

Date:	16 January 2023
Author:	EBIC
Status:	Final
Distribution:	Public
Classification:	Public

WHITE PAPER

Recent insights into the mode of action of seaweed-based plant biostimulants

Executive summary

In this white paper, the European Biostimulants Industry Council (EBIC) reviews the science on the mode of action of seaweed-based plant biostimulants. Contrary to earlier hypotheses, recent research has shown that the effects of seaweed extracts on plants and their metabolism appear to be largely a function of other biomolecules (carbohydrates, polyphenols, etc.), which modulate gene expression and induce metabolic changes in plants that lead to the observed biostimulant effects.

Introduction

Plant biostimulants are defined as fertilising products the function of which is to stimulate plant nutrition processes independently of the product's nutrient content with the aim of improving the following characteristics of the plant or the plant rhizosphere: nutrient use efficiency, tolerance to abiotic stress, quality traits, or the availability of confined nutrients in the soil or rhizosphere (EU, 2019). Plant biostimulants are thus defined by their function, and may incorporate a wide range of components in their formulation (Yakhin et al., 2017).

One of the most common components of plant biostimulants are seaweed extracts, which are currently obtained from brown (*Ascophyllum nodosum*, *Laminaria* spp., *Macrocystis* spp., etc.), red (*Kappaphycus alvarezii*, *Palmaria* spp., *Gracilaria* spp., etc.), and green seaweeds (*Ulva* spp. and *Enteromorpha* spp.) (Goñi et al., 2020; Sujeeth et al., 2022), subject to a variety of extraction processes: alkaline, neutral or acidic aqueous extractions, disruption by milling under high or low pressure with subsequent addition of an acid, alkali or water; rupture of the cells through low temperatures and high pressure; and crushing of frozen seaweeds in order to obtain a suspension of fine particles (Baltazar et al., 2021).

The global market for non-microbial plant biostimulants, including those based on seaweed extracts, was estimated to be 2540 million dollars in 2021, and was projected to reach 2830 million dollars in 2022, with a cumulative aggregated growth rate of 11,3-11,6% (Dunham-Trimmer 2022, pers. com.). Plant biostimulants based on seaweed extracts accounted for 758 million dollars in 2021 (Dunham-Trimmer 2022, pers. com.), and roughly account for 29,8-33,5% of the global plant biostimulants market (North Sea Farm Foundation, 2018, Dunham-Trimmer 2022, pers. com.). With the application of the new Fertilising Products Regulation in July 2022, which allows the placement of plant biostimulants in the European Union Single Market (EU, 2019), the use of seaweed-based plant biostimulants is expected to increase in the coming years.

Seaweeds have been used since antiquity either directly or in composted form as a soil amendment to improve the productivity of crops in coastal regions. The observed benefits on the growth, vigor and yield of plant crops were traditionally attributed to the supply of essential

nutrients and to improved soil texture and water holding capacity. The first liquid seaweed extracts produced from 1950 were historically known as plant strengtheners, promoting plant growth and crop yield regardless of the fertiliser content of the extract (Craigie, 2011). While some early studies implied that plant hormones in seaweed extracts could explain their bioactivity, later studies have clarified that the effects observed in plants are due to the stimulation of molecular pathways in plants themselves, and not due to plant hormones.

This scientific review looks at the state of the art of scientists' understanding of how products containing seaweed extracts benefit plants, with a focus on products with plant biostimulant functions.

Until the early 2010s, studies that characterised the effects of seaweed-based plant biostimulants often misattributed their mode of action

As plant biostimulants started to become the subject of intensive research over the past decades (Brown and Saa, 2015; Yakhin et al., 2017), seaweed-based plant biostimulants were shown to stimulate rooting of camellia cuttings (Ferrante et al., 2013) and root growth of rocket plants (Vernieri et al., 2005), among other plant biostimulant effects in many different crops. These studies also demonstrated that they could significantly increase chlorophyll content and reduce nitrate content in the leaves of rocket plants (Vernieri et al., 2005). The hypothesis was that the biostimulant probably affected nitrogen metabolism in the plant, speeding up the incorporation of nitrate through the activation of related enzymes. The high chlorophyll content could increase photosynthetic processes and indirectly stimulate nitrate reduction, because the two physiological pathways are tightly coupled (Vernieri et al., 2005). Some of these studies also claimed that certain seaweed extracts contained plant hormones, which could account for the biological effects observed when applied to plants (reviewed by Baltazar et al., 2021).

