IECAG 2021

Proceedings

Effects of exogenous natural or synthetic auxins on tomato transplant production†

Moncada A. [1]**, Vetrano F.* and Miceli A.**

[1] Dipartimento di Scienze agrarie, Alimentari e Forestali, Università di Palermo, Viale delle Scienze 4, 90129 Palermo; alessandra.moncada@unipa.it; alessandro.miceli@unipa.it
* Correspondence: filippo.vetrano@unipa.it (F.V.); Tel.: +39 091 238 62219 (A.M.); +39 091 238 62229 (F.V.)
† Presented at the 1st International Electronic Conference on Agronomy, 3–17 May 2021; Available online: https://sciforum.net/conference/IECAG2021

Abstract: The research aimed to evaluate the use of synthetic or natural auxins on the growth of tomato seedlings. The seeds of *Solanum Lycopersicum* "Marmande" were sown in polystyrene plug plant trays (104 cells). Two doses of natural or synthetic exogenous auxins (200 ppm and 100 ppm) were supplied to the substrate through the irrigation water with an ebb and flow system 3, 11, and 17 days after sowing (05, 11, and 13th BBCH growth stage, respectively). A commercial biostimulant based on *Ecklonia maxima* extracts (Basfoliar® Kelp SL Compo) was used as a source of natural auxin while 1-naphthaleneacetic acid NAA was used as a synthetic auxin. Seedlings supplied only with water were used as a control. The treatments had significant effects on many morphological and physiological parameters (plant height, stem diameter, plant fresh and dry weight, leaf number and area, stomatal conductance, plant water use, and water use efficiency). Seedlings treated with both doses of exogenous auxin provided via *Ecklonia maxima* extracts increased their fresh and dry weight by 31% and 37% respectively and were taller and leafier than the control seedlings. The use of NAA had a negative effect on plant height and stem fresh and dry weight but did not alter the other morpho-physiological parameters as compared to the control seedlings. The treatments with auxins from algae extract during nursery growth improved the performance of tomato seedlings but the benefits could be probably ascribed not only to auxins themselves but to the synergic effect of the other organic compounds contained in the product (amino acids, proteins, carbohydrates, and vitamins)

Keywords: Vegetable nursery; Plant growth regulators; *Solanum Lycopersicum*; Seedlings; *Ecklonia maxima*; Basfoliar® Kelp

Citation: Lastname, F.; Lastname, F.; Lastname, F. Title. *Proceedings* **2021**, *68*, x. https://doi.org/10.3390/xxxxx

Published: date

1. Introduction

The supply of fertilizers to vegetable plants begins in the nursery for transplant production where the plug plant trays with small volumes of substrate poor in nutrients require an accurate fertility management to achieve well developed seedlings that can guarantee a good performance after transplanting. The administration of fertilizers on plants raised in containers with a small volume of substrate requires daily irrigations that can leech the fertilizers determining severe environmental issues [1]. The use of plant growth promoters or biostimulants could be a sustainable technique that allows the production of high-quality transplants even with the reduction of mineral fertilizers [2]. Plant growth promotion can be achieved using microorganisms (bacteria, mycorrhizae, or other fungi), organic (algae extracts, humic acids, protein hydrolysates) or synthetic (plant growth regulators) products which may exert different functions (biofertilizers, biocontrol, phytostimulator, etc.) [3,4]. The effects of plant growth promoters are mainly linked to hormonal effects or hormonal homeostasis determined by the presence or the production of compounds with plant growth regulator effects (phytohormones) of natural and/or synthetic origin. Among the phytohormones, auxins are universally recognized as rooting hormones and synthetic auxins (IAA, IBA, NAA) are commonly used in the propagation by cuttings. Recent researches have shown that seed priming with synthetic hormones improves seed germination, seedling vigor, and promotes plant height and growth after transplanting even in strong stress condition [1,5–7]. A wide range of mechanism related to plant development and growth could be controlled by exogenous auxin supplementation. These effects may vary according to the species or the growth stage but generally the application of auxins reduces root elongation and concurrently stimulates lateral root formation [8]. The exogenous synthetic auxins increase both the surface area and length of lateral roots [9,10], and this can improve seedling growth and transplant quality as a well-developed root system has greater access to water and substrate nutrients allowing a proper growth of the shoot, lessening transplanting stress and speeding up post-transplanting growth. Increasing agricultural practice sustainability is one of the more actual goal of agriculture and the use of natural sources of plant growth regulators could help achieving this goal. Thus, the research aimed to evaluate the use of synthetic or natural auxins on the nursery growth of tomato transplants.

