Brazilian Journal of Biology®

ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

Original Article

Seed priming with *Ascophyllum nodosum* (L.) Le Jolis extract macroalgae in soybean

Condicionamento de sementes com extrato de macroalga *Ascophyllum nodosum* (L.) Le Jolis em soja

A. F. R. Oliveira[a], M. P. da Silva[a], C. C. Santos[a*], E. C. de Oliveira Júnior[b], L. S. Novaes[c], L. O. M. Martins[a], H. L. dos Santos[a], J. M. Silverio[a] and S. P. Q. Scalon[a]

[a]Universidade Federal da Grande Dourados – UFGD, Faculdade de Ciências Agrárias, Dourados, MS, Brasil
[b]Serviço Nacional de Aprendizagem Industrial - SENAI, Laboratório de Tecnologia de Sementes, Dourados, MS, Brasil
[c]Centro Universitário da Grande Dourados - UNIGRAN, Departamento de Ciências Agrárias, Dourados, MS, Brasil

Abstract

Seed priming with biostimulant for soybean is a promising practice contributing positively to the physiological quality and vigor of seedlings, but there are little studies regarding protocols of bioinputs, such as *Ascophyllum nodosum* L. seaweed extract. We aimed to evaluate the effect of doses of *A. nodosum* macroalgae in seed priming and its impact on germination and seedling vigor of soybean. Seeds were subjected to priming with *A. nodosum* extract (ANE) at doses of 0, 1, 2, 3, 4, and 5 mL kg seed^{-1} during 15 min. Priming with ANE did not influence the first count and seed germination. Soybean seedlings from priming with 5 mL kg^{-1} ANE showed higher growth and shoot and root dry biomass. We observed increase 18% for shoot dry matter with priming of 5 mL kg^{-1} ANE compared to untreated seeds. The responses were variable for the emergence speed index in function ANE and did not influence the photochemical processes in photosystem II. Seed priming with ANE contributed in higher chlorophyll index. ANE showed a biostimulant effect on soybean seedlings, providing better growth and biomass characteristics, being promising in seed priming, but further studies are suggested in order to increase information regarding its use protocol for soybean.

Keywords: *Glycine max* L., bioinputs, biostimulant, germination, Photosystem II.

Resumo

O condicionamento de sementes com bioestimulante para soja é uma prática promissora contribuindo positivamente na qualidade fisiológica e vigor das plântulas, mas existem poucos estudos quanto a protocolos de bioinsumos como o extrato da alga *Ascophyllum nodosum* L. Objetivamos avaliar o efeito de doses de extrato de *A. nodosum* no condicionamento de sementes e seu impacto na germinação e vigor de plântulas de soja. As sementes foram submetidas ao condicionamento com extrato de *A. nodosum* (ANE) nas doses de 0, 1, 2, 3, 4 e 5 mL kg semente^{-1} durante 15 minutos. O condicionamento com ANE não influenciou a primeira contagem e a germinação das sementes. Plântulas de soja provenientes do condicionamento com 5 mL kg^{-1} de ANE tiveram maior crescimento e biomassa da parte aérea e da raiz. Observamos aumento de 18% na matéria seca da parte aérea com condicionamento de 5 mL kg^{-1} de ANE em comparação as sementes não tratadas. As respostas foram variáveis para o índice de velocidade de emergência em função do ANE e não influenciaram os processos fotoquímicos no fotossistema II. O condicionamento de sementes com ANE contribuiu em maior índice de clorofila. O ANE apresentou efeito bioestimulante em plântulas de soja, proporcionando melhores características de crescimento e biomassa, sendo promissor no condicionamento de sementes, mas sugerimos novos estudos com intuito de aumentar as informações quanto ao seu protocolo de uso para soja.

Palavras-chave: *Glycine max* L., bioinsumos, bioestimulante, germinação, Fotossistema II.

1. Introduction

Soybean [*Glycine max* (L.) Merr.] is the grain crop of greatest socioeconomic interest in the World. Currently, Brazil is considered the largest producer of soybean, with an estimated production for 2023 of 151.5 million tons, surpassing the previous harvest of 2021/2022 by 25.8 million tons more (CONAB, 2023). According to the Brazilian Institute of Geography and Statistics (IBGE, 2023), the 2021/2022 Season, Brazil has a planted area of 41,107.606 hectares and production that same year closed at 119,523.533 tons, obtaining average yield of 2.923 kg ha^{-1}.

*e-mail: cleber_frs@yahoo.com.br
Received: May 24, 2024 – Accepted: June 27, 2024

Considering the significant representation of soybean, studies are necessary to enhance its productivity, and seedling vigor is important characteristic in the formation for production stand. In this sense, seed priming with biostimulant or biofertilizer products is a promising practice, as it contributes to seed physiology, stimulating germination, emergence and vigor of seedlings (Cardarelli et al., 2022) through hormonal stimulation and participation of other compounds that act on plant metabolism (González-Pérez et al., 2022; Gupta et al., 2022; Villa e Vila et al., 2023).

In Brazil, Ministry of Agriculture and Livestock published Decree No. 10,375 (Brasil, 2020), which establishes the National Bioinputs Program, encouraging farmers to use products of plants, animal or microbial origin in their production processes, which, in addition to achieve sustainable development goals, also favors the bioeconomy. Among the commercial products that qualify as bioinputs, we can mention those based in seaweed extract (macroalgae).

