Plant Physiology and Biochemistry 159 (2021) 37–42

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Short communication

Improvement of moss photosynthesis by humic acids from Antarctic tundra soil

Mi Young Byun [a], Dockyu Kim [a,*], Ui Joung Youn [a,b], Seulah Lee [a], Hyoungseok Lee [a,b,**]

[a] Division of Life Sciences, Korea Polar Research Institute, Incheon, 21990, Republic of Korea
[b] Polar Science, University of Science & Technology, Incheon, 21990, Republic of Korea

ARTICLE INFO

Keywords:
Antarctic
Humic acids
Humic substances
Photosynthetic activity
Plant growth promoting

ABSTRACT

There have been several published reports regarding the growth promoting effect of humic acids (HA) on vascular plants; however, the effect of HA on bryophytes is still unknown. Due to the ecological importance of mosses, which dominate the Antarctic flora, we assessed the effectiveness of HA as a biostimulant using three moss species: Antarctic *Ceratodon purpureus* KMA5038, Arctic *Bryum* sp. KMR5045, and *Physcomitrella patens* which inhabits temperate regions. Natural HA (KS1-3_HA) were extracted through acidic precipitation of alkaline extracts from Antarctic tundra soil. Spectroscopic structural properties of KS1-3_HA were characterized and determined to possess several functional groups such as hydroxyl (R–OH) and carboxyl (R–COOH), implying they could have a growth-related biological function. For two polar mosses, increasing HA concentrations correlated with increased growth and photosynthesis. The efficiency for temperate moss increased at lower concentrations tested, but rather began to reduce at the highest HA concentration, indicating that effective concentrations of HA vary depending on the moss species and habitat. Based on these results, Antarctic HA may have ecological role in enhancing the growth and photosynthesis of Antarctic mosses. We believe this is the first study to establish a positive physiological effect of HA on mosses and hope it may serve as a basis for studying the role of HA in preserving the terrestrial ecosystem of Antarctica.

1. Introduction

Enormous amounts of soil organic matter (SOM) is stored in the Arctic and Antarctic tundra soils as compared to temperate regions due to low-level microbial degradative activity resulting from low air temperatures (Davidson and Janssens 2006). Humic substances (HS), which are formed by condensation of the lignin and small organic compounds originating from dead biota, are the largest constituent of SOM. The detailed structure of HS depends on both the plant and soil sources available and the specific formation conditions, yet the average properties and characteristics are remarkably similar (Abakumov and Alekseev 2018; García et al., 2019). The International Humic Substances Society (IHSS) defines HS as complex and heterogeneous mixtures of polydisperse materials, some of which have aromatic nuclei with hydroxyl (-OH), carbonyl (C═O), and carboxyl (-COOH) groups. These functional groups are the major contributors to surface charges, the reactivity of HS, and are responsible for the cation exchange capacity of soils (Lipczynska-Kochany 2018; García et al., 2019). HS are classified into three major groups based on their solubility in acids: the most insoluble humin, insoluble humic acids (HA), and soluble fulvic acids (Lipczynska-Kochany 2018).

HS in polar tundra soils are composed of materials with low lignin content, which are mostly derived from grasses, mosses, and lichens (Abakumov and Alekseev 2018). Although their structure is large and complex, which leads to a rather slow microbial HS decomposition rate, HS are thought to be inherently highly sensitive to temperature rising, as their activation energy for decomposition is higher (Lehmann and Kleber 2015). Therefore, rising temperatures associated with global climate change may accelerate HS decomposition rates in polar tundra soils (Park et al., 2015; Kim et al., 2018). Polymeric HS and HS-derived small compounds, produced through microbial decomposition, regulate the growth of plants and microorganisms through various and continuous interactions within soils (Lehmann and Kleber 2015; Lipczynska-Kochany 2018). Considering their large contribution to SOM, higher

Abbreviations: commercial HA, CHA; humic acids, HA; humic substances, HS; soil organic matter, SOM.

* Corresponding author.
** Corresponding author. Division of Life Sciences, Korea Polar Research Institute, Incheon, 21990, Republic of Korea.
E-mail addresses: envimic@kopri.re.kr (D. Kim), soulaid@kopri.re.kr (H. Lee).

https://doi.org/10.1016/j.plaphy.2020.12.007
Received 28 August 2020; Accepted 7 December 2020
Available online 9 December 2020
0981-9428/© 2020 Elsevier Masson SAS. All rights reserved.

temperature sensitivity, and conformational flexibility, even small increase in the HS decomposition rate can result in a significant change in the soil ecosystem of the polar tundra. However, little attention has been given to the ecological impact of increasing temperatures in the maritime Antarctic tundra on HS decomposition.