2. Materials and Methods

A nursery trial was carried out in a greenhouse situated at the Department of Agricultural, Food, and Forest Sciences (University of Palermo, Italy; 38°6′28″ N 13°21′3″ E; altitude 49 m above sea level) during autumn 2020. Seeds of *Solanum lycopersicum* 'Marmande' (Vilmorin, La Ménitré, France) were sown (27 October 2020) into polystyrene trays (104 cells) filled with a commercial substrate (SER CA-V7 Special semine, Vigorplant Italia srl, Fombio, Italy, containing 800 g m^{-3} of a mineral fertilizer NPK 12-11-18). The trays were kept in the dark at 22 - 24 °C until seed germination and were then moved onto benches in the greenhouse for seedling growth. The substrate was treated with 200 ppm or 100 ppm of natural or synthetic exogenous auxins three times: when the radicle emerged from seed, the first true leaf was fully open and the 3rd leaf was unfolded (5-11-13th BBCH growth stage) [11]. The auxin solutions were supplied through the irrigation water by an ebb and flow system. A control treatment was obtained supplying only water throughout the seedling growth The source of synthetic auxin was 1-naphthaleneacetic acid (NAA) (Cifo Radicante in polvere NUFARM ITALIA Srl, Milano, Italia), whereas the natural source of auxins was a commercial biostimulant based on *Ecklonia maxima* extracts (Basfoliar® Kelp SL, Compo Expert Italia Srl, Cesano Maderno (MB), Italia) that contains 11 mg L^{-1} of natural auxins as well as amino acids, carbohydrates, proteins, vitamins and trace of cytokinins (0.04%). Two fertilization were performed by sub-fertigating the trays with a nutrient solution of 2 g L^{-1} of a water-soluble NPK fertilizer (20-20-20) after 13 and 20 days from sowing. Plantlets were also sub-irrigated as needed until they were ready for transplant (twice a week on average including fertigation).

During seedling growth, plant water use (PWU) and plant water use efficiency (WUE) were calculated from the amount of water consumed by the plug plants, measured for each irrigation and fertigation event by weighing all the seedling trays with a digital balance before refilling the reservoir and after drainage of the exceeding nutrient solution. Plant water use was calculated as fresh biomass produced per unit of fertigation water, PWU (g FW L^{-1} H_2O) = plant fresh weight (g)/H_2O (L), and plant water use efficiency was calculated as the dry weight production expressed per liter of applied fertigation water, WUE (g DW L^{-1} H_2O) = plant dry weight (g)/H_2O (L). From these last values and the volume of nutrient solution consumed by the plants, the total N uptake during the crop cycle was estimated and the Nitrogen Use Efficiency [12] was calculated as NUE (g DW g^{-1} N) = plant total dry weight (g)/plant N uptake (g).

Leaf stomatal conductance was measured one week before seedlings were ready for transplanting, using a diffusion porometer (AP4, Delta-T Devices Ltd., Cambridge, England) on two young unshaded leaves of 20 seedlings for each species and each replicate.

Leaf color of each seedling was measured on the upper part of 2 randomly selected leaves, using a colorimeter (CR-400, Minolta corporation, Ltd., Osaka, Japan) that measured L* (lightness), a* (positive values for reddish colors and negative values for greenish colors) and b* (positive values for yellowish colors and negative values for bluish colors). These components were used to calculate Hue angle (h°) and Chroma (C*) as $h° = 180° + \arctan(b^*/a^*)$ [13] and $C^* = (a^{*2} + b^{*2})^{1/2}$.

