhance photosynthetic processes, especially under environmental stress conditions which impair the photochemical reactions or modify the chemical composition of thylakoid membranes in chloroplasts, was also demonstrated in certain crop plant species (Godoy et al., 2021; Tompa et al., 2022).

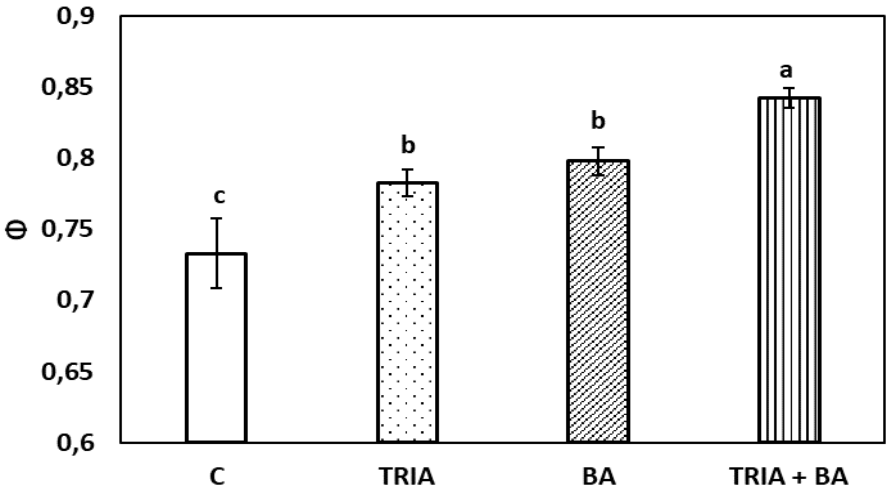

Fig. 4. Influence of 1 µM triacontanol (TRIA), 2 µM benzyladenine (BA) and their combination (TRIA + BA) on the effective quantum yield of photosystem II (Φ) in leaves of St. John's wort plantlets. C – control group. Vertical bars show the ± standard errors from means (n = 7), and the different letters represent significant differences at $p < 0.05$

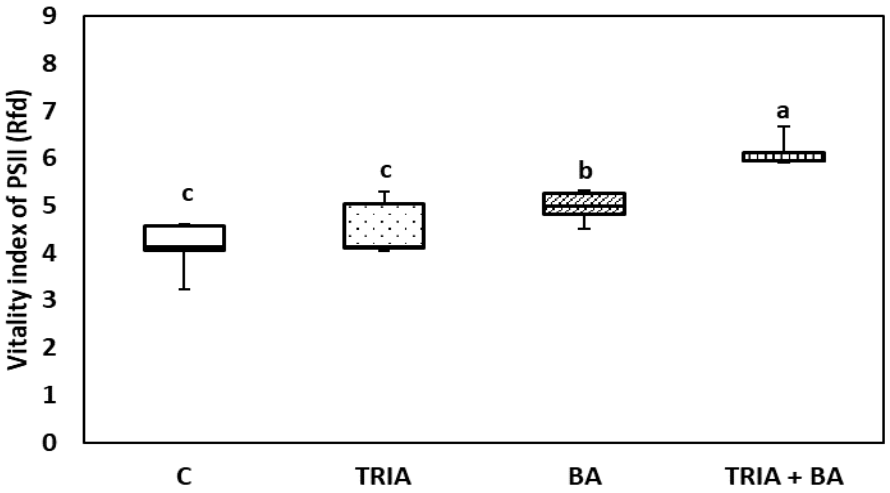

Fig. 5. Vitality index of the photosynthetic apparatus in leaves of St. John's wort plantlets treated with 1 µM triacontanol (TRIA), 2 µM benzyladenine (BA) and their combination (TRIA + BA). C – control group; PSII – photosystem II; Rfd – relative fluorescence decrease, obtained from measurements of induced chlorophyll fluorescence. Vertical bars show the ± standard errors from means (n = 7), and the different letters represent significant differences at $p < 0.05$

For BA there is also evidence for its beneficial influence on the development of the photosynthetic apparatus of leaves (Haisel et al., 2006), but we could not find any evidence on the possible interrelations between Tria and BA concerning any step of the photosynthetic process.