Plant growth regulation is critical to the production of cultivated crops (Bechtold and Field 2018). Biostimulants may be utilized in the crop production process to increase yields (Parađiković et al., 2018) and HS have been widely recognized as effective biostimulants providing a broad range of useful functions for plants (Canellas and Olivares 2014; Shah et al., 2018) based on their unique characteristics (Trevisan et al., 2010). Several studies have focused on the various physiological and biochemical properties of these materials and attempted to explain their importance on soil fertility. The diverse benefits of HS include chelating metal ions (Canellas et al., 2015) and improving root development and morphology. Specifically, HS promote lateral root formation and dry root weight (Trevisan et al., 2010) through mediation of phyto-regulation at the root system (Jannin et al., 2012; Olaetxea et al., 2016). In the rhizosphere, HS release structural molecules that affect hormone bioactivity (Canellas and Olivares 2014) and trigger a variety of molecular processes in plant cells, enhancing the plant's tolerance to abiotic stresses (García et al., 2016; Van Oosten et al., 2017). On the other hand, HS also can enhance shoot growth without releasing structural molecules by affecting the natural hormonal balance in roots through a complex signaling network (Mora et al., 2014).

Recent studies analyzed the effects of HA on increase of maize root biomass, through protein profiling, which displayed the changes of the energy metabolism-associated proteins (Nunes et al., 2019). In addition, proteomics analysis of *Arabidopsis* roots treated with HS also represented the stimulation in energy metabolism and protein synthesis (Roomi et al., 2018). It has been suggested that HS regulate plant growth through a complex and sophisticated mechanism partially linked to auxin activity, but also via auxin-independent signaling pathways (Canellas and Olivares 2014). These studies revealed HS-induced changes in plant cells but have not revealed their mode of action.

Bryophytes are an intermediary between primitive algae and terrestrial plants and have important evolutionary significance (Prigge and Bezanilla 2010). They are informally referred to as mosses and have a simple structure and short life history, making them ideally suited for studying the mechanism and functions of signaling pathways (Kofuji and Hasebe 2014). In the Antarctic, where there are few flowering plants, bryophytes dominate the flora and are important primary producers (Ochyra et al., 2008). However, studies on the effects of HA on plants have been limited to horticultural crops and flowering plants, and the effect of HA on bryophytes is unknown. In this study, we examined the growth-promoting effects of HA on mosses, including Antarctic and Arctic species, and a moss species from temperate regions that has served as a model organism. We investigated the structural similarity between KS1-3_HA derived from Antarctica and commercially available HA and applied both HA sources to three moss species and evaluated their effect on growth and photosynthetic efficiency.

2. Materials and methods

2.1. Soil sampling and HA extraction

A soil sampling site covered with lichens and mosses, designated KS1 (=KGL04-03, 62°13′ 47″ S, 58°46′ 54″ W), was selected at Kaya Hill near the King Sejong Station on Barton Peninsula of King George Islands, maritime Antarctica. HS-rich tundra soil (KS1-3) was collected in January of 2015. The sample was subjected to extraction using 0.5 N NaOH solution. Insoluble HA was precipitated by acidification (pH 2.0 with 5.0 N HCl) of the NaOH extract. Finally, Antarctic natural HA (KS1-3_HA) was obtained by freeze-drying the pelleted HA paste (Kim et al., 2018). Commercial HA (CHA) purchased from Sigma-Aldrich (Cat. No. 53680) was used as a control.

2.2. Structural characterization of HA

The structures of KS1-3_HA and CHA were examined by Fourier-transform infrared (FTIR) and solid-state ^{13}C-nuclear magnetic resonance (NMR) spectroscopy. The FTIR spectrum was recorded on a Thermo Scientific Nicolet 6700 FTIR spectrometer using a potassium bromide disk prepared from powdered HA (2%, w/w) mixed with dry KBr. The one-dimensional solid-state cross-polarization magic angle spinning ^{13}C nuclear magnetic resonance (^{13}C-CP/MAS NMR) spectrum of powdered HA was measured with a Bruker Avance 500 MHz system. Elemental analyses (C, H, O, N, and S) were performed with each powdered HA by Flash2000 (Thermo Fisher Scientific).

2.3. Moss growth conditions

Ceratodon purpureus KMA5038 was collected in the Antarctic, near the King Sejong Station in January of 2015. *Bryum* sp. KMR5045 was collected in the Arctic, in the vicinity of the Arctic Dasan Station (78°55′ N, 11°56′ E) on Ny-Ålesund, Spitsbergen Island in the Svalbard archipelago, Norway in July of 2014. The dried gametophore tissues were sterilized with 0.2% (v/v) NaClO solution and washed with distilled water several times. Sterilized tissues of KMA5038, KMR5045, and *Physcomitrella patens* were put on Knop solid media [1.84 mM KH_2PO_4, 2.08 mM $MgSO_4 \cdot 7H_2O$, 3.35 mM KCl, 4.23 mM $Ca(NO_3)_2 \cdot 4H_2O$, 45 μM $FeSO_4 \cdot 7H_2O$]. Protonema derived from the gametophores were subcultured using the T 10 basic ULTRA-TURRAX (IKA). For the analysis of the influence of HA on moss growth, ground protonema was dotted onto media in a 24-well plate (catalog No. 30024, SPL Life Sciences, Korea), supplemented with various concentrations (0–0.05%, w/v) of KS1-3_HA and CHA, in 0.1 N NaOH. Plates were covered and sealed with plastic wrap to prevent drying, and then incubated at 20 °C with 16 h/8 h light/dark cycles for 3 weeks.