However, the earlier assumption that plant hormones native to certain seaweeds are mainly responsible for the bioactivities observed in extract treated plants was revised by Wally et al (2013a; 2013b). This study analysed commercial seaweed extracts from several commonly used seaweed species using analytical instrumentation with high sensitivity and accuracy (UPLC-ESI-MS/MS); and showed undetectable or very low levels of plant hormones. The authors suggested that seaweed extracts would be unlikely to induce any phytohormone-responsive phenotypes at field application because the minimum concentrations required for phenotypic changes in the plant are reported to be significantly higher than concentrations reported to be found in some commercial seaweed extracts.

The consensus in plant science is that the minimum concentrations of plant hormones required for phenotypic changes within plants are 100 nM for auxin (Doyle et al., 2019; Kramer and Ackelsberg, 2015; Robert et al., 2010), 10 nM for cytokinin (Shen et al., 2014; Street et al., 2016; Werner et al., 2001), or 100 nM for brassinosteroids (Zhou et al., 2013). These minimum concentrations, when considered with studies showing that very low (if any) phytohormones are found in seaweed extracts, demonstrate that when these extracts are applied to a crop, any natural phytohormones contained in them would be far below the threshold levels required for physiological effects.

Since the early 2010s, more precise analytical techniques have shed light on the carbohydrate compounds and metabolic pathways involved in the mode of action of seaweed-based plant biostimulants

More recent studies have started to provide a greater understanding of the molecular mechanisms involved in the plant biostimulant effects of seaweed extracts. Over the years, it became clearer that the effects of seaweed-based plant biostimulants vary depending on the type of seaweed used (e.g. brown, green or red), the spatiotemporal source of the seaweed raw material, the conditions in the areas where the seaweed is harvested, and the manufacturing process, including the temperature and pH conditions of the extraction process (Goñi et al., 2018; Staykov et al., 2021).

For example, it was shown that the capacity of *A. nodosum* extracts to enhance tolerance to abiotic stress was largely associated with (a) the type of extract (b) the biochemical characteristics of the extract and (c) the type of stress involved (Guinan et al., 2013). Extracts obtained under different conditions (i.e., temperature) contained different bioactive molecules that led to different effects when the extract was applied to plants (Guinan et al., 2013). An extract of *A. nodosum* obtained at temperatures higher than 125°C was shown to improve abiotic stress tolerance, and this could potentially be explained by its high polyphenol content and high antioxidant activity (Guinan et al., 2013).

Likewise, three commercial extracts of *A. nodosum* obtained under different extraction conditions were shown to provide different degrees of drought stress tolerance when applied to tomato plants (Goñi et al., 2018). The study highlighted that there is still “very little data published linking chemical composition with biostimulant activity and changes at the molecular level within the plant” (Goñi et al., 2018), although more recent reviews have associated key carbohydrates from seaweed extracts to drought stress tolerance and improved growth in plants (Goñi et al., 2020; Sujeeth et al., 2022).

“Molecular priming” has been proposed as a mechanism for inducing tolerance against abiotic and oxidative stresses in crop plants, using seaweed extracts such as *A. nodosum* (Kerchev et al., 2020). For example, an extract of *A. nodosum* obtained under high temperature and high pressure conditions was shown to prevent oxidative stress damage (Omidbakhshfard et al., 2020). A transcriptome analysis demonstrated that plants treated with the seaweed extract had prevented the expression of genes associated with reactive oxygen species (ROS), genes associated with ROS-induced programmed cell death, or autophagy-related genes, which in contrast were upregulated in untreated stressed plants. Metabolomic analyses showed that plants treated with this particular seaweed extract had higher levels of several primary metabolites, including maltose and raffinose, which are known to contribute to stress protection. Lipid profiling analyses revealed that alterations in lipids following the application of the seaweed extract were associated with lower cell death and chloroplast degradation. In addition, the seaweed extract stimulated growth by upregulating photosynthesis, hormone signaling and growth-related genes (Omidbakhshfard et al., 2020).