Ascophyllum nodosum (L.) Le Jolis (Fucaceae, Phaeophyceae) is considered a rich source of organic matter (Soares et al., 2020; Ammar et al., 2022), and are found in greater quantities in the region of the North East Atlantic Ocean (Hurtado et al., 2008; Pereira et al., 2020). *A. nodosum* extract is used in agriculture, even in the organic systems (Silva et al., 2016), and is commercially available in liquid form through its extract or even in product formulations, which may or not contain other ingredients (Kumari et al., 2023). In composition of Acadian® and others products based of *A. nodosum* contains hormones, that stimulate germination (Mahapatra et al., 2022; Engel et al., 2023), in addition to elicitor bioactive compounds and readily available nutrients (Ma et al., 2022; Santos et al., 2023), promoted the growth. In literature, we did not find recommended doses for soybean seed with *A. nodosum*.

However, research regarding protocols of *A. nodosum* extract on soybean seeds and others crops priming for biostimulant purposes is still insufficient, starting from the ANE dose to be used, and we hypothesized that the use of ANE favors seed germination and the production of photoassimilates, and contributes the photochemical efficiency. We aimed to evaluate the effect of doses of *A. nodosum* extract on soybean seed priming and its impact on germination and seedling vigor.

2. Material and Methods

2.1. General characterization and seed priming with ANE

The experiments were conducted in the Laboratory and in field condition, being carried out in the Seed Technology Laboratory, of the Faculty of Technology of the National Service for Industrial Learning (Fatec SENAI), and the field trial was carried out at the Faculty of Agricultural Sciences (22° 11' 51.5" S, 54° 56' 04.3" W), in the Federal University of Grande Dourados (UFGD), both in Dourados – MS, Brazil.

Seeds cv. BMX FIBRA IPRO 64i61 (100% purity, Season 2022) were subjected to priming with commercially formulated *Ascophyllum nodosum* extract (ANE) Acadian® at doses of 0 (control), 1, 2, 3, 4, and 5 mL kg seed^{-1}. The design used was completely randomized, with four replications of 50 seeds. For the priming, the seeds were placed in plastic packaging, adding the corresponding dose (wet way), homogenizing them, and leaving them to rest for 15 minutes.

The *A. nodosum* extraction process was alkali extraction (Villa e Vila et al., 2023). The ANE was commercially formulated by Acadian® (Acadian Seaplants Ltd., Canada) is composed with fresh seaweed of *A. nodosum*, potassium hydroxide, 0.5% citric acid as complexing agent, and water, with the following specifications according to the manufacturer: appearance = dark brown viscous liquid; density = 1.16 g mL^{-1}, odor = marine; water solubility = 100%; pH = 8.0; organic carbon = 69.60 g L^{-1}, and K_2O= 61.48 g L^{-1}.

In addition, Polo and Mata (2018), described that Acadian contains the following chemical and amino acid composition according to the manufacturer: total organic matter, total nitrogen, phosphorous (P_2O_5) were 14.2, 0.34, and 0.3, respectively, expressed in %, and S= 3.950 ppm, Ca= 0.17 ppm, Mg= 230 ppm, Fe= 40.2 ppm, B= 64.5 ppm, Mn= 3.60 ppm and Zn= 7.48 ppm. According to their authors, the amino acid composition (%) were: alanine= 0.08, aspartic acid= 0.17, glutamic acid= 0.27, glycine= 0.06, isoleucine= 0.03, leucine= 0.08, lysine= 0.03, methionine= 0.02, phenylalanine= 0.05, proline= 0.06, tryptophan= 0.01, tyrosine= 0.03, valine= 0.06. Additionally, it contains fucoidans, mannitol, and alginic acid (Pandey et al., 2023) and betaines (Kumari et al., 2023).

2.2. Assessments

The following tests were carried out:

a) Germination – four repetitions of 50 seeds were used, which were placed on Germitest® paper moistened with distilled water equivalent to 2.5 times the matter of the dry paper. The paper rolls were kept in a BOD (Biochemical Oxygen Demand) chamber regulated at temperature of 25 ± 2 ºC in the presence of white light, and counting was performed at 8 days after sowing, in accordance with the percentage of normal seedling, i.e., shoot and root system development (Brasil, 2009).
b) First count - was conducted together with the germination test, which the paper rolls were placed in a B.O.D. chamber regulated at a temperature of 25 ± 2 ºC in the presence of white light. The counting of normal seedlings was performed at 5 days after sowing (Brasil, 2009).
c) Phenotypic responses: photographic records and visual observations of the seedlings were carried out 8 days after sowing.
d) Seedling length – ten seedlings were measured randomly, with the shoot length, determined by the distance from the collar to the apical bud and the length of the root determined by the measurement of the insertion to the tip of the primary root, using a millimeter ruler, results expressed in centimeters seedling^{-1}.
e) Seedlings matter – the seedlings were sectioned to separate shoots and roots, and weighed on a millesimal precision scale (0.0001 g). Subsequently, they were packed in Kraft paper bags and dried in an oven with

forced air circulation at 60 ºC ± 5, for 72 hours, and the dry matter were weighed on a precision scale, and the results expressed in grams seedling^{-1}.