2.4. Chlorophyll fluorescence measurements

Prior to chlorophyll fluorescence measurement, each plate containing moss was transferred to acclimate for 30 min in the dark chamber and submitted to the chlorophyll fluorescence system using Maxi-Imaging-PAM (WALZ, Germany) to measure the maximum (Fm) and minimum (Fo) fluorescence. For rapid light curves (RLC), plates were exposed to 13 intensities of actinic light: 0, 11, 21, 36, 56, 81, 111, 146, 186, 231, 281, 336, and 396 μmol photons m^{-2} s^{-1}, each with a 30 s irradiance step. Chlorophyll fluorescence parameters were extracted from the digital images using analytical software (Imaging Win, Walz, Germany). For each level of actinic light, Fv/Fm and the relative electron transport rate (rETR) from the delivered actinic irradiance and $rETR = E \times \Delta F/Fm' \times 0.84 \times 0.5$ were calculated (Liu et al., 2020). The experiments were conducted four biologically independent experiments for each moss species.

2.5. Statistical analysis

Data were analyzed by Excel and GraphPad Prism 8.4.3 software. Data were expressed as mean ± standard deviation from four technical replicates. The analysis of variance (multifactor ANOVA) was followed by the mean comparison test ($p < 0.05$, Tukey-HSD).

3. Results and discussion

3.1. Structural characterization of CHA and KS1-3_HA

HA contain aromatic and aliphatic compounds, amino acids, and polysaccharide residues. The aromatic rings carry two functional groups, hydroxyl (R–OH) and carboxyl (R–COOH), probably the most important players in plant nutrition and growth (García et al., 2019). Commercial HA and KS1-3_HA had similar FTIR spectral patterns with respect to several major functional groups (Fig. 1A): 3500–3000 cm^{-1} for R–COOH, R–OH and R–NH, 3000–2800 cm^{-1} for aliphatic C–C groups, 1725–1600 cm^{-1} for C=O of R–COO–R′ and R–CO–R′ groups, 1320–1200 cm^{-1} for C–O of R–OH and R–COOH, and 1180–1000 cm^{-1} for C–O of R-O-R' (Ribeiro et al., 2001; Kar et al., 2011). It seems likely that the presence of R–OH and R–COOH as the main groups in HAs contributes to the structural similarity between KS1-3_HA and CHA, and confers their ability to complex with ions such as Mg^{2+}, Ca^{2+}, Fe^{2+}, Fe^{3+}, Cu^{2+}, Zn^{2+}, and Mn^{2+}. The higher cation exchange capacity in soil that results from such complexes could, in turn, increase the interaction with cations and the bioavailability of metal ions important for plant nutrition (García et al., 2019).

Solid-state NMR spectra contain a wealth of information about molecular properties, allowing researchers to detect different constituents in amorphous materials such as HA, and to predict differences or similarities in biological functions between two materials. As shown in Fig. 1B, the solid-state ^{13}C-NMR spectra patterns of CHA and KS1-3_HA were similar. These were, specifically, 0–50 ppm (aliphatic area), 50–100 ppm [OCH-R, OCH_2-R, R-(Cl, Br), and R–N], 120–140 ppm [protonated aromatic (Ar–Hs) and unsaturated (R–C=C-R′) carbons], 145–160 ppm [Ar-O-R (R = H or alkyls) carbons], 160–190 ppm (R–COO–R′ carboxylic groups), and 190–230 ppm (R–CO–R′ carbonylic groups). HAs from the two sources differed in that KS1-3_HA had stronger carbon signals at 50–100 ppm and 160–180 ppm, while CHA had stronger signals at 120–140 ppm. The lower content of aromatic fragments in KS1-3_HA agrees with an earlier finding that HAs derived from Antarctic soils have a lower proportion of aromatic fragments than those from temperate climates (Abakumov and Alekseev 2018). We assume that the KS1 soil sample contains HA that is typical for polar tundra soil under mosses. Both FTIR and ^{13}C-NMR spectroscopy showed that, despite differences in peak intensities of R–OH and R–COOH (R = alkyls or Ars) functional groups, KS1-3_HA and CHA share similar structures.