One month after sowing, the seedlings were ready for transplanting (14–15th BBCH growth stage), so four replicated samples of 20 seedlings for each treatment were randomly selected and analyzed to evaluate their morphological characteristics (stem diameter, seedling height and leaf number). Then, the seedlings were separated into leaves, stem, and roots, weighed, and dried to a constant weight at 85 °C to determine the fresh and dry biomass and the shoot/root ratio for both fresh and dry weight. Before drying, the leaves were scanned at 350 dpi (Epson Perfection 4180 Photo, Seiko Epson Corp., Suwa, Japan) to obtain digital images that were analyzed with the ImageJ 1.52a software (National Institutes Health, Bethesda, MD, USA) to measure the leaf area. The specific leaf area (SLA cm^2 g^{-1} DW) was estimated as the leaf area/leaf dry weight.

The experimental design consisted of four replicated samples of 20 seedlings each for every treatment, randomly assigned in four blocks. A one-way ANOVA was performed to evaluate the effects of natural and synthetic auxins treatments on tomato seedlings. The mean values were compared by the least significant differences (LSD) test at $p \leq 5\%$ to identify the significant differences among treatments.

3. Results

During the experiment, the average temperature outside the greenhouse ranged between 10.1 ± 0.3 °C (night) and 31.4 ± 0.6 °C (day), and the average net solar radiation at noon was 451 W·m^{-2}, with a day length that ranged between 7 and 8 h. Inside the greenhouse, the air temperature was on average 17.6 ± 1.7 °C and ranged between 38.8 °C (day) and 10.2 °C (night), whereas the relative humidity was 81.3 ± 1.4% and ranged between 72.5% and 100%; the light intensity at noon was 39063 ± 2451 lux and ranged from 58728 to 1286 lux as a function of the cloudiness.

All the morphological parameters were influenced by the treatments. The height of the plants and the diameter of the stem were greater than control using 100 ppm of natural auxins from algae extract (18.1 cm and 2.65 mm, respectively), whereas both level of NAA reduced by 33% on average compared to the control the height of the seedlings but had no significant effect on stem diameter (Table 1).

Table 1. Effect of natural (AE Algae Extract from Basfoliar® Kelp SL) and synthetic (NAA) exogenous auxin on morphological parameters of tomato seedlings.

Source of auxin (ppm)	Height (cm)	Stem (mm)	Fresh Weight (g seedling $^{-1}$)					Dry Weight (mg seedling $^{-1}$)					Dry matter %
			Total	Leaves	Stem	Root	Shoot/root	Total	Leaves	Stem	Root	Shoot/root	
Control	**15.7 b**	**2.47 bc**	**1.77 b**	**0.57 b**	**1.01 b**	**0.22 c**	**7.2 ab**	**117.7** c	50.0 bc	45.0 b	22.7 cd	4.2 a	6.6 b
AE 200	17.1 ab	2.58 ab	2.40 a	0.78 a	1.28 a	0.33 a	6.2 bc	171.8 a	70.3 a	63.5 a	38.0 a	3.5 b	7.2 a
NAA 200	12.2 c	2.36 c	1.72 b	0.60 b	0.77 c	0.29 ab	4.7 d	114.8 c	53.5 b	36.8 c	24.5 c	3.7 ab	6.7 ab
AE 100	18.1 a	2.65 a	2.43 a	0.79 a	1.38 a	0.28 ab	7.7 a	161.4 b	67.3 a	63.5 a	30.7 b	4.3 a	6.6 b
NAA 100	12.0 c	2.53 b	1.61 b	0.59 b	0.79 c	0.26 bc	5.5 cd	101.9 d	46.5 c	34.8 c	20.7 d	3.9 ab	6.3 b

Data within a column followed by the same letters do not differ significantly at $p \leq 0.05$ according to LSD Test. Significance: ns = not significant. All percentage data were subjected to angular transformation.