The experiment under field conditions was carried in greenhouse with a 150 microns transparent plastic cover, in which seed previously treated with ANE doses similar to the laboratory procedure were sown in expanded polystyrene trays previously filled with coarse sand, in a completely randomized design, with four replications of 50 seeds. Irrigation was carried out daily in two shifts.

During the field test (August, 2023), the average values of maximum and minimum temperatures of 25 and 23 °C, and maximum and minimum relative humidity of 75 and 61%, respectively, were recorded using a thermo-hygrometer.

At the end of twelve days after sowing, the percentage of normal seedlings was calculated (Nakagawa, 1999) and assessments of chlorophyll index and photochemical processes in photosystem II.

f) Emergence measurements were carried out daily, until stabilization, which occurred ten days after sowing.
g) Emergence speed index – the number of normal emergency seedlings was counted daily and calculated using the Formula 1 proposed to Maguire (1962):

$$ESI = \frac{E1}{N1} + \frac{E2}{N2} + \ldots + \frac{En}{Nn} \quad (1)$$

Whereas:
ESI = emergency speed index.
E_1, E_2, ... E_n = number of normal seedlings computed in the first count, second, and in the last count.
N_1, N_2, ... N_n = number of days from sowing to the first, second and last count.

h) Chlorophyll index and photochemical processes – was measured in ten seedlings per replicate in each treatment using a portable chlorophyll meter SPAD 502 (Soil Plant Analyzer Development) in fully expanded leaves, from 8 to 11 am. Subsequently, the same leaves were subjected to dark conditions using adapter clips for 30 minutes. Using portable fluorometer OS-30p (Opti Sciences Chlorophyll Fluorometer, Hudson, USA), under flash 1,500 mmol photons m^{-2} s^{-1} the potential photochemical quantum efficiency of PSII - F_v/F_m was determined, and the absorbed energy conversion efficiency - F_v/F_0 and the maximum yield of non-photochemical processes - F_0/F_m were calculated.

2.3. Data analysis

The data were subjected to the Shapiro-Wilk normality test. All data were subjected to analysis of variance (F test, $p \leq 0.05$), and when significant, the means were compared using the Scott-Knott test $p \leq 0.05$ ± standard error, using SISVAR software.

3. Results

3.1. ANE on seed germination and soybean seedling vigor

We observed that soybean seedlings had better phenotypic responses of growth and formation of vegetative organs with increase in ANE doses on seed priming, since the emission and opening of cotyledons occurred, especially with 5 mL kg^{-1} ANE (Figure 1).

The first count and seed germination (normal seedlings) were not influenced by ANE doses (Figure 2a and 2b). We observed that root and shoot length (Figure 2c and 2d) was influenced by doses, with higher values (18.14 and 12.66 cm seedling^{-1}, respectively) with 5 mL kg^{-1} ANE, promoting an increase of 17 and 15%, respectively, compared to seedlings from untreated seed.

Shoot fresh matter was higher (0.693 g seedling^{-1}) in seedlings from priming with 5 mL kg^{-1} ANE (Figure 3a), while that of the root was higher in function of priming with 2 and 5 mL kg^{-1} ANE, with values of 0.171 and 0.185 g seedling^{-1}, respectively (Figure 3b). Shoot dry matter were not influenced by the ANE doses ($p > 0.05$) (Figure 3c), but observed increase 18% with priming with 5 mL kg^{-1} ANE compared to untreated seed. Root dry matter was higher (0.016 g seedling^{-1}) in seedlings from priming with 5 mL kg^{-1} ANE, statistically differing from the other doses (Figure 3d).

3.2. ANE on emergence, chlorophyll index, and photochemical processes in soybean seedling

The emergence of soybean seedlings was not influenced by ANE doses (Figure 4a), while the emergency speed index was influenced, with higher values without, and with 3 or 5 mL kg^{-1} ANE (Figure 4b). Chlorophyll index was lower only in seedlings from seeds untreated with ANE (Figure 4c), while the photochemical processes in photosystem II were not influenced by ANE doses (Figure 4d-e-f).

4. Discussion

We partially accept the initial hypothesis and although seed priming with ANE did not contribute to germination indicators of soybean, favored an increase in growth and

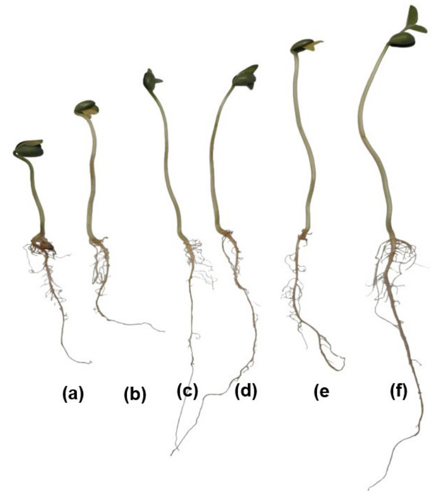

Figure 1. Phenotypic responses of soybean seedlings from cv. BMX FIBRA IPRO 64i61 treated with *Ascophyllum nodosum* extract. (a)= 0 mL kg^{-1}, (b)= 1 mL kg^{-1}, (c)= 2 mL kg^{-1}, (d)= 3 mL kg^{-1}, (e)= 4 mL kg^{-1}, (f)= 5 mL kg^{-1} ANE.