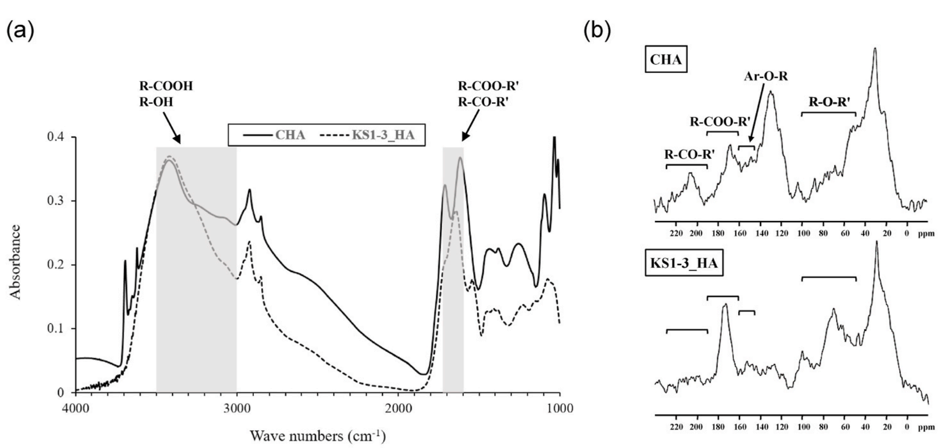
To further confirm the similarity between components of CHA and KS1-3_HA, elemental analysis was performed considering five elements (nitrogen, carbon, hydrogen, sulphur, and oxygen) (Fig. 1C). Among the analyzed elements, carbon was measured as a major component in both samples (42% and 36% in CHA and KS1-3_HA, respectively). Similar levels of hydrogen, sulphur, and oxygen were detected between CHA and KS1-3_HA, while somewhat higher amount of nitrogen was present in KS1-3_HA. Considering the comparable amounts of carbon, hydrogen, and oxygen, which account for the majority of elemental composition of HA, the acquired data unequivocally support that KS1-3_HA has element contents highly similar to those of CHA.

3.2. Effects of HAs on growth and photosynthesis of mosses

We examined the effects of HA from the Antarctic region on growth and photosynthesis on three moss species: an Arctic moss *Bryum* sp. KMR5045, an Antarctic moss *C. purpureus* KMA5038, and the model moss *P. patens*, which is adapted to temperate climates. The growth and photosynthetic efficiency of moss species were observed and compared in response to the addition of HA to the growth medium. CHA was used as a control to compare the activity of KS1-3_HA. Maximum quantum yield (Fv/Fm) and the electron transport rate (ETR) parameters were used to represent the photosynthetic efficiency of each treatment. As shown in Fig. 2, KS1-3_HA positively affected growth of all three mosses.

Photosynthesis is a critical metabolic process in plants and serves as the source by which plants acquire the energy they need to live (Hohmann-Marriott and Blankenship 2011). HA can promote biochemical and molecular changes that impact photosynthesis in various types of plants. Exogenous HA have been reported to improve photosynthesis and antioxidant defense in maize seedling (Zhang et al., 2014). Also, HA derived from sediments had positive effects on photosynthesis and chloroplast ultrastructure in chrysanthemum (Fan et al., 2014) and creeping bentgrass exhibited enhanced net photosynthesis rates in response to HA application (Liu et al., 1998). Furthermore, HA treatment improved photosynthesis efficiency of rapeseed plants under water stress conditions by enhancing the gas exchange and electron transport flux (Lotfi et al., 2018). Finally, HA promoted photosynthesis and yield of *Plantago ovate*, an important medicinal plant, under high salinity condition (Gholami et al., 2013). These results suggest damages induced by abiotic stress can be mitigated by application of HA.

When KS1-3_HA was included in the medium, the biomass of Arctic *Bryum* sp. and Antarctic *C. purpureus* appeared to increase compared to the control group. However, quantitative analysis of these results was very difficult because the biomass was so small that it was impossible to weigh the moss. In addition, as the HA concentration increased, the background color of the medium became dark, which hindered the visual inspection of the growth of moss (Fig. 2a–c). Therefore, Fv/Fm and RLC were investigated to find out the effect of HA on photosynthesis of mosses. Compared to the control, Fv/Fm was significantly higher in the moss grown in HA containing medium for all three species. The Fv/Fm pattern according to the HA concentration was slightly different for each



(c)

(unit: wt%)

Sample	C	H	O	N	S	atomic N/C	atomic H/C	atomic O/C
CHA	42.2	3.2	26.4	0.9	0.3	0.02	0.08	0.63
KS1-3_HA	35.9	4.4	27.8	3.6	0.4	0.10	0.12	0.77

Fig. 1. FTIR (A) spectra, ^{13}C-NMR (B) spectra, and elemental compositions (C) of KS1-3_HA and CHA. The gray shaded boxes in (A) and square brackets in (B) indicate the common functional groups between KS1-3_HA and CHA. Each calculated element content (wt%) in (C) is a mean of two measurements.