A follow up study testing the effect of the same *A. nodosum* extract on the model plant *Arabidopsis thaliana*, tomato and pepper, showed that the extract was able to mitigate oxidative stress responses in all three plants species (Staykov et al., 2021). It reduced the appearance of oxidative stress lesions by hindering the accumulation of ROS in stressed tissues, it mitigated the negative impact of stress on photosynthetic parameters, and it reconfigured the metabolic profile of treated and stressed plants to a pattern that was very different from that of untreated stressed plants (Staykov et al., 2021).

Another recent study by Rasul *et al.*, (2021) with the same *A. nodosum* extract demonstrated that it could protect plants from severe drought stress. This study showed that a foliar treatment using the specific extract of *A. nodosum* prior to a stress event can induce gene expression changes in the treated plants. The seaweed extract treatment downregulated or repressed the expression of the stress-responsive negative growth regulator-RESPONSIVE TO DESSICATION 26 (*RD26*) gene and induced higher expression of Histone H4 (*HIS4*), which is crucial for maintaining the shoot apical meristem and growth under severe drought. The study concluded that this seaweed extract has the potential to improve crop tolerance to challenges arising from climate change (Rasul *et al.*, 2021).

Similarly, a commercial plant biostimulant based on an extract of *A. nodosum* obtained through a cold extraction process was shown to increase proline levels, upregulate the ethylene signaling pathway and downregulate the jasmonic acid pathway, leading to better growth of *A. thaliana* plants under drought stress (Fleming *et al.*, 2019). Another study with this seaweed extract applied to *A. thaliana* plants subject to heat stress demonstrates that it upregulated several genes involved in transcription regulation and ethylene signaling pathways, it reduced the accumulation of ROS, preserved cell membrane integrity and increased chlorophyll content (Cocetta *et al.*, 2022).

Another *A. nodosum* extract was shown to promote the growth of maize plants under phosphorus (P) deficiency conditions by a variety of mechanisms, none of which were related to plant hormones (Shukla and Prithviraj, 2021). Seeds were treated with the seaweed extract, and 7-day-old seedlings were grown under P deficiency conditions for 14 days. At the end of this period, the root and shoot biomass of treated plants was higher than that of untreated plants, and they had higher nitrogen and phosphorus content. Chlorophyll content was also significantly increased by the extract with respect to untreated plants, and anthocyanin content was reduced. Treated plants had significantly less electrolyte leakage because of an improvement in membrane stability, and they showed lower accumulation of ROS. The extract also modified the biochemical profile of treated plants, with higher concentrations of total soluble sugars and amino acids, and lower concentrations of phenolic compounds and flavonoids. A transcriptomic analysis showed the induction of P homeostasis genes, sucrose transporters, glucose translocators and other genes related to carbon assimilation, genes involved in lipid metabolism and the downregulation of genes involved in secondary metabolism that helped treated plants reduce the detrimental effects of P deficiency (Shukla and Prithviraj, 2021).

In terms of improving nutrient efficiency, an extract from *A. nodosum* was shown to allow for up to 27% reduction in nitrogen fertiliser applications while maintaining or increasing crop yield (Goñi *et al.*, 2021). This extract was composed of carbohydrates (26%), polyphenols (12,3%), other organic components (18%), and ash (43,7%), and it was applied as a foliar spray or a coating for granular fertiliser to *A. thaliana* and barley plants. A gene expression analysis showed an upregulation of three membrane nitrate transporters in the roots of treated barley plants, which led to a 17,9-72,2% increase of nitrate content in the shoots. The application of the seaweed extract also enhanced nitrate reductase and glutamine synthase activities, and it increased the content of free amino acids (glutamate, glutamine, aspartate, proline), soluble protein and photosynthetic pigments (chlorophylls and carotenoids) in treated barley plants. The study concluded that further basic research to investigate the mode of action of the biostimulant would help understand the optimal conditions for use, which could be tested on other important crops to assess the potential contribution of this product to achieving the EU Green Deal target of a 20% reduction in nitrogen use in agriculture (Goñi *et al.*, 2021).