Total fresh plant weight (FW) was on average 2.41 g in the tomato seedlings treated with the algae extract, 29.5% higher than the other treatments. The main part of the biomass was represented by the stem and the leaves that were heavier than those of control and NAA-treated seedlings (0.78 g of fresh leaves and 1.33 g of fresh stem on average). The treatments with natural and synthetic auxins also affected the fresh weight of the roots which had a higher weight when treated with highest level of natural auxins (0.33 g seedling^{-1}) and gradually decreased to the lowest value (0. 22 g seedling^{-1}) recorded in control seedlings. The modification of fresh biomass partitioning due to the treatments was shown by the modification of shoot/root fresh weight ratio (Table 3). The seedlings with a higher S/R were those treated with the lower dose of algae extract (7.67); for the others, especially for the plants treated with NAA at the maximum dose, the allocation of biomass was greater in the roots. The treatments also affected the dry weight (DW) of tomato seedlings. Those treated with natural auxins at the highest dose weighed 171.75 mg DW seedling^{-1} (+46.0% than control) due to the highest dry biomass of leaves (70.25 mg seedling^{-1}), stem (63.5 mg seedling^{-1}) and roots (38 mg seedling^{-1}), followed by the treatment with 100 ppm of natural auxins (161.42 mg seedling^{-1}) that recorded also the highest dry biomass in leaves and stem. The seedlings treated with 200 ppm of NAA had a significantly lower dry biomass accumulation and did not differ from control, whereas the lowest dose of NAA accumulated 13.4% less total dry biomass than control, mainly due to the reduction of stem dry biomass (-22.7% than control). Regarding the shoot/root ratio, the higher proportion of roots was collected with the Basfoliar treatment at 100 ppm (4.26) and with the control seedling (4.19). The dry matter percentage was significantly higher than control only with the highest dose of natural auxins (7.17%).

The seedlings with the highest stomatal conductance were those of control treatment and those treated with NAA (343.1 mmol m^2 s^{-1}, on average), while the use of natural auxins determined a significant reduction (50%) of the stomatal conductance (171.5 mmol m^2 s^{-1} on average) (Table 2).

Table 2. Effect of natural (AE Algae Extract from Basfoliar® Kelp SL) and synthetic (NAA) exogenous auxin on stomatal conductance, plant water use (PWU), water use efficiency (WUE) and nitrogen use efficiency (NUE) of tomato seedlings.

Source of auxin (ppm)	Stomatal conductance (mmol m^2 s^{-1})		PWU (g FW L^{-1} H_2O)		WUE (g DW L^{-1} H_2O)		NUE (g DW g^{-1} N)	
Control	**361.4**	**a**	**50.6**	c	3.4	c	9.2	c
AE 200	171.0	b	67.0	a	4.8	a	12.9	a
NAA 200	289.0	a	54.4	bc	3.6	c	9.8	b
AE 100	172.0	b	65.8	a	4.4	b	12.0	a
NAA 100	378.9	a	57.4	b	3.6	c	9.8	b

Data within a column followed by the same letters do not differ significantly at $p \leq 0.05$ according to LSD Test. Significance: ns = not significant.

The control seedlings produced 50.6 g of fresh biomass with one liter of water; treating the seedlings with 100 or 200 ppm natural auxins increased by 31% fresh biomass per liter of water consumed on average, while a lower increase of PWU was recorded using NAA (+ 10% on average for 100 and 200 ppm of NAA). The WUE increased as increasing the level of natural auxins by 29.4% (100 ppm) and 41.2% (200 ppm) compared to control seedlings (3.4 g DW L^{-1} H_2O) whereas this parameter was not affected by NAA (3.6 g DW L^{-1} H_2O on average for 100 and 200 ppm NAA). NUE was highest in the tomato seedling treated with algae extract (12.4 g DW g^{-1} N on average, +34.6% than control). The NAA treatments had a lower effect on NUE but increased by 6.1% on average this parameter compared to the control (Table 2).

The highest number of leaves (5 leaves plant^{-1} on average) and the greatest leaf area per plant (44.33 cm^2 plant^{-1}) were found in tomato seedling treated with natural auxins from algae extracts (Table 2); no differences were observed among the other treatments (4.56 leaves plant^{-1} and 33.19 cm^2 plant^{-1} on average) (Table 3). The chromatic characteristics of the tomato leaves (L*, Chroma and Hue) were influenced by the treatments. The leaf color of NAA-treated tomato seedlings was lighter (L* 50.5 on average) than the other treatments but did not significantly differ from control seedlings as regards Chroma and Hue angle.

