

Review

## SEAWEEDS IN VITICULTURE: A REVIEW FOCUSED ON GRAPE QUALITY

### ALGAS MARINHAS NA VITICULTURA: UMA REVISÃO FOCADA NA QUALIDADE DA UVA

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#### SUMMARY

Cell walls of seaweeds contain a wide number of organic and inorganic constituents, of which polysaccharides have important biological activity. Some researchers suggest that polysaccharides from seaweeds can behave as biotic elicitors in viticulture, triggering the synthesis of phenolic compounds in leaves and grape berries. The mechanism of action of seaweeds after a foliar application to grapevines is not fully understood but it is discussed in this review. An overview of the recent research focused on the effects of seaweeds foliar applications on grapevine productivity, on grape and wine quality is included as well as a short-term future perspective for the research in this field.

#### RESUMO

As paredes celulares das algas marinhas contêm um elevado número de constituintes orgânicos e inorgânicos, dos quais os polissacarídeos têm importante atividade biológica. Alguns investigadores sugerem que os polissacarídeos de algas marinhas podem comportar-se como elicitadores bióticos em viticultura, desencadeando a síntese de compostos fenólicos em folhas e uvas. Os mecanismos de ação após a aplicação foliar de algas marinhas em videiras não foram totalmente estudados, mas são discutidos nesta revisão. É incluída uma visão geral dos trabalhos de investigação recentes sobre os efeitos das algas marinhas aplicadas, por via foliar, na produtividade da vinha, na qualidade da uva e do vinho, assim como uma perspectiva de estudo futuro de curto prazo para a investigação neste campo.

**Key words:** amino acids, seaweeds, polysaccharides, elicitation, foliar fertilizer, phenolic compounds, volatile compounds.

**Palavras-chave:** aminoácidos, algas marinhas, polissacarídeos, elicitação, fertilizante foliar, compostos fenólicos, compostos voláteis.

#### INTRODUCTION

Intensive food production for human and animal consumption, based on conventional agricultural systems, can lead to the indiscriminate use of agrochemicals, generating a negative effect on the conservation of biodiversity worldwide (Hannah *et al.*, 2013; Provost and Pedneault, 2016; Adhikari *et al.*, 2019; Banerjee *et al.*, 2019; Gutiérrez-Gamboa *et al.*, 2019a; Neset *et al.*, 2019). These negative impacts include greater contamination of soils that decrease their fertility, as well as water pollution (Parris, 2011). On the other hand, and during the past decades, there has been a massive increase in marine eutrophication globally (Howarth, 2008), mainly driven by an excess of nitrogen and phosphorus coming from anthropogenic activities that can leach into groundwater or move into waterways via surface runoff (Parris, 2011; Ngatia *et al.*, 2019). Eutrophication leads to an excessive

algae production, blooms of harmful algae, including an increase of the frequency of anoxic events, and the death of several fish species (Ngatia *et al.*, 2019). These conditions lead to economic losses for fish and oyster producers, including losses of recreational amenities, such as beaches and lakes (Ngatia *et al.*, 2019).

Crop fertilization using seaweeds dates back to ancient times. However, the development of agrochemicals at the end of the 19<sup>th</sup> century made the use of natural and organic products less popular (Craigie, 2011). Due to the aforementioned problems and the current global situation in relation to the demand for organic fertilizers in developed countries, an opportunity for the use of algae in viticulture has been opened, as they have been widely used in other horticultural crops with the aim to improve quality and disease resistance (Battacharyya *et al.*, 2015). Based on this, the

utilization of algae in viticulture is currently under study and there are few reports in the literature assessing its effect on avoiding grapevine diseases and improving grape and wine quality (Aziz *et al.*, 2003; Sharma *et al.*, 2014; Zhang *et al.*, 2016; Frioni *et al.*, 2018, 2019; Mondello *et al.*, 2018; Gutiérrez-Gamboa *et al.*, 2019b; Salvi *et al.*, 2019; Taskos *et al.*, 2019; Gutiérrez-Gamboa, 2020; Gutiérrez-Gamboa *et al.*, 2020a,b,c; Thankaraj *et al.*, 2020).