The evidence accumulated over the past years suggests that the mode of action of seaweed-based plant biostimulants is dependent on their physicochemical characteristics, which are derived from their processing conditions. A recent study showed that there is a link between the lower molecular size of carbohydrates inside *A. nodosum* extracts and enhanced heat stress tolerance in tomato plants at reproductive stage (Carmody et al., 2020). Likewise, a comparative analysis found significant differences in the efficacy of three commercial plant biostimulants based on *A. nodosum* extracts in improving nitrogen use efficiency in winter wheat. The results emphasized the role of processing conditions in the production of plant biostimulants with optimized effectiveness to solve specific agronomic problems (Langowski et al., 2022).

New studies have confirmed that plant hormone concentrations in seaweed extracts are too low to invoke physiological responses in plants, particularly considering the low application rates of the products under field conditions (reviewed by Baltazar et al., 2021). While certain plant hormones have been reported to be present in some seaweed extracts at very low concentrations (nanogram or picogram per mL), they have also been below the detection level in other extracts, which may suggest their potential absence from many seaweed plant biostimulants (Baltazar et al., 2021). Therefore, it is the in-plant modulation of phytohormone pathways mediated by the seaweed extract application that may promote plant growth and development under stress conditions (Deolu-Ajayi et al., 2022).

A recent review from Sujeeth et al. (2022) also looked at the various functional studies which demonstrated that the trace concentrations of plant hormones found in some seaweed extracts are not responsible for the effects observed on treated plants. These studies were done with phytohormone insensitive and biosynthetic mutants, and the results suggested that plant phenotypic alterations (growth effects) were potentially due to endogenous hormone-associated changes, rather than to the effects of exogenous phytohormones present within the seaweed extracts. On the other hand, unique chemical compounds abundantly present in seaweeds may potentially induce non-hormonal pathways or endogenous growth hormone signalling in treated plants, as a part of other regulatory networks to modulate plant growth (Sujeeth et al., 2022). The review also summarised the recent advances in determining the genetic and molecular mechanisms activated by seaweed-based plant biostimulants, their influence on transcriptome reconfiguration, metabolite adjustment and ultimately stress protection, improved nutrient uptake, plant growth and performance. Another recent study demonstrated an improvement in N uptake and differential expression of genetic markers involved in nitrate perception and transport in plants treated with a seaweed extracts (Langowski et al., 2022)

A. nodosum extracts are the most widely studied and best characterized seaweed-based plant biostimulants, both in terms of their phenotypic effects on crops and the molecular mechanisms involved (Baltazar et al., 2021). In contrast, the mechanisms of other seaweed extracts remain largely unexplored. A functional study with an extract of a green seaweed, *Ulva intestinalis*, demonstrated that the growth effects observed in *A. thaliana* were not due to plant hormones in the extract (Ghaderiardakani et al., 2019), and a crude extract of *Ulva* sp. composed of several polysaccharides was shown to promote the growth of *A. thaliana* in a dose- and light intensity-dependent manner (Shefer et al., 2022). On the other hand, cell wall polysaccharides from red seaweeds, which are present in their extracts, have been shown to activate signaling pathways and basal metabolism to stimulate cell division and growth in treated plants (González et al., 2013). An extract of a red seaweed, *Kappaphycus alvarezii*, led to improved plant growth, abiotic stress mitigation and upregulation of stress-responsive genes such as *WCK-1*, *TaWRKY10*, *TdCAT* and *TdSOD* when applied to common wheat (*Triticum spp.*)

(Patel et al., 2018). Over expression of gene transcripts for fatty acid metabolism, starch synthesis, nutrient transport and metabolism, as well as cell cycle and division, were also observed in *Zea mays* following treatment with a *K. alvarezii* extract (Kumar et al., 2020). Further research is required to characterize the molecular signaling networks modulated by other seaweed-based plant biostimulants, in order to determine their mode of action and efficacy.

Overall, the most recent studies are suggesting that certain carbohydrates or other non-hormone compounds found in seaweeds, which are absent from terrestrial plants, may be responsible for the specific modes of action of seaweed extracts as plant biostimulants (Deolu-Ajayi et al., 2022). More specifically, growing evidence highlights how seaweed carbohydrates are the essential components in eliciting plant biostimulant activity in most commercial seaweed extracts. Over the past 10 years, several research articles have described the biological activity of some polysaccharides and oligosaccharides extracted from seaweeds and their roles enhancing nutrient use efficiency or abiotic stress tolerance (Carmody et al., 2020; Goñi et al., 2020; Sujeeth et al., 2022). Because the content and molecular weight of these bioactive carbohydrates would be determined by the extraction conditions of the seaweed extract, we can expect that this variability explains the different bioactivities observed in commercial extracts. Further insights into the mode of action of seaweed-based plant biostimulants will enable product improvement and the synergistic application of seaweed extracts combined with other plant biostimulant technologies to face current and future agricultural production challenges.