On the contrary, the seedlings treated with auxins from the algae extract had leaves with a more greenish (higher Hue angle) and a less vivid color (lower Chroma) (Table 3).

Table 3. Effect of natural (AE Algae Extract from Basfoliar® Kelp SL) and synthetic (NAA) exogenous auxin on the leaf characteristics of tomato seedling.

Source of auxin (ppm)	Number of leaves		Leaf area (cm^2 plant^{-1})		L*		Chroma		Hue	
Control	4.55	b	34.47	b	49.09	b	39.03	ab	123.15	bc
AE 200	5.00	a	44.61	a	49.02	b	37.26	b	124.41	a
NAA 200	4.60	b	33.09	b	50.40	a	39.47	ab	121.92	c
AE 100	4.98	a	44.05	a	48.70	b	37.50	b	124.00	ab
NAA 100	4.53	b	32.02	b	50.72	a	40.72	a	121.93	c

Data within a column followed by the same letters do not differ significantly at $p \leq 0.05$ according to LSD Test. Significance: ns = not significant.

4. Discussion

All the analyzed parameters of the tomato seedlings (stomatal conductivity, PUE, WUE, NUE, morphological parameters) were positively influenced by the radical treatment with auxins from *Ecklonia maxima* extracts while the treatment with synthetic NAA auxin determined no effect or some negative effects with the lowest NAA level.

Cellular auxin levels depend on the rate of anabolism, catabolism, transport and conjugation at any given time in a tissue. *Ecklonia maxima* contains phenolic compounds that influence the metabolism and concentration of active forms of auxins in plants [14–17]. Particularly, phenolic inhibitors of auxin-oxidase such as phenolic compounds, chlorogenic acid and rutin have been shown to enhance auxin activities [18]. Some of these compounds act as alternative substrates for oxidative enzyme and thus protect auxins from oxidative breakdown. Aremu et al., [19] stated that *Ecklonia maxima*-treated plants had more endogenous auxin. According to Wilson and Van Staden [20], some phenolics may protect auxins from decarboxylation thereby enhancing the concentration of active forms of this hormone. In our study, the effect of NAA was not very influential; we think that the explanation of a better answer to the *Ecklonia maxima* treatments is due to a synergistic action of the auxin content and of all the other components of the product: amino acids, proteins, carbohydrates and vitamins. In conclusion, the results showed that the supplementation of natural auxins from *Ecklonia maxima* extract improved tomato seedling quality and promoted shoot and root growth, whereas the same quantity of a synthetic auxin have no effect or a negative effect. Thus, the positive effect of the natural source of auxins could be probably ascribed not only to auxins themselves but to the synergic effect of the other organic compounds contained in the algae extract (amino acids, proteins, carbohydrates, and vitamins).

Author Contributions: authors contributed equally.

Funding: This research received no external funding.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Miceli, A.; Vetrano, F.; Moncada, A. Effects of Foliar Application of Gibberellic Acid on the Salt Tolerance of Tomato and Sweet Pepper Transplants. *Horticulturae* **2020**, *6*, 93.
2. Moncada, A.; Miceli, A.; Vetrano, F. Use of plant growth-promoting rhizobacteria (PGPR) and organic fertilization for soilless cultivation of basil. *Sci. Hortic. (Amsterdam).* **2021**, *275*, 109733, doi:https://doi.org/10.1016/j.scienta.2020.109733.
3. Zandi, P.; Basu, S.K. Role of Plant Growth-Promoting Rhizobacteria (PGPR) as BioFertilizers in Stabilizing Agricultural Ecosystems. In *Organic Farming for Sustainable Agriculture*; Nandwani D., Ed.; Springer, 2016; pp. 71–87.
4. Singh, J.S.; Pandey, V.C.; Singh, D.P. Efficient soil microorganisms: a new dimension for sustainable agriculture and environmental development. *Agric. Ecosyst. Environ.* **2011**, *140*, 339–353.
5. Tsegay, B.A.; Andargie, M. Seed Priming with Gibberellic Acid (GA 3) Alleviates Salinity Induced Inhibition of Germination and Seedling Growth of Zea mays L., Pisum sativum Var. abyssinicum A. Braun and Lathyrus sativus L. *J. Crop Sci. Bio-*