Seaweed extracts contain several substances that promote plant growth, such as auxins, cytokinins, betaines and gibberellins, as well as organic substances, such as amino acids, macronutrients and trace elements, which can improve crop yield and quality (Khan *et al.*, 2009; Craigie, 2011; Arioli *et al.*, 2015; Battacharyya *et al.*, 2015). Therefore, the aim of this work was to revise recent research that has evaluated the effects of seaweed extracts on grapevine productivity and grape and wine quality, and to propose a short-term future perspective for the research in this field.

#### **SPECIES OF ALGAE USED AS EXTRACTS AND THEIR COMPOSITION**

Seaweeds are a diverse group of photosynthetic eukaryotic organisms that cover about 10,000 species of red, brown and green algae (Khan *et al.*, 2009). Respect to their abundance and distribution, brown algae (Phaeophyceae) are the most used for commercial extract preparation with several applications in horticulture (Battacharyya *et al.*, 2015). Most of the biostimulant products elaborated from algae are manufactured from a single species of brown algae (*Ascophyllum nodosum*), which is abundantly distributed along the Northwest coast of Europe and the Northeast coast of North America (Craigie, 2011). However, several commercial extracts currently available in the markets are made from other species of brown algae, such as *Ecklonia maxima*, *Durvillaea potatorum*, *D. antarctica*, *Himantalia elongata*, *Laminaria digitata*, *L. hyperborea*, *Macrocystis pyrifera*, and *Sargassum spp.*, although other minor species, such as *Fucus serratus*, *Ulva intestinalis*, *U. lactuca* and *Kappaphycus alvarezii* are also used (Craigie, 2011; Sharma *et al.*, 2014; Gutiérrez-Gamboa, 2020).

Commercial extracts made from brown algae (*Ascophyllum nodosum*) contain a wide range of inorganic and organic components (Verkleij, 1992; Sharma *et al.*, 2014) including nitrogen, phosphorus, potassium, calcium, iron, magnesium, zinc, sodium and sulfur (Fleurence, 2004; Rayirath *et al.*, 2009). Brown algae extracts also contain varying amounts of amino acids (Fleurence, 2004; Rayirath *et al.*, 2009; Blunden *et al.*, 2010; MacKinnon *et al.*, 2010; Gutiérrez-Gamboa *et al.*, 2020c) and only between 3 to 15% of their dry

weight in proteins, representing a lower content than that found in green and red algae, which varies from 10 to 47% of their dry weight (Fleurence, 1999, 2004; Nagahama *et al.*, 2009; Sharma *et al.*, 2014; Gutiérrez-Gamboa, 2020).

Polysaccharides are one of the main components of commercial extracts of most algae species (Sharma *et al.*, 2014). These components can represent up to 30 or 40% of the dry weight of the seaweed extract (Rayirath *et al.*, 2009). In the case of brown algae extracts, the most common polysaccharides are alginates, fucoidans, laminates and glucans (Khan *et al.*, 2009). Fucoidans have different molecular structure according to their varying degrees of methylation, sulfation and branching (Battacharyya *et al.*, 2015). Depending on the chemical and physical methods used during raw material extraction, harvest season and species of algae, the structure of fucose-containing polymers varies (Craigie, 2011). Similarly, alginates that are polymers of D-mannuronic and L-guluronic acids that have been shown to promote plant growth (Yabur *et al.*, 2007), vary in viscosity according to the species of algae used (Battacharyya *et al.*, 2015). Other important components of seaweed extracts are laminarins, well known elicitors that are used in viticulture and that have an effect in modulating the defense responses of plants against fungal and bacterial pathogens (Mercier *et al.*, 2001; Aziz *et al.*, 2003; Gauthier *et al.*, 2014; Nesler *et al.*, 2015; Romanazzi *et al.*, 2016; Garde-Cerdán *et al.*, 2017; Lemaître-Guillier *et al.*, 2017).

Brown algae, especially *Ecklonia stolonifera*, *Fucus vesiculosus*, *F. serratus* and *Ascophyllum nodosum* can reach high concentrations of phenolic compounds even up to 30 g/100 g of extract (Audibert *et al.*, 2010; Keyrouz *et al.*, 2011; Balboa *et al.*, 2013; Battacharyya *et al.*, 2015). Phenolic compounds play a primary role in the structural components of algae cell walls protecting cells and their components (Nakamura *et al.*, 1996; Wang *et al.*, 2009). Phlorotannins are oligomers of phloroglucinol existing in brown algae have been found to be more efficient antioxidants compared to catechin, ascorbic acid, epigallocatechin gallate, resveratrol and tocopherol (Shibata *et al.*, 2003).