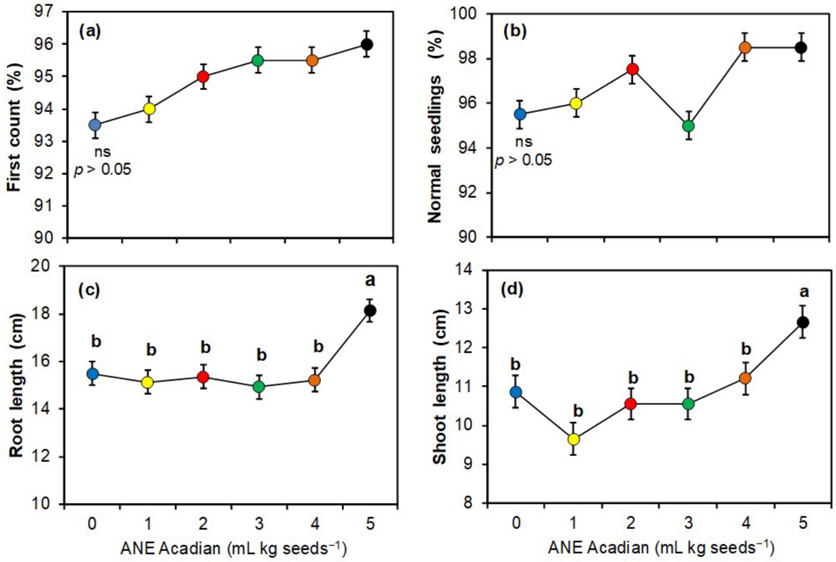

Figure 2. First count (a), normal seedlings (b), root length (c), and shoot length (d) of soybean seedlings from cv. BMX FIBRA IPRO 64i61 treated with *Ascophyllum nodosum* extract (ANE Acadian). Different letters between markers indicates significative effect by the Scott-Knott test ± standard error ($p \leq 0.05$). ns= not significant ($p > 0.05$).

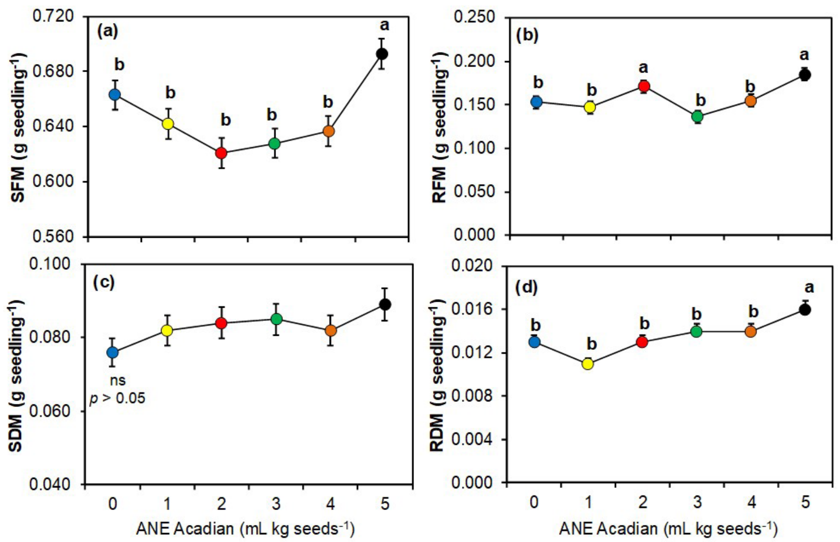

Figure 3. Shoot fresh matter – SFM (a) and roots fresh matter – RFM (b), shoot dry matter – SDM (c) and roots dry matter – RDM (d) in soybean seedlings from seeds cv. BMX FIBRA IPRO 64i61 treated with *Ascophyllum nodosum* extract (ANE Acadian). Different letters between markers indicates significative effect by the Scott-Knott test ± standard error ($p \leq 0.05$). ns= not significant ($p > 0.05$).

vigor. The fact that ANE did not influence germination characteristics may be associated with intrinsic aspects of the seed itself, i.e., its reserves and physiological and technological quality, since the minimum value of normal seedlings from untreated seeds was 95.5%, considered high. Considering the positive effects on phenotypic responses with 5 mL kg^{-1} ANE, demonstrating physiological and nutritional effect for seedlings, we suggest that new studies with this product testing higher doses be carried out, and we raise the following question – will the effect be positive, neutral or negative?

Conversely, the influence of ANE on increase in the ESI values may be associated with what has been reported in other studies due to the presence of hormones contained in macroalgae, such as cytokinin and auxin (Frioni et al., 2018; Bertoldo et al., 2023), and other bioactive compounds (Rajendran et al., 2022), which can accelerate root protrusion. However, we found in our study that the effect