Under certain conditions (prolongued illumination of leaves with oversaturating white light beams), parameters of the induced chlorophyll fluorescence may be used to evaluate the overall functionality of the photosynthetic apparatus under the given developmental conditions (Lichtenthaler et al., 2005), with the determination of a physiological marker known as the vitality index of photosystem II (PSII), expressed as the relative fluorescence decrease (Rfd). When St. John's wort plants were treated separately with Tria and BA, only BA led to a significant increase in the vitality index of PSII, but when Tria was combined with BA, this increment was even more pronounced (Rfd increased from about 4 in the control group to 5 under the influence of BA and further to the value of 6 in the leaves of plants receiving both BA and Tria, **Fig. 5**). This is another parameter that demonstrates the existence of an interaction between Tria and BA in the up-regulation of photosynthetic processes, in this case Tria further enhances the stimulating capacity of BA.

Influences on carotenoid pigment content

Changes in the photosynthetic pigment content of leaves are usually reliable biochemical markers of many external influences exerted on plant metabolism. Treatments with Tria and BA did not cause any significant change in the chlorophyll *a* and chlorophyll *b* content of St. John's wort leaves (their interval remaining around 1.67 ± 0.24 mg g^{-1} for chlorophyll *a* and 0.56 ± 0.04 mg g^{-1} for chlorophyll *b*, data not shown), but the carotenoid pigment content was increased with the same degree by all of the three types of treatments (with Tria, with BA and with their combination).

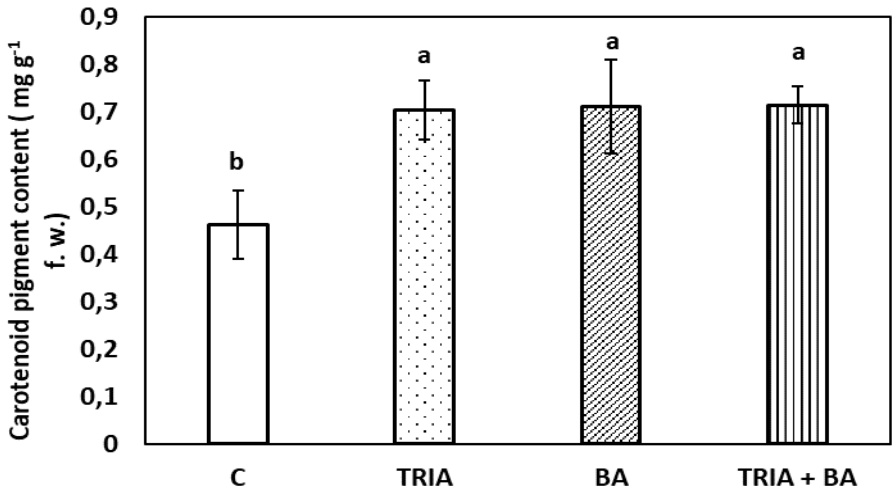

Fig. 6. Influence of 1 µM triacontanol (TRIA), 2 µM benzyladenine (BA) and their combination (TRIA + BA) on the carotenoid pigment content in leaves of St. John's wort plantlets. C – control group; f.w. – fresh weight. Vertical bars show the ± standard errors from means (n = 7), and the different letters represent significant differences at $p < 0.05$