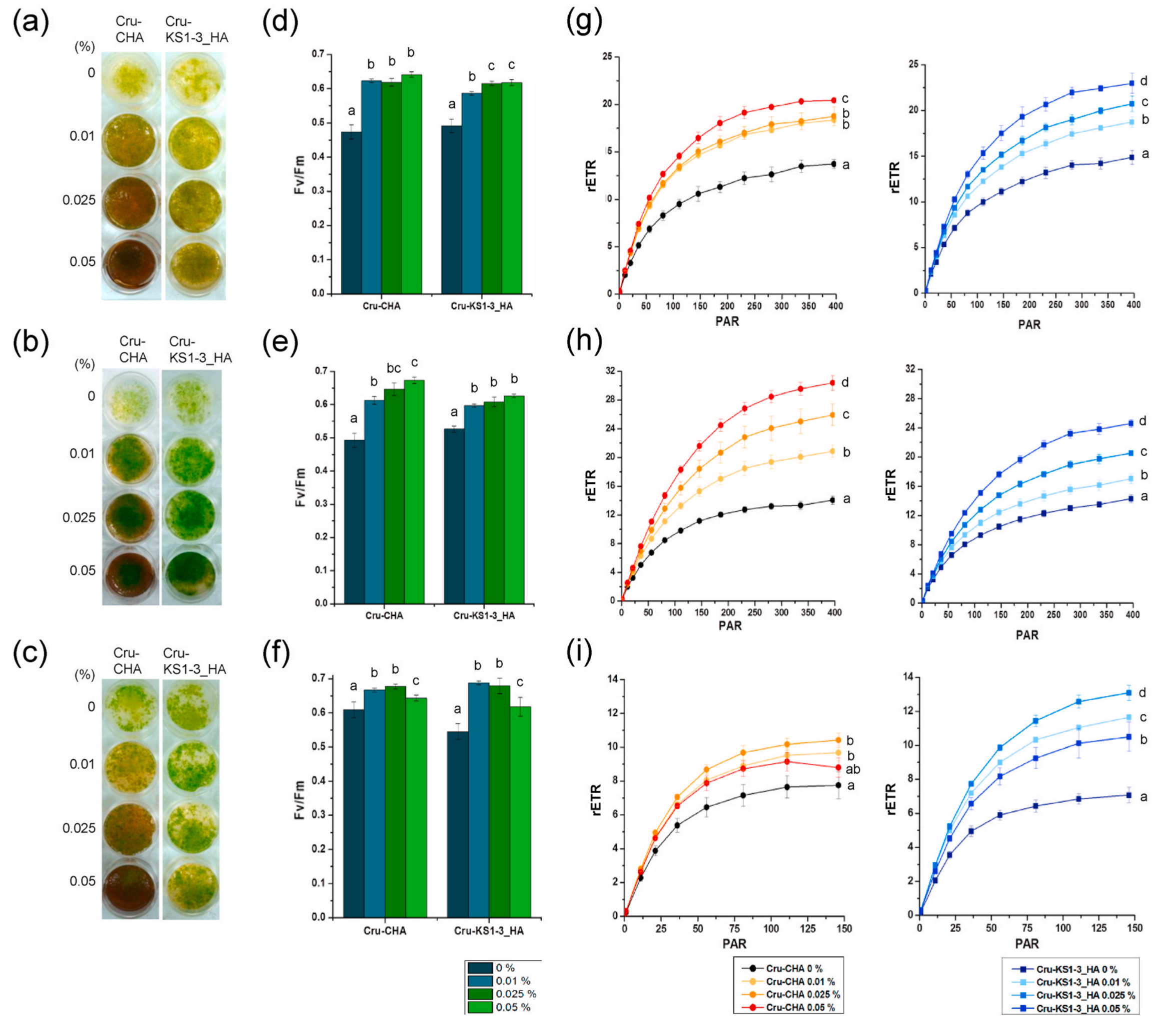


Fig. 2. Effects of HA on the growth and photosynthetic efficiency of three moss species from different regions. Changes of the growth and photosynthetic parameters of Arctic *Bryum* sp. KMR5045 (a, d, and g), Antarctic *Ceratodon purpureus* KMA5038 (b, e, and h), and temperate *Physcomitrella patens* (c, f, and i). Photosynthetic parameters were displayed as maximum quantum yield (Fv/Fm) and the relative electron transport rate (rETR) analyzed using chlorophyll fluorescence system. Prefix Cru-refers to crude HS solution in 0.1 N NaOH, neither degraded nor transformed. Different lowercase letters show a significance differentiated by ANOVA followed by Tukey's test, $p < 0.05$ ($n = 4$).

species. As the HA concentration increased, the Fv/Fm values of Arctic *Bryum* sp. and Antarctic *C. purpureus* were gradually increased or remained elevated (Fig. 2d–e). In the case of *P. patens*, however, Fv/Fm values increased similarly to polar moss up to 0.025% HA, but when the HA increased to 0.05%, the values decreased (Fig. 2f). Moreover, a significant increase in RLC and rETR by HA treatment was also observed in 3 mosses in a manner similar to Fv/Fm (Table 1; Fig. 2g-i).