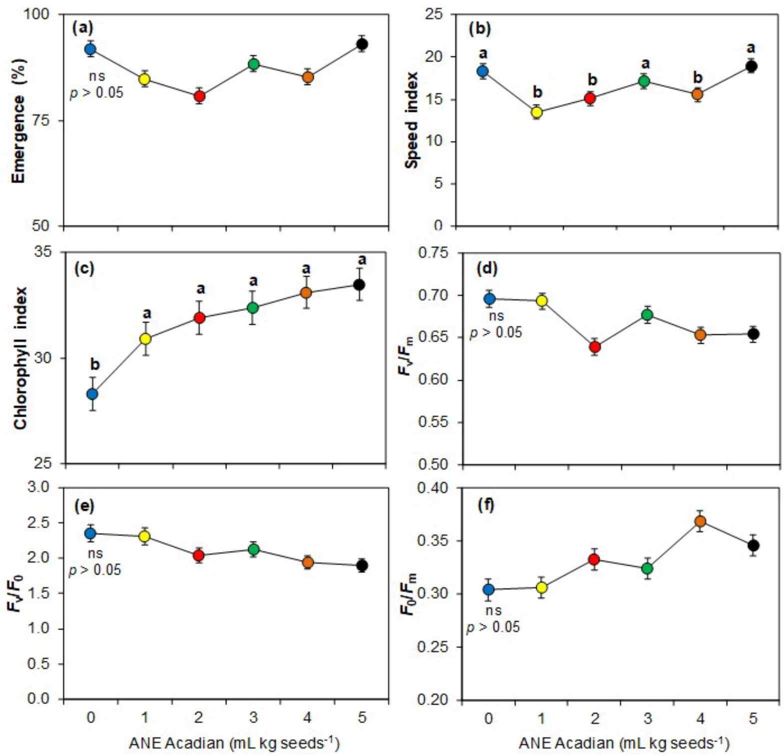

Figure 4. Emergence (a), emergence speed index (b), chlorophyll index (**c**), potential photochemical quantum efficiency of PSII - F_v/F_m (d), absorbed energy conversion efficiency - F_v/F_0 (e), and maximum yield of non-photochemical processes - F_0/F_m (f) in soybean seedlings from seeds cv. BMX FIBRA IPRO 64i61 treated with *Ascophyllum nodosum* extract (ANE Acadian). Different letters between markers indicates significative effect by the Scott-Knott test ± standard error ($p \leq 0.05$). ^{ns}= not significant ($p > 0.05$).

of ANE on emergence indicators is still variable, since untreated seeds with the product also showed better ESI results. We also emphasize that the set of characteristics evaluated must be analyzed and not individually, as we observed that the product contributed to the vigor of soybean seedlings.

Better root growth with 5 mL kg^{-1} ANE is associated with the presence of hormones in ANE. Its composition contains auxins and cytokinins, and organic elements such as polysaccharides, playing a physiological role in plants (Arioli et al., 2015; Saeger et al., 2020). This is because auxin stimulates the expansion of the root system (Sosnowski et al., 2023) and other bioactive compounds, as carriers, connect to certain macro and micronutrients in ANE and others types of extracts, acting on mineral metabolism and plant growth (Chanda et al., 2019; Pereira et al., 2020; Rajendran et al., 2022). Similarly, Gomes et al. (2018) reported that *Passiflora actinia* Hook. seedlings had linear increase in the rooting according to the concentrations 10, 20, 30, and 40% ANE. Furthermore, although we have not evaluated it, because ANE contains amino acids, and other elicitors compounds in its composition, such as alanine, aspartic acid, glutamic acid and glycine (Polo and Mata, 2018), stimulate proline (Santos et al., 2023), and presented fucoidans and mannitol (Bernardes et al., 2023), it may contribute to alleviating the effect of isolated or multiple abiotic stresses.

In addition, growth responses of soybean seedlings are also associated with the fact that ANE and others types contributes to the modulation of hormone gene expression, such as gibberellin and cytokinin, which participate in cell division and formation vegetative organs (Ali et al., 2019; Saeger et al., 2020). According to these same authors, this mechanism can also stimulate plant growth and, therefore, result in an increase in shoot matter, fact observed in tomato seedlings, similar to growth and biomass in our study.

According to Saeger et al. (2020) reported in their work containing an overview on the pathways modulated by ANEs that promote plant growth reported that treatment with ANE induced activity of promoters or hormone-responsive genes fused to a reporter such as β-glucuronidase in roots and sprouts, indicating significant upregulation of the cytokinin response. Similarly, Machado et al. (2017), studying lettuce var. Serena, observed that the application of Asco® fertilizer, containing 50% ANE + other minerals and inert ingredients, promoted greater shoot fresh matter.

ANE contains bioactive compounds in its composition, including mannitol, fucoidans, alginate and readily available mineral elements, such as macro and micronutrients (Pereira et al., 2020; Pandey et al., 2023). Although during germination, the main source of nutrition to the germinating seedling is derived from the seed reserves for biomass accumulation, the nitrogen, phosphorus, potassium and other minerals are important, and participate in the protein synthesis and production ATP and NADPH, energy substrate for cellular respiration (Andrade et al., 2022; Jiaying et al., 2022; Santos et al., 2023).

The gradual increase in the chlorophyll index with ANE can be explained owing nitrogen and magnesium contents, readily available in the product, which contribute to the nutrition and metabolism of seedlings. According to Goni et al. (2018) the influence on chlorophyll content in leaves may indicate an increase in the photosynthetic efficiency of plants. This result can be attributed to bioactive molecules, also considered elicitors, present in brown algae extract, such as alginate, fucose, mannitol and laminarin (Shukla et al., 2021; Rajendran et al., 2022), which according to these same authors, favor the synthesis of carbohydrates, efficiency of nitrogen metabolism and act on the antioxidant defense system. Corroborating our study, Villa e Vila et al. (2023) describe that the chlorophyll content in tomato's was higher in plants that received ANE application.