Seaweed extracts also contain several phytohormones, including auxins, cytokinins, gibberellins, abscisic acid and brassinosteroids (Stirk and Van Staden, 1996; Stirk *et al.*, 2004; Stirk *et al.*, 2014; Battacharyya *et al.*, 2015; Gutiérrez-Gamboa, 2020). Brown algae such as *Alaria esculenta*, *Ascophyllum nodosum*, *Ectocarpus siliculosus*, *Fucus serratus*, *F. spiralis*, *F. vesiculosus*, *Halidrys siliquosa*, *Laminaria digitata*, *L. hyperborea*, *L. saccharina* and *Pilayella littoralis* contain osmolytes such as mannitol, an important protective compound that is activated in response to abiotic factors (Battacharyya *et al.*,

2015; Gutiérrez-Gamboa, 2020). Mannitol is also known as a chelating agent, which explains why algae can release otherwise unavailable elements from the soil for plant crop use (Reed *et al.*, 1985). Additionally, considerable amounts of melatonin have been documented in the brown algae *Pterygophora californica* (Fries and Thorén-Tolling, 1978).

One of the unique characteristics of *Ascophyllum nodosum* is its mutualistic association with the fungal endophyte *Mycosphaerella ascophylli* (Fries and Thorén-Tolling, 1978; Fries, 1979; Garbary and Gautam, 1989; Craigie, 2011; Shukla *et al.*, 2019) that protects *A. nodosum* from desiccation (Garbary and Gautam, 1989). Interestingly, it has been showed that fungal sterols derived from *M. ascophylli* present in extracts made from *A. nodosum* can mitigate salinity stress in plants (Shukla *et al.*, 2019).

## PROCEDURES FOR SEAWEED BIOSTIMULANT PRODUCTION

Seaweed composition provides an excellent opportunity for the biostimulant industry (Godlewska *et al.*, 2016). Extraction is the most important and starting step in isolating different types of components that condition seaweed extract bioactivity (Jeong *et al.*, 2004; Godlewska *et al.*, 2016; Shukla *et al.*, 2019). Extraction efficiency is mostly reduced by the presence of algae cell walls, which can be affected by solvent composition, temperature, time, and pH (Godlewska *et al.*, 2016). Different extraction techniques have been used to maximize biologically active compounds from seaweeds such as acid and alkaline hydrolysis, microwave assisted extraction, supercritical fluid extraction, pressurized liquid extraction, Soxhlet extraction, enzyme-assisted extraction (EAE), and ultrasound-assisted extraction (UAE) (Godlewska *et al.*, 2016; Shukla *et al.*, 2019). These extraction techniques use different solvents such as ethanol, acetone, methanol-toluene, methanol, petroleum ether, ethyl acetate, dichloromethane, and butanol (Ganesan *et al.*, 2008; Godlewska *et al.*, 2016). Some of abovementioned techniques require the use of expensive and toxic solvents, nevertheless simpler procedures such as seaweed boiling and soaking as methods for obtaining extracts have been successfully developed (Godlewska *et al.*, 2016).

In water based extractions, raw materials are washed with fresh water to remove sand, stones and other debris, later chopped and oven dried at a temperature lower to 80 °C (Sharma *et al.*, 2014). The solid residues are then separated using different filtration methods based on the end use of the biostimulant to be produced (Shukla *et al.*, 2019). This process has several advantages over the normal extraction procedure in terms of acid, alkali,

and enzymatic reaction (Saravana *et al.*, 2018). Biostimulants obtained through water or acid hydrolysis are reported to be rich in phytohormone-like activity (Blunden *et al.*, 2010; Shukla *et al.*, 2019). The main advantages of this technique is that only water is used as an extraction solvent, it is environmentally friendly and it does not require organic solvent (Godlewska *et al.*, 2016; Saravana *et al.*, 2018).

In acid hydrolysis extractions, seaweed biomass is treated with sulfuric or hydrochloric acid under temperature conditions (40 to 50 °C) over a period of time (30 min, approximately) (Shukla *et al.*, 2019). This process is usually used for the extraction of fucose-containing sulfated polysaccharides and also may include calcium addition to promote alginate precipitation during purification (Chizhov *et al.*, 1999; Ale *et al.*, 2012). The process of acid hydrolysis induces a remotion of complex phenolic compounds but an increase in the de-polymerization of polysaccharides (Flórez-Fernández *et al.*, 2018).