Conclusion

Many scientific publications over the past 10 years have demonstrated that plant hormones are not responsible for the plant biostimulant effects observed in crops when applying seaweed extracts. In fact, recent studies have demonstrated that seaweed extracts modulate gene expression and induce metabolic changes in treated plants, thereby enhancing nutrient use efficiency, abiotic stress tolerance and providing other biostimulant effects. These molecular mechanisms have proven to be independent of any plant growth hormones that may (or may not) be present in seaweed extracts. At this time, the most likely candidates for the biostimulant effects of seaweed extracts are the complex carbohydrate compounds found in these extracts, although additional non-hormone compounds may also be involved, independently of, or in combination with, carbohydrate compounds. More research is required in this area to fully elucidate the changes and pathways induced by seaweed extracts at the molecular level.

ABOUT EBIC



The European Biostimulants Industry Council (EBIC) promotes the contribution of plant biostimulants to make agriculture more sustainable and resilient and in doing so promotes the growth and development of the European Biostimulants Industry. Our mission is to ensure biostimulant technologies are valued as integral to sustainable agriculture, while securing an enabling regulatory framework for all of them.

For more information about this topic, please contact Sara García Figuera (sara.gfiguera@prospero.ag)

References

- Baltazar, M., Correia, S., Guinan, K.J., Sujeeth, N., Bragança, R. & Gonçalves, B. (2021) Recent Advances in the Molecular Effects of Biostimulants in Plants: An Overview. *Biomolecules*, 11. <https://doi.org/10.3390/biom11081096>.
- Brown, P. & Saa, S. (2015) Biostimulants in agriculture. *Frontiers in Plant Science*, 6, 671. <https://doi.org/10.3389/fpls.2015.00671>.
- Carmody, N., Goñi, O., Łangowski, Ł. & O'Connell, S. (2020) Ascophyllum nodosum Extract Biostimulant Processing and Its Impact on Enhancing Heat Stress Tolerance During Tomato Fruit Set. *Frontiers in Plant Science*, 11. <https://doi.org/10.3389/fpls.2020.00807>.
- Cocetta, G., Landoni, M., Pilu, R., Repiso, C., Nolasco, J., Alajarin, M., et al. (2022) Priming Treatments with Biostimulants to Cope the Short-Term Heat Stress Response: A Transcriptomic Profile Evaluation. *Plants*, 11. <https://doi.org/10.3390/plants11091130>.
- Craigie, J.S. (2011) Seaweed extract stimuli in plant science and agriculture. *Journal of Applied Phycology*, 23, 371–393. <https://doi.org/10.1007/s10811-010-9560-4>.
- Deolu-Ajayi, A.O., Meer, I.M. van der, Werf, A. van der & Karlova, R. (2022) The power of seaweeds as plant biostimulants to boost crop production under abiotic stress. *Plant, Cell & Environment*, 45, 2537–2553. <https://doi.org/10.1111/pce.14391>.
- Doyle, S.M., Rigal, A., Grones, P., Karady, M., Barange, D.K., Majda, M., et al. (2019) A role for the auxin precursor anthranilic acid in root gravitropism via regulation of PIN-FORMED protein polarity and relocalisation in Arabidopsis. *New Phytologist*, 223, 1420–1432. <https://doi.org/10.1111/nph.15877>.
- EU (2019) *Regulation (EU) 2019/1009 of the European Parliament and of the Council of 5 June 2019 laying down rules on the making available on the market of EU fertilising products and amending Regulations (EC) No 1069/2009 and (EC) No 1107/2009 and repealing Regulation (EC) No 2003/2003*.
- Ferrante, A., Trivellini, A., Vernieri, P. & Piaggese, A. (2013) Application of Actiwave® for improving the rooting of camellia cuttings. *Acta Horticulturae*, 213–218. <https://doi.org/10.17660/ActaHortic.2013.1009.25>.
- Fleming, T.R., Fleming, C.C., Levy, C.C.B., Repiso, C., Hennequart, F., Nolasco, J.B., et al. (2019) Biostimulants enhance growth and drought tolerance in Arabidopsis thaliana and exhibit chemical priming action. *Annals of Applied Biology*, 174, 153–165. <https://doi.org/10.1111/aab.12482>.
- Ghaderiardakani, F., Collas, E., Damiano, D.K., Tagg, K., Graham, N.S. & Coates, J.C. (2019) Effects of green seaweed extract on Arabidopsis early development suggest roles for