We believe that seed priming with ANE mainly participates in the initial processes associated with germination and formation of normal seedlings, not having a direct impact on photochemical processes in photosystem II in the experimental period. Possibly, if applied via foliar or soil application, with the seedling emerged, the responses would have been more expressive, making additional studies necessary in the field phase, considering appropriate positioning based on soybean phenological stages.

ANE has shown promise on soybean seed priming, as it contributed to some of the characteristics evaluated, especially growth and biomass with 5 mL kg^{-1}, demonstrating that ANE has a biostimulant and biofertilizer action for the species, being a bioinputs available on the market. However, there are still gaps regarding its positioning, and we suggest future studies using seeds with lower physiological and technological quality, that could possibly be more expressive and even present improvements the vigor, contributing to the seed market. Another point that must be highlighted is the economic aspect of the use of ANE, since their use can contribute to maintaining or improving the physiological and nutritional quality of seeds, seedlings and plants, especially under adverse conditions, losses of production.

Future studies should be carried out with the aim of adding information to soybean seed priming protocols with bioinputs, such as other forms of priming, immersion in a solution containing ANE, other extracts or algae, immersion times, dilution of extract, resting time after seed homogenization, and higher concentrations de ANE, need to be studied and their effect on plant development in the field, strengthening information on phycology applied to agriculture.

5. Conclusions

The use of *Ascophyllum nodosum* extract was beneficial for soybean seedlings, with seed priming with 5 mL kg $seed^{-1}$ contributing to growth, biomass production and phenotypic responses. Seed priming with 5 mL kg^{-1} ANE contributed to the emergence speed index, and contribute in increase of chlorophyll index in the seedlings.

Acknowledgements

The authors thank CAPES and CNPq, for granting the scholarships, and the FUNDECT, for financial support. We thank the members of the Plant Ecophysiology Study Group (GEEP) who contributed to the evaluation of the field experiment.

References

ALI, O., RAMSUBHAG, A. and JAYARAMAN, J., 2019. Biostimulatory activities of *Ascophyllum nodosum* extract in tomato and sweet pepper crops in a tropical environment. *PLoS One*, vol. 14, no. 5, pp. e0216710. http://doi.org/10.1371/journal.pone.0216710. PMid:31086398.

AMMAR, E.E., AIOUB, A.A.A., ELESAWY, A.E., KARKOUR, A.M., MOUHAMED, M.S., AMER, A.A. and EL-SHERSHABY, N.A., 2022. Algae as bio-fertilizers: between current situation and future prospective. *Saudi Journal of Biological Sciences*, vol. 29, no. 5, pp. 3083-3096. http://doi.org/10.1016/j.sjbs.2022.03.020. PMid:35360501.

ANDRADE, R.A., SILVA, A.P., RAPOSO, R.W.C., ROCHA, J.L.A., RODRIGUES, A.A. and RODRIGUES, J.B.B., 2022. Eficiência nutricional de fósforo e enxofre pelo abacaxizeiro 'Pérola' em função de doses de P e S. *Scientia Plena*, vol. 18, no. 7, pp. 1-9. http://doi.org/10.14808/sci.plena.2022.070203.

ARIOLI, T., MATTNER, S. and WINBERG, P., 2015. Applications of seaweed extracts in Australian agriculture: past, present and future. *Journal of Applied Phycology*, vol. 27, no. 5, pp. 2007-2015. http://doi.org/10.1007/s10811-015-0574-9. PMid:26435578.

BERNARDES, R.S., SANTOS, S.C., SANTOS, C.C., HEID, D.M., VIEIRA, M.C. and TORALES, E.P., 2023. *Ascophyllum nodosum* seaweed extract and mineral nitrogen in *Alibertia edulis* seedlings. *Revista Brasileira de Engenharia Agrícola e Ambiental*, vol. 27, no. 3, pp. 173-180. http://doi.org/10.1590/1807-1929/agriambi.v27n3p173-180.

BERTOLDO, G., CHIODI, C., LUCIA, M.C.D., BORELLA, M., RAVI, S., BAGLIERI, A., LUCENTI, P., GANASULA, B.K., MULAGALA, C., SQUARTINI, A., CONCHERI, G., MAGRO, F., CAMPAGNA, G., STEVANATO, P. and NARDI, S., 2023. Brown seaweed extract (BSE) application influences auxin and ABA-related gene expression, root development, and sugar yield in *Beta vulgaris* L. *Plants*, vol. 12, no. 4, pp. 843. http://doi.org/10.3390/plants12040843. PMid:36840191.

BRASIL. *Decreto Nº 10.375, de 26 de maio de 2020. Institui o Programa Nacional de Bioinsumos e o Conselho Estratégico do Programa Nacional de Bioinsumos*. Diário Oficial da União, Brasilia, 27 mai.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento, 2009. *Regras para análise de sementes*. Brasília: MAPA/ACS, 395 p.

CARDARELLI, M., WOO, S.L., ROUPHAEL, Y. and COLLA, G., 2022. Seed treatments with microorganisms can have a biostimulant effect by influencing germination and seedling growth of crops. *Plants*, vol. 11, no. 3, pp. 259. http://doi.org/10.3390/plants11030259. PMid:35161239.