When applied together, Tria and BA did not lead to a more increased carotenoid content than in the case when they were applied separately on different plants, indicating that these two bioactive compounds do not interact in the metabolism of carotenes and xantho-phylls (**Fig. 6**). The increment of carotenoid pigment content could be beneficial under very high photon flux densities and under several other environmental stress conditions which induce photooxidative damage in the photo-synthetic apparatus. It was demonstrated that carotenoids act as valuable non-enzymatic antioxidants by protecting the photosynthetic membranes from severe damages caused by over-generation of singlet oxygen and other reactive oxygen species (Garcia-Garcia et al., 2020). In experiments conducted with spinach, 1 µM Tria did not induce an increment in the carotenoid pigment content of leaves, but managed to restore the original carotenoid content in plants exposed to salt stress generated with 150 mM sodium chloride (Tompa et al., 2022). In the case of BA, experiments with bean, tobacco, sugar beet and maize plantlets showed that 10 µM BA could increase the chlorophyll pigment content of chloroplasts when plants were exposed to drought stress. The increment caused by water deficiency in the content of the carotenoids implied in the protective xanthophyll cycle was further enhanced when plantlets were treated with BA, demonstrating its implication in the stimulation of the protective capacity of carotenoids against photooxidative damages (Haisel et al., 2006).

Conclusions

Interaction between exogenously applied triacontanol and benzyladenine enhances photo-synthetic performance and biomass production of St. John's wort plantlets, beyond the stimulation exerted separately by the two growth regulators. Shoot height and carotenoid pigment content of leaves are also increased both by 1 µM Tria and 2 µM BA, but the combined treatment with these compounds does not lead to further stimulation of stem elongation and accumulation of carotenoids. Synergism of Tria and BA in the up-regulation of specific metabolic and developmental processes may be exploited to optimize the

cultivation of this medicinal plant and this may be a prerequisite to increase the content of active compounds in the herbal product. For this later purpose further investigations are needed concerning the influence of different concentrations of triacontanol and benzyl-adenine, applied separately or together, on more physiological processes that are relevant for the secondary metabolic pathways involved in the biosynthesis of the desired pharmaco-logically active products.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. Avato P, Raffo F, Guglielmi G, Vitali C, Rosato A (2004) Extracts from St. John's wort and their antimicrobial activity. Phytother Res 18(3):230–232. doi: 10. 1002/ptr.1430
2. Edwards SE, Rocha IC, Williamson EM, Heinrich M (2015) Phytopharmacy: an evidence-based guide to herbal medicinal products. John Wiley & Sons Ltd, Singapore, pp 335–339
3. El-Ghamery AA, Mousa MA (2017) Investigation on the effect of benzyladenine on the germination, radicle growth and meristematic cells of *Nigella sativa* L. and *Allium cepa* L. Ann Agric Sci 62(1):11–21. doi: 10.1016/j.aoas.2016.11.002
4. Garcia-Garcia AL, Garcia-Machado FJ, Borges AA, Morales-Sierra S, Boto A, Jimenez-Arias D (2020) Pure organic active compounds against abiotic stress: a biostimulant overview. Front Plant Sci 11:575829. doi: 10.3389/fpls.2020.575829
5. Godoy F, Olivos-Hernandez K, Stange C, Handford M (2021) Abiotic stress in crop species: improving tolerance by applying plant metabolites. Plants 10:186–195. doi: 10.3390/ plants10020186
6. Haisel D, Pospisilova J, Synkova H, Schnablova R, Batkova P (2006) Effects of abscisic acid or benzyladenine on pigment contents, chlorophyll fluorescence and chloroplast ultrastructure during water stress and after rehydration. Photosyn-thetica 44(4):606–614. doi: 10.1007/ s11099-006-0079-5
7. Hoagland DR, Arnon DI (1950) The water-culture method for growing plants without soil. Calif Agric Exp Stat Circ 347:1–32
8. Hwang I, Sheen J, Muller B (2012) Cytokinin signaling networks. Annu Rev Plant Biol 63: 353–380. doi: 10.1146/ annurev-arplant-042811-105503
9. Islam S, Mohammad F (2020) Triacontanol as a dynamic growth regulator for plants under diverse environmental conditions. Physiol Mol Biol Plants 26(5):871–883. doi: 10.1007/ s12298-020-00815-0
10. Karakas O, Toker Z, Tilkat E, Ozen HC, Onay A (2009) Effects of different concentrations of benzylaminopurine on shoot regeneration and hypericin content in *Hypericum triquetrifolium* Turra. Nat Prod Res 23(16):1459–1465. doi: 10.1080/ 14786410701664528
11. Kasper S, Caraci F, Forti B, Drago F, Aguglia E (2012) Efficacy and tolerability of Hypericum extract for the treatment of mild to moderate depression. Eur Neuropsych 20(11):747–756. doi: 10.1016/ j.euroneuro.2010.07.005
12. Li S-M, Zheng H-X, Zhang X-S, Sui N (2021) Cytokinins as central regulators during plant growth and stress response. Plant Cell Rep 40:271–282. doi: 10.1007/ s00299-020-02612-1