technol. **2018**, *21*, 261–267.
6. Spaepen, S.; Vanderleyden, J.; Remans, R. Indole-3-acetic acid in microbial and microorganism-plant signaling. *FEMS Microbiol. Rev.* **2007**, *31*, 425–448.
7. Vacheron, J.; Desbrosses, G.; Bouffaud, M.-L.; Touraine, B.; Moënne-Loccoz, Y.; Muller, D.; Legendre, L.; Wisniewski-Dyé, F.; Prigent-Combaret, C. Plant growth-promoting rhizobacteria and root system functioning. *Front. Plant Sci.* **2013**, *4*, 356.
8. Davies, P.J. *Plant hormones: biosynthesis, signal transduction, action!*; Springer Science & Business Media, 2004; ISBN 1402026854.
9. Li, Z.; Zhang, X.; Zhao, Y.; Li, Y.; Zhang, G.; Peng, Z.; Zhang, J. Enhancing auxin accumulation in maize root tips improves root growth and dwarfs plant height. *Plant Biotechnol. J.* **2018**, *16*, 86–99.
10. Pitts, R.J.; Cernac, A.; Estelle, M. Auxin and ethylene promote root hair elongation in Arabidopsis. *Plant J.* **1998**, *16*, 553–560.
11. Feller, C.; Bleiholder, H.; Buhr, L.; Hack, H.; Hess, M.; Klose, R.; Meier, U.; Stauss, R.; Boom, T. van den; Weber, E. Phanologische Entwicklungsstadien von Gemusepflanzen II. Fruchtgemuse und Hulsenfruchte. *Nachrichtenblatt des Dtsch. Pflanzenschutzdienstes* **1995**, *47*, 217–232.
12. Baligar, V.C.; Fageria, N.K. Nutrient Use Efficiency in Plants: An Overview. *Nutr. Use Effic. From Basics to Adv.* **2015**, *32*, 1–14, doi:10.1007/978-81-322-2169-2_1.
13. McGuire, R.G. Reporting of Objective Color Measurements. *HortScience HortSci 27*, 1254–1255, doi:10.21273/HORTSCI.27.12.1254.
14. Peer, W.A.; Murphy, A.S. Flavonoids and auxin transport: modulators or regulators? *Trends Plant Sci.* **2007**, *12*, 556–563.
15. Yuan, C.L.; Mou, C.X.; Wu, W.L.; Guo, Y. Bin Effect of different fertilization treatments on indole-3-acetic acid producing bacteria in soil. *J. Soils Sediments* **2011**, *11*, 322–329, doi:10.1007/s11368-010-0315-2.
16. De Klerk, G.-J.; Guan, H.; Huisman, P.; Marinova, S. Effects of phenolic compounds on adventitious root formation and oxidative decarboxylation of applied indoleacetic acid in Malus 'Jork 9.' *Plant Growth Regul.* **2011**, *63*, 175–185.
17. da Silva, J.A.T.; Dobránszki, J.; Ross, S. Phloroglucinol in plant tissue culture. *Vitr. Cell. Dev. Biol.* **2013**, *49*, 1–16.
18. Gaspar, T.; Kevers, C.; Penel, C.; Greppin, H.; Reid, D.M.; Thorpe, T.A. Plant hormones and plant growth regulators in plant tissue culture. *Vitr. Cell. Dev. Biol.* **1996**, *32*, 272–289.
19. Aremu, A.O.; Masondo, N.A.; Rengasamy, K.R.R.; Amoo, S.O.; Gruz, J.; Bíba, O.; Šubrtová, M.; Pěnčík, A.; Novák, O.; Doležal, K.; et al. Physiological role of phenolic biostimulants isolated from brown seaweed Ecklonia maxima on plant growth and development. *Planta* **2015**, *241*, 1313–1324, doi:10.1007/s00425-015-2256-x.
20. Wilson, Pj.; Staden, J. van Rhizocaline, rooting co-factors, and the concept of promoters and inhibitors of adventitious rooting-a review. *Ann. Bot.* **1990**, *66*, 479–490.