CHANDA, M.J., MERGHOUB, N. and EL ARROUSSI, H., 2019. Microalgae polysaccharides: the new sustainable bioactive products for the development of plant biostimulants? *World Journal of Microbiology & Biotechnology*, vol. 35, no. 11, pp. 177. http://doi.org/10.1007/s11274-019-2745-3. PMid:31696403.

COMPANHIA NACIONAL DE ABASTECIMENTO – CONAB, 2023. *Acompanhamento da Safra Brasileira de Grãos*. Safra 2021/22. 6ºlevantamento - vol. 2. [viewed 24 March 2023]. 97 p. Available from: https://www.conab.gov.br/info-agro/safras/graos/boletim-da-safra-de

ENGEL, D.C.H., FELTRIM, D., RODRIGUES, M., BAPTISTELLA, J.L.C. and MAZZAFERA, P., 2023. Algae extract increases seed production of soybean plants and alters nitrogen metabolism. *Agriculture*, vol. 13, no. 7, pp. 1296. http://doi.org/10.3390/agriculture13071296.

FRIONI, T., SABBATINI, P., TOMBESI, S., NORRIE, J., PONI, S., GATTI, M. and PALLIOTTI, A., 2018. Effects of a biostimulant derived from the brown seaweed *Ascophyllum nodosum* on ripening dynamics and fruit quality of grapevines. *Scientia Horticulturae*, vol. 232, pp. 97-106. http://doi.org/10.1016/j.scienta.2017.12.054.

GOMES, E.N., VIEIRA, L.M., TOMASI, J.C., TOMAZZOLI, M.M., GRUNENNVALDT, R.L., FAGUNDES, C.M. and MACHADO, R.C.B., 2018. Brown seaweed extract enhances rooting and roots growth on *Passiflora actinia* Hook stem cuttings. *Ornamental Horticulture (Campinas)*, vol. 24, no. 3, pp. 269-276. http://doi.org/10.14295/oh.v24i3.1221.

GOÑI, O., QUILLE, P. and O'CONNELL, S., 2018. *Ascophyllum nodosum* extract biostimulants and their role in enhancing tolerance to drought stress in tomato plants. *Plant Physiology and Biochemistry*, vol. 126, pp. 63-73. http://doi.org/10.1016/j.plaphy.2018.02.024. PMid:29501894.

GONZÁLEZ-PÉREZ, B.K., RIVAS-CASTILLO, A.M., VALDES-CALDERÓN, A. and GAYOSSO-MORALES, A., 2022. Microalgae as biostimulants: a new approach in agriculture. *World Journal of Microbiology & Biotechnology*, vol. 38, no. 1, pp. 4. http://doi.org/10.1007/s11274-021-03192-2. PMid:34825262.

GUPTA, S., DOLEZAL, K., KULKARNI, M.G., BALÁZS, E. and STADEN, J.V., 2022. Role of non-microbial biostimulants in regulation of seed germination and seedling establishment. *Plant Growth Regulation*, vol. 97, no. 2, pp. 271-313. http://doi.org/10.1007/s10725-021-00794-6.

HURTADO, A.Q., YUNQUE, D.A., TIBUBOS, K. and CRITCHLEY, A., 2008. Use of Acadian marine plant extract powder from *Ascophyllum nodosum* in tissue culture of *Kappaphycus* varieties. *Journal of Applied Phycology*, vol. 21, no. 6, pp. 633-639. http://doi.org/10.1007/s10811-008-9395-4.

INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA – IBGE [online], 2023 [viewed 24 March 2023]. Available from: https://sidra.ibge.gov.br/tabela/7832#resultado

JIAYING, M., TINGTING, C., JIE, L., WEIMENG, F., BAOHUA, F., GUANGYAN, L., HUBO, L., JUNCAI, L., ZHIHAI, W., LONGXING, T. and GUANFU, F., 2022. Functions of nitrogen, phosphorus and potassium in energy status and their influences on rice growth and development. *Rice Science*, vol. 29, no. 2, pp. 166-179. http://doi.org/10.1016/j.rsci.2022.01.005.

KUMARI, S., SEHRAWAT, K.D., PHOGAT, D., SEHRAWAT, A.R., CHAUDHARY, R., SUSHKOVA, S.N., VOLOSHINA, M.S., RAJPUT, V.D., SHMARAEVA, A.N., MARC, R.A. and SHENDE, S.S., 2023. *Ascophyllum nodosum* (L.) Le Jolis, a pivotal biostimulant toward sustainable agriculture: a comprehensive review. *Agriculture*, vol. 13, no. 6, pp. 1179. http://doi.org/10.3390/agriculture13061179.

MA, C., SONG, W., YANG, J., REN, C., DU, H., TANG, T., QIN, S., LIU, Z. and CUI, H., 2022. The role and mechanism of commercial macroalgae for soil conditioner and nutrient uptake catalyzer. *Plant Growth Regulation*, vol. 97, no. 3, pp. 455-476. http://doi.org/10.1007/s10725-022-00819-8.

MACHADO, R., GAI, V.F. and HOJO, E.T.D., 2017. Uso de *Ascophyllum nodosum* e fertilizantes em diferentes cultivares de alface. *Cultivando o Saber (Cumaná)*, vol. 10, no. 1, pp. 29-38.

MAGUIRE, J.D., 1962. Speed of germination-aid in selection and evaluation for seedling emergence and vigor. *Crop Science*, vol. 2, no. 2, pp. 176-177. http://doi.org/10.2135/cropsci1962.0011183X000200020033x.