Most commercial biostimulants produced from brown algae are produced by alkaline hydrolysis (Craigie, 2011; Briceño-Domínguez *et al.*, 2014). This method involves extracting seaweeds in sodium or potassium hydroxide solutions at temperatures less than 100°C (Shukla *et al.*, 2019). Seaweed viscosity produced from *Macrocystis pyrifera* increases with increasing pH and temperature to a maximum which occurs at pH 10 and 80 °C (Briceño-Domínguez *et al.*, 2014). Alkaline hydrolysis also breaks down complex polysaccharides into smaller, lower-molecular-weight oligomers (Shukla *et al.*, 2019). Some water-soluble fractions and certain oligo and polysaccharides of importance could be lost (Sharma *et al.*, 2014), and new compounds not initially present in the algae biomass could be formed (El Boukhari *et al.*, 2020). Therefore, further separation and purification steps are necessary to maximize the extraction yield (Sharma *et al.*, 2014).

UAE appears as an interesting alternative to solve the problems mentioned above (El Boukhari *et al.*, 2020). UAE has allowed to optimize the extraction time of carrageenans and alginates from different seaweed species, without altering their chemical structure and molar mass distribution (Kadam *et al.*, 2015a; Youssouf *et al.*, 2017; El Boukhari *et al.*, 2020). In this way, extraction yields of 143.12 mg GAE/g<sub>db</sub>, 87.06 mg/g<sub>db</sub> and 128.54 mg/g<sub>db</sub> were obtained for total phenolics, fucose and uronic acid respectively at optimized extraction conditions of extraction time (25 min), acid concentration (0.03 M HCl) and ultrasonic amplitude (114 μm) (Kadam *et al.*, 2015b). UAE technology may also enhance the extraction of high molecular weight phenolic compounds from brown seaweeds (Kadam *et al.*,

2015a). Despite these improvements, the level of variability of extraction methodologies is still significant as to respond to industrial requirements (El Boukhari *et al.*, 2020).

EAE is a promising biotechnological application that has been widely used to improve the extraction efficiency of bioactive components (Sabeena *et al.*, 2020). EAE increases yield, high specificity, and preserves protein properties and it is a fast, scalable, and environmentally friendly method (El Boukhari *et al.*, 2020; Sabeena *et al.*, 2020). Protein extraction yield can be maximized by the optimization of certain physicochemical parameters such as hydrolysis time, pH, and temperature (Vásquez *et al.*, 2019; El Boukhari *et al.*, 2020). Enzymatic extracts obtained by hydrolyzing seaweeds with different carbohydrases and proteases presented significant antioxidant and antimicrobial activity (Sabeena *et al.*, 2020). However, some published reports show excessive extraction time and low yields compared to the extracts obtained from conventional methods using chemicals (Okolie *et al.*, 2019; Vásquez *et al.*, 2019).

It is important to note that when trying to extract a wide number of bioactive compounds, several interactions may occur within the mixture leading to both synergistic and antagonistic effects (El Boukhari *et al.*, 2020). For this reason, seaweed extraction techniques should be performed always considering biomass features, biostimulant's mode of application, crop's type, and the desired physiological effect such as abiotic stress tolerance, improvement of soil fertility or enhancement of fruit quality.

## PHYSIOLOGICAL ASPECTS OF SEAWEED FOLIAR APPLICATIONS

### Plant tissue interactions after seaweed applications

Through evolution plants have developed passive and active mechanisms to defend themselves against pathogens (Saigne-Soulard *et al.*, 2015). Passive mechanisms, such as structural barriers and antifungal compounds contribute to delay disease development (Saigne-Soulard *et al.*, 2015). Similarly, plants produce a wide number of secondary metabolites, such as terpenes, steroids, phenolics, pathogenesis-related (PR) proteins and alkaloids, developing active defense mechanisms, which play a key role in plant defense and adaptation to the environment (Ramirez-Estrada *et al.*, 2016). These mechanisms are induced only if the plants recognize the pathogen by the perception of signal molecules called elicitors (Delaunoy *et al.*, 2014; Saigne-Soulard *et al.*, 2015). Elicitors comprise a wide range of abiotic and biotic components (Delaunoy *et al.*, 2014). Abiotic elicitors can be physical stimuli or chemical