Canellas et al. (2015) summarized reports on the ability of HS to act as biostimulants on horticultural crops. Various sources and doses of HA had variable effects on soil fertility and yield of rice plants (Mindari et al., 2018). Also, in two species of *Pinus*, nitrogen assimilation displayed distinct responses to HS, suggesting that the different environmental conditions these two species inhabit may be related to their divergent responses (Panuccio et al., 2001). These previously published data indicate that effective concentrations of HA vary depending on the species of plants. As shown in Fig. 2, KS1-3_HA had different growth promoting effects depending on the moss species. The substance showed positive correlations between concentration and photosynthetic yield for *Bryum* sp. and *C. purpureus*, but photosynthetic efficiency was reduced at the highest concentrations for *P. patens*. Consistent with prior studies, these results demonstrate that effective HA concentrations may be species-dependent.

HA promotes plant growth by triggering physiological and

Table 1
Effect of different concentrations of CHA and KS1-3_HA on ETR at the last step of rapid light curves for *Bryum* sp. KMR5045, *Ceratodon purpureus* KMA5038, and *Physcomitrella patens*.

HA Concentration	*Bryum* sp. KMR5045		*Ceratodon purpureus* KMA5038		*Physcomitrell patens*	
	CHA	KS1-3_HA	CHA	KS1-3_HA	CHA	KS1-3_HA
0%	13.73 ± 0.46^{a}	15.20 ± 0.92^{a}	13.65 ± 0.96^{a}	14.30 ± 0.54^{a}	7.78 ± 0.79^{a}	7.03 ± 0.42^{a}
0.01%	18.35 ± 0.06^{b}	18.93 ± 0.60^{b}	20.83 ± 0.88^{b}	17.03 ± 0.71^{b}	9.65 ± 0.51^{ab}	10.48 ± 0.78^{b}
0.025%	18.63 ± 0.98^{b}	21.15 ± 1.09^{c}	25.93 ± 1.52^{c}	20.38 ± 0.36^{c}	10.49 ± 0.37^{b}	11.61 ± 0.06^{c}
0.05%	20.73 ± 0.59^{c}	23.10 ± 0.94^{d}	29.68 ± 1.68^{d}	24.43 ± 0.56^{d}	8.78 ± 0.58^{b}	13.05 ± 0.41^{d}

Light intensity was 400 μmol m^{-2} s^{-1} for *Bryum* sp. KMR5045 and *C. purpureus* KMA5038, and 150 μmol m^{-2} s^{-1} for *P. patens*. Each value is mean ± standard deviation of four technical replications. Different lowercase letters show a significance differentiated by ANOVA followed by Tukey's test, $p < 0.05$ ($n = 4$), between treatments.

biochemical changes (Trevisan et al., 2010). Photosynthesis serves as the primary source by which plants acquire the energy they need (Hohmann-Marriott and Blankenship 2011). HAs have been reported to have positive effects on photosynthesis in previous reports. The application of HS at suitable concentrations alleviated damages induced by the salt stress in *Plantago ovate*, probably via the enhanced nutrient uptakes and induced physiological changes (Gholami et al., 2013). The FHA fertilizer improved the ultrastructure of the thylakoids, which accelerated the rate of photon to be absorbed then promoted the photosynthesis in chrysanthemum (Fan et al., 2014). Antarctica is an extreme environment and hostile to its flora dominated by bryophytes (Ochyra et al., 2008). HS, decomposed by microbes in the soil, is the only organic nutrient supplied to the terrestrial plants of Antarctica (Schmidt et al., 2011). In this study, HA from Antarctic soil had a positive effect on the photosynthetic efficiency of moss. It is not yet known whether this phenomenon is due to the enhancement of nutrient uptake or a change in the structure of chloroplast thylakoids in cells. Further works is needed to explore the mechanism, such as analysis of the organic matter composition of HA and mosses, and ultrastructural observation of chloroplast.

4. Conclusion

Antarctic tundra humic acids (KS1-3_HA) were characterized to possess several functional groups, such as Ar-OH and Ar-COOH, which were reported to be involved in growth promotion of vascular plants. When KS1-3_HA was assessed with three moss species inhabiting Antarctic, Arctic, and temperate regions, growth and photosynthesis of the mosses enhanced with increasing HA concentrations. However, it had different growth promoting effects at higher concentrations depending on the moss species, showing positive correlations between concentration and photosynthetic yield for the Antarctic and Arctic mosses. The photosynthetic efficiency of temperate moss was reduced at the highest concentrations. In conclusion, we investigated the effects of Antarctic HA on growth and photosynthesis of mosses, a dominant part of the polar flora. We suggest that Antarctic HA have a major ecological role in the moss ecosystem of the Antarctic by enhancing their growth and photosynthetic activity. To the best of our knowledge, this is the first study to establish a positive relationship between HA and the physiological response of mosses and may provide a basis for evaluating the role of HA in the terrestrial ecosystem of Antarctica. On the other hand, the results of this study are limited in that it is the approach in a controlled laboratory condition, subsequently it is insufficient to understand natural phenomena occurring in an actual ecosystem. In the next step, studies on the more detailed structure of HS existing in the Antarctic field, the relationship between its existence and the surrounding microflora, and the identification of substances that directly affect the growth of moss are necessary.