- hormone signalling in plant responses to algal fertilisers. *Scientific Reports*, 9, 1983. <https://doi.org/10.1038/s41598-018-38093-2>.
- Goñi, O., Łangowski, Ł., Feeney, E., Quille, P. & O'Connell, S. (2021) Reducing Nitrogen Input in Barley Crops While Maintaining Yields Using an Engineered Biostimulant Derived From *Ascophyllum nodosum* to Enhance Nitrogen Use Efficiency. *Frontiers in Plant Science*, 12. <https://doi.org/10.3389/fpls.2021.664682>.
- Goñi, O., Quille, P. & O'Connell, S. (2018) *Ascophyllum nodosum* extract biostimulants and their role in enhancing tolerance to drought stress in tomato plants. *Plant Physiology and Biochemistry*, 126, 63–73. <https://doi.org/10.1016/j.plaphy.2018.02.024>.
- Goñi, O., Quille, P. & O'Connell, S. (2020) Seaweed Carbohydrates. In: Geelen, D. and Xu, L. (Eds.) *The Chemical Biology of Plant Biostimulants*. John Wiley & Sons Ltd., pp. 57–95.
- González, A., Castro, J., Vera, J. & Moenne, A. (2013) Seaweed Oligosaccharides Stimulate Plant Growth by Enhancing Carbon and Nitrogen Assimilation, Basal Metabolism, and Cell Division. *Journal of Plant Growth Regulation*, 32, 443–448. <https://doi.org/10.1007/s00344-012-9309-1>.
- Guinan, K.J., Sujeeth, N., Copeland, R.B., Jones, P.W., O'Brien, N.M., Sharma, H.S.S., et al. (2013) Discrete roles for extracts of *Ascophyllum nodosum* in enhancing plant growth and tolerance to abiotic and biotic stresses. *Acta Horticulturae*, 127–135. <https://doi.org/10.17660/ActaHortic.2013.1009.15>.
- Kerchev, P., Meer, T. van der, Sujeeth, N., Verlee, A., Stevens, C.V., Van Breusegem, F., et al. (2020) Molecular priming as an approach to induce tolerance against abiotic and oxidative stresses in crop plants. *Biotechnology Advances*, 40, 107503. <https://doi.org/10.1016/j.biotechadv.2019.107503>.
- Kramer, E.M. & Ackelsberg, E.M. (2015) Auxin metabolism rates and implications for plant development. *Frontiers in Plant Science*, 6. <https://doi.org/10.3389/fpls.2015.00150>.
- Kumar, R., Trivedi, K., Anand, K.G.V. & Ghosh, A. (2020) Science behind biostimulant action of seaweed extract on growth and crop yield: insights into transcriptional changes in roots of maize treated with *Kappaphycus alvarezii* seaweed extract under soil moisture stressed conditions. *Journal of Applied Phycology*, 32, 599–613. <https://doi.org/10.1007/s10811-019-01938-y>.
- Łangowski, Ł., Goñi, O., Ikuyinminu, E., Feeney, E. & O'Connell, S. (2022) Investigation of the direct effect of a precision *Ascophyllum nodosum* biostimulant on nitrogen use efficiency in wheat seedlings. *Plant Physiology and Biochemistry*, 179, 44–57. <https://doi.org/10.1016/j.plaphy.2022.03.006>.
- North Sea Farm Foundation (2018) Identification of the seaweed biostimulant market (phase 1).
- Omidbakhshfard, M.A., Sujeeth, N., Gupta, S., Omranian, N., Guinan, K.J., Brotman, Y., et al. (2020) A Biostimulant Obtained from the Seaweed *Ascophyllum nodosum* Protects