13. Lichtenthaler HK, Buschmann C, Knapp M (2005) How to correctly determine the different chlorophyll fluorescence parameters and the chlorophyll fluorescence decrease ratio Rfd of leaves with PAM fluorometer. Photosynthetica 43:379–393. doi: 10.1007/s11099-005-0062-6
14. Mortensen T, Shen S, Shen F, Walsh MK, Sims RC, Miller CD (2012) Investigating the effectiveness of St. John's wort herb as an antimicrobial agent against mycobacteria. Phytother Res 26(9):1327–1333. doi: 10.1002/ptr.3716
15. Naeem M, Masroor M, Khan A, Moinuddin M (2012) Triacontanol: a potent growth regulator in agriculture. J Plant Interact 7(2):129–142. doi: 10.1080/17429145.2011. 619281
16. Nahrstedt A, Butterweck V (2010) Lessons learned from herbal medicinal products: the example of St. John's wort. J Nat Prod 73(5):1015–1021. doi: 10.1021/np.1000329
17. Oshchepkov MS, Kalistratova AV, Savelieva EM, Romanov GA, Bystrova NA, Kochetkov KA (2020) Natural and synthetic cytokinins and their applications in biotechnology, agrochemistry and medicine. Russ Chem Rev 89(8):787-810. doi: 10.1070/RCR4921
18. Rademacher W (2015) Plant growth regulators: background and uses in plant production. J Plant Growth Regul 34:845–872. doi: 10.1007/s00344-015-9541-6
19. Russo E, Scicchitano F, Whalley BJ, Mazzitello C, Ciriaco M, Esposito S, Patane M, Upton R, Pugliese M, Chimirri S, Mammi M, Palleria C, De Sarro G (2014) *Hypericum perforatum*: pharmacokinetics, mechanism of action, tolerability and clinical drug-drug interactions. Phytother Res 28(5):643–655. doi: 10. 1002/ptr.5050
20. Shrivastava M, Dwivedi LK (2015) Therapeutic potential of *Hypericum perforatum*: a review. Intl J Pharmac Sci Res 6(12):1000–1007. doi: 10.13040/IJPSR. 0975-8232.6(12). 1000.07
21. Tompa B, Fodorpataki L (2021) Influence of triacontanol and salt stress on the growth and metabolism of spinach. Acta Univ Sap Agric Environ 13:65–76. doi: 10.2478/ ausae-2021- 0006
22. Tompa B, Balint J, Fodorpataki L (2022) Enhancement of biomass production, salinity tolerance and nutraceutical content of spinach (*Spinacia oleracea* L.) with the cuticular wax constituent triacontanol. J Appl Bot Food Qual 95:121–128. doi: 10.5073/JABFQ.2022. 095.00X
23. Tsukagoshi S, Yamori W (2020) Beneficial effects of various environmental stresses on vegetables and medicinal plants for the production of high value-added plants. In: Pessarakli M (ed) Handbook of plant and crop stress. CRC Press, Boca Raton, USA, pp 909–918
24. Wellburn AR (1994) The spectral determination of chlorophylls *a* and *b*, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. J Plant Physiol 144:307–313. doi: 10.1016/S0176-1617-81192-2
25. Zaid A, Asgher M, Wani IA, Wani SH (2020) Role of triacontanol in overcoming environmental stresses. In: Roychoudhury A, Tripathi DK (eds) Protective chemical agents in the amelioration of plant abiotic stress. John Wiley & Sons Ltd, Croydon, UK, pp 491–509.