Contributions

Dockyu Kim and Hyoungseok Lee conceived and designed the study. Mi Young Byun and Dockyu Kim performed the experiments. Mi Young Byun, Dockyu Kim, Ui Joung Youn, Seulah Lee, and Hyoungseok Lee wrote, reviewed, and edited the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

5. Acknowledgements

This work was supported by a grant (PE20170) funded by the Korea Polar Research Institute.

References

Abakumov, E., Alekseev, I., 2018. Stability of soil organic matter in cryosols of the maritime Antarctic: insights from ^{13}C NMR and electron spin resonance spectroscopy. Solid Earth 9, 1329–1339. https://doi.org/10.5194/se-9-1329-2018.

Bechtold, U., Field, B., 2018. Molecular mechanisms controlling plant growth during abiotic stress. J. Exp. Bot. 69, 2753–2758. https://doi.org/10.1093/jxb/ery157.

Canellas, L.P., Olivares, F.L., 2014. Physiological responses to humic substances as plant growth promoter. Chem Biol Technol Agric 1, 3. https://doi.org/10.1186/2196-5641-1-3.

Canellas, L.P., Olivares, F.L., Aguiar, N.O., Jones, D.L., et al., 2015. Humic and fulvic acids as biostimulants in horticulture. Sci. Hortic. 196, 15–27. https://doi.org/10.1016/j.scienta.2015.09.013.

Davidson, E.A., Janssens, I.A., 2006. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. Nature 440, 165–173. https://doi.org/10.1038/nature04514.

Fan, H.M., Wang, X.W., Sun, X., Li, Y.Y., et al., 2014. Effects of humic acid derived from sediments on growth, photosynthesis and chloroplast ultrastructure in chrysanthemum. Sci. Hortic. 177, 118–123. https://doi.org/10.1016/j.scienta.2014.05.010.

Garcia, A.C., de Castro, T.A.V., Santos, L.A., Tavares, O.C.H., et al., 2019. Structure-property-function relationship of humic substances in modulating the root growth of plants: a review. J. Environ. Qual. 48, 1622–1632. https://doi.org/10.2134/jeq2019.01.0027.

Garcia, A.C., Santos, L.A., de Souza, L.G., Tavares, O.C., et al., 2016. Vermicompost humic acids modulate the accumulation and metabolism of ros in rice plants. J. Plant Physiol. 192, 56–63. https://doi.org/10.1016/j.jplph.2016.01.008.

Gholami, H., Samavat, S., Ardebili, Z.O., 2013. The alleviating effects of humic substances on photosynthesis and yield of Plantago ovate in salinity conditions. Int Res J Appl Sci 4, 1683–1686.

Hohmann-Marriott, M.F., Blankenship, R.E., 2011. Evolution of photosynthesis. Annu. Rev. Plant Biol. 62, 515–548. https://doi.org/10.1146/annurev-arplant-042110-103811.

Jannin, L., Arkoun, M., Ourry, A., Laine, P., et al., 2012. Microarray analysis of humic acid effects on Brassica napus growth: involvement of N, C and S metabolisms. Plant Soil 359, 297–319. https://doi.org/10.1007/s11104-012-1191-x.

Kar, S., Maity, J.P., Jean, J.S., Liu, C.C., et al., 2011. Role of organic matter and humic substances in the binding and mobility of arsenic in a Gangetic aquifer. J Environ Sci Health A Tox Hazard Subst Environ Eng 46, 1231–1238. https://doi.org/10.1080/10934529.2011.598796.

Kim, D., Park, H.J., Kim, J.H., Youn, U.J., et al., 2018. Passive warming effect on soil microbial community and humic substance degradation in maritime Antarctic region. J. Basic Microbiol. 58, 513–522. https://doi.org/10.1002/jobm.201700470.

Kofuji, R., Hasebe, M., 2014. Eight types of stem cells in the life cycle of the moss Physcomitrella patens. Curr. Opin. Plant Biol. 17, 13–21. https://doi.org/10.1016/j.pbi.2013.10.007.

Lehmann, J., Kleber, M., 2015. The contentious nature of soil organic matter. Nature 528, 60–68. https://doi.org/10.1038/nature16069.

Lipczynska-Kochany, E., 2018. Humic substances, their microbial interactions and effects on biological transformations of organic pollutants in water and soil: a review. Chemosphere 202, 420–437. https://doi.org/10.1016/j.chemosphere.2018.03.104.

Liu, C.H., Cooper, R.J., Bowman, D.C., 1998. Humic acid application affects photosynthesis, root development, and nutrient content of creeping bentgrass. Hortscience 33, 1023–1025. https://doi.org/10.21273/Hortsci.33.6.1023.