MAHAPATRA, D.M., SATAPATHY, K.C. and PANDA, B., 2022. Biofertilizers and nanofertilizers for sustainable agriculture: phycoprospects and challenges. *The Science of the Total Environment*, vol. 803, pp. 149990. http://doi.org/10.1016/j.scitotenv.2021.149990. PMid:34492488.

NAKAGAWA, J., 1999. Testes de vigor baseados na avaliação das plântulas. In: VIEIRA, R.D.; CARVALHO, N.M. *Teste de vigor em sementes*. Jaboticabal: FUNEP, pp. 49-85.

PANDEY, D., NAESS, G., FONSECA, A.J.M., MAIA, M.R.G., CABRITA, A.R.J. and KHANAL, P., 2023. Differential impacts of postharvest hydrothermal treatments on chemical composition and in vitro digestibility of two brown macroalgae (Fucales, Phaeophyceae), *Ascophyllum nodosum* and *Fucus vesiculosus*, for animal feed applications. *Journal of Applied Phycology*, vol. 35, no. 5, pp. 2511-2529. http://doi.org/10.1007/s10811-023-03044-6.

PEREIRA, L., MORRISON, L., SHUKLA, P.S. and CRITCHLEY, A.T., 2020. A concise review of the brown macroalga *Ascophyllum nodosum* (Linnaeus) Le Jolis. *Journal of Applied Phycology*, vol. 32, no. 6, pp. 3561-3584. http://doi.org/10.1007/s10811-020-02246-6.

POLO, J. and MATA, P., 2018. Evaluation of a biostimulant (Pepton) based in enzymatic hydrolyzed animal protein in comparison to seaweed extracts on root development, vegetative growth, flowering, and yield of gold cherry tomatoes grown under low stress ambient field conditions. *Frontiers in Plant Science*, vol. 8, pp. 2261. http://doi.org/10.3389/fpls.2017.02261. PMid:29403513.

RAJENDRAN, R., JAGMOHAN, S., JAYARAJ, P., ALI, O., RAMSUBHAG, A. and JAYARAMAN, J., 2022. Effects of *Ascophyllum nodosum* extract on sweet pepper plants as an organic biostimulant in grow box home garden conditions. *Journal of Applied Phycology*, vol. 34, no. 1, pp. 647-657. http://doi.org/10.1007/s10811-021-02611-z.

SAEGER, J., PRAET, S.V., VEREECKE, D., PARK, J., JACQUES, S., HAN, T. and DEPUYDT, S., 2020. Toward the molecular understanding of the action mechanism of *Ascophyllum nodosum* extracts on plants. *Journal of Applied Phycology*, vol. 32, no. 1, pp. 573-597. http://doi.org/10.1007/s10811-019-01903-9.

SANTOS, C.C., SILVA, A.A.S., OLIVEIRA, C.H.C., SILVERIO, J.M., DIAS, A.S., LINNÉ, J.A., SCALON, S.P.Q. and ALOVISI, A.M.T., 2023. *Ascophyllum nodosum* seaweed extract in *Inga edulis* seedlings under drought and the potential of phenotypic plasticity. *Journal of Applied Phycology*, vol. 35, no. 6, pp. 3123-3135. http://doi.org/10.1007/s10811-023-03094-w.

SHUKLA, P.S., BORZA, T., CRITCHLEY, A.T. and PRITHIVIRAJ, B., 2021. Seaweed-based compounds and products for sustainable protection against plant pathogens. *Marine Drugs*, vol. 19, no. 2, pp. 59. http://doi.org/10.3390/md19020059. PMid:33504049.

SILVA, C.C., ARRAIS, I.G., ALMEIDA, J.P.N., DANTAS, L.L.G.R., FRANCISCO, S.O. and MENDONÇA, V., 2016. Seaweed extract of *Ascophyllum nodosum* (L.) Le Jolis in production of rootstock *Anonna glabra* L. *Agrária*, vol. 39, no. 2, pp. 234-241. http://doi.org/10.19084/RCA15057.

SOARES, C., SVARC-GAJIC, J., OLIVA-TELES, M., PINTO, E., NASTIC, N., SAVIC, S., ALMEIDA, A. and DELERUE-MATOS, C., 2020. Mineral composition of subcritical water extracts of *Saccorhiza polyschides*, a brow seaweed used as fertilizer in the north of Portugal. *Journal of Marine Science and Engineering*, vol. 8, no. 4, pp. 244. http://doi.org/10.3390/jmse8040244.

SOSNOWSKI, J., TRUBA, M. and VASILEVA, V., 2023. The impact of auxin and cytokinin on the growth and development of selected crops. *Agriculture*, vol. 13, no. 3, pp. 724. http://doi.org/10.3390/agriculture13030724.

VILLA E VILA, V., REZENDE, R., MARQUES, P.A.A., WENNECK, G.S., NOCCHI, R.C.F., TERAS, D.S. and ANDREAN, A.F.B., 2023. Seaweed extract of *Ascophyllum nodosum* applied in tomato crop as a biostimulant for improving growth, yield and soil fertility in subtropical condition. *Journal of Applied Phycology*, vol. 35, no. 5, pp. 2531-2541. http://doi.org/10.1007/s10811-023-03060-6.