Liu, P.C., Peacock, W.J., Wang, L., Furbank, R., et al., 2020. Leaf growth in early development is key to biomass heterosis in Arabidopsis. J. Exp. Bot. 71, 2439–2450. https://doi.org/10.1093/jxb/eraa006.

Lotfi, R., Kalaji, H.M., Valizadeh, G.R., Behrozyar, E.K., et al., 2018. Effects of humic acid on photosynthetic efficiency of rapeseed plants growing under different watering conditions. Photosynthetica 56, 962–970. https://doi.org/10.1007/s11099-017-0745-9.

Mindari, W., Sasongko, P.E., Kusuma, Z., Syekhfani, et al., 2018. Efficiency of various sources and doses of humic acid on physical and chemical properties of saline soil and growth and yield of rice. In: Paper Presented at the AIP Conference Proceedings, vol. 1. https://doi.org/10.1063/1.5061854, 030001.

Mora, V., Bacaicoa, E., Baigorri, R., Zamarreno, A.M., et al., 2014. NO and IAA key regulators in the shoot growth promoting action of humic acid in Cucumis sativus L. J. Plant Growth Regul. 33, 430–439. https://doi.org/10.1007/s00344-013-9394-9.

Nunes, R.O., Domiciano, G.A., Alves, W.S., Melo, A.C.A., et al., 2019. Evaluation of the effects of humic acids on maize root architecture by label-free proteomics analysis. Sci. Rep. 9, 1–11. https://doi.org/10.1038/s41598-019-48509-2.

Ochyra, R., Lewis, S., Bednarek-Ochyra, H., 2008. The Illustrated Moss Flora of antarctica. Cambridge University Press.

Olaetxea, M., Mora, V., Garcia, A.C., Santos, L.A., et al., 2016. Root-shoot signaling crosstalk involved in the shoot growth promoting action of rhizospheric humic acids. Plant Signal. Behav. 11, e1161878 https://doi.org/10.1080/15592324.2016.1161878.

Panuccio, M.R., Muscolo, A., Nardi, S., 2001. Effect of humic substances on nitrogen uptake and assimilation in two species of pinus. J. Plant Nutr. 24, 693–704. https://doi.org/10.1081/Pln-100103663.

Parađiković, N., Teklić, T., Zeljković, S., Lisjak, M., et al., 2018. Biostimulants research in some horticultural plant species—a review. Food Energy Secur 8. https://doi.org/10.1002/fes3.162.

Park, H.J., Chae, N., Sul, W.J., Lee, B.Y., et al., 2015. Temporal changes in soil bacterial diversity and humic substances degradation in subarctic tundra soil. Microb. Ecol. 69, 668–675. https://doi.org/10.1007/s00248-014-0499-x.

Prigge, M.J., Bezanilla, M., 2010. Evolutionary crossroads in developmental biology: Physcomitrella patens. Development 137, 3535–3543. https://doi.org/10.1242/dev.049023.

Ribeiro, J.S., Ok, S.S., Garrigues, S., de la Guardia, M., 2001. FTIR tentative characterization of humic acids extracted from organic materials. Spectrosc. Lett. 34, 179–190. https://doi.org/10.1081/Sl-100002007.

Roomi, S., Masi, A., Conselvan, G.B., Trevisan, S., et al., 2018. Protein profiling of Arabidopsis roots treated with humic substances: insights into the metabolic and interactome networks. Front. Plant Sci. 9, 1812. https://doi.org/10.3389/fpls.2018.01812.

Schmidt, M.W., Torn, M.S., Abiven, S., Dittmar, T., et al., 2011. Persistence of soil organic matter as an ecosystem property. Nature 478, 49–56. https://doi.org/10.1038/nature10386.

Shah, Z.H., Rehman, H.M., Akhtar, T., Alsamadany, H., et al., 2018. Humic substances: determining potential molecular regulatory processes in plants. Front. Plant Sci. 9, 263. https://doi.org/10.3389/fpls.2018.00263.

Trevisan, S., Francioso, O., Quaggiotti, S., Nardi, S., et al., 2010. Humic substances biological activity at the plant-soil interface: from environmental aspects to molecular factors. Plant Signal. Behav. 5, 635–643. https://doi.org/10.4161/psb.5.6.11211.

Van Oosten, M.J., Pepe, O., De Pascale, S., Silletti, S., et al., 2017. The role of biostimulants and bioeffectors as alleviators of abiotic stress in crop plants. Chem Biol Technol Agric 4. https://doi.org/10.1186/s40538-017-0089-5.

Zhang, L.X., Lai, J.H., Gao, M., Ashraf, M., 2014. Exogenous glycinebetaine and humic acid improve growth, nitrogen status, photosynthesis, and antioxidant defense system and confer tolerance to nitrogen stress in maize seedlings. J. Plant Interact. 9, 159–166. https://doi.org/10.1080/17429145.2013.791379.