compounds, while biotic elicitors usually refer to molecules secreted by microorganisms, derived from cell walls of fungi, bacteria and host plants or from seaweeds (Saigne-Soulard *et al.*, 2015). R-genes (resistance genes) convey disease resistance against pathogens in plants by producing leucine-rich proteins (R proteins) (Liu *et al.*, 2017). These genes may be involved in elicitor binding and pathogen recognition and could initiate signaling pathways that activate various defenses against pathogens (Marone *et al.*, 2013). Once the elicitor is recognized by the R-gene complex, signaling pathways are initiated resulting in the activation of defense responses (Malik *et al.*, 2020). A transient change in permeability is generated in the plasma membrane, stimulating the inflow of  $Ca^{2+}$  and  $H^{+}$  ions into the cells and an outflow of  $K^{+}$  and  $Cl^{-}$  ions, which leads to an extracellular alkalinization and a depolarization of the plasma membrane (Bigard *et al.*, 2015). This activates the oxidative response of the plant and the signaling of other defense reactions (Malik *et al.*, 2020). These reactions are essential to initiate the signaling network that will activate all plant defense responses (Hammond-Kosack and Jones, 1996; Malik *et al.*, 2020).

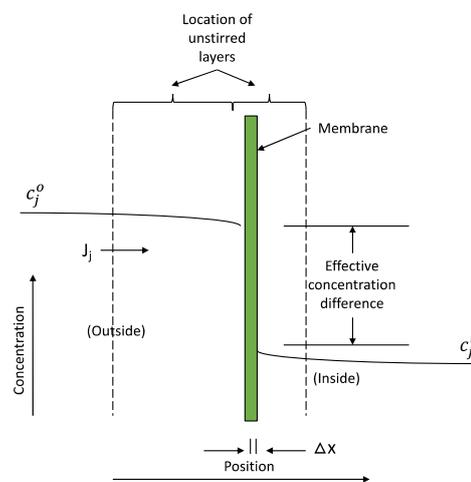
Seaweeds contain bioactive substances such as polysaccharides, proteins, lipids, and polyphenols with a diversity of antibacterial, antiviral, and antifungal activity (Peso-Echarri *et al.*, 2012). Cell walls of seaweeds contain a wide range of polysaccharides, such as  $\beta$ -(1 $\rightarrow$ 3)-glucans that are formed by neutral sugars and acids, and can act as elicitors when applied to plant tissues (Battacharyya *et al.*, 2015). Preliminary studies reported that a linear  $\beta$ -(1 $\rightarrow$ 3)-glucans applied to tobacco (*Nicotiana tabacum*) induced immunity through a series of reactions, such as the production of Reactive Oxygen Species (ROS), the expression of defense genes, a strong enzymatic activity of phenylalanine ammonia lyase, caffeic acid *O*-methyltransferase and lipoxygenase, and the accumulation of salicylic acid and PR proteins of family 1, 2, 3 and 5 in the leaves (Klarzynski *et al.*, 2000; Ménard *et al.*, 2004). Aziz *et al.* (2003) showed that a laminarin extract from *Laminaria digitata* applied to grapevine leaves reduced infection by *Botrytis cinerea* and *Plasmopara viticola* in greenhouse trials. The authors explained this effect based on extract implications on the calcium influx, alkalization of the extracellular medium, production of ROS, expression of defense genes and the accumulation of stilbene phytoalexins, such as resveratrol and viniferin. The accumulation of stilbenes has been recently confirmed in a field trial carried out after the application of an *Ascophyllum nodosum* extract on the 'Tempranillo Blanco' variety (Gutiérrez-Gamboa, 2020). In addition, Garde-Cerdán *et al.* (2017) reported that a treatment based on laminarin

and *Saccharomyces* extracts applied to grapevines decreased the content of several amino acids in grape berries. Probably, the induction of defense mechanisms in plants through exogenous elicitation may result in a physiological cost that involves the consumption of some amino acids as enzymatic substrates or the compartmentalization of these compounds in reserve organs as a survival mechanism (Gutiérrez-Gamboa *et al.*, 2017a). Pathogenic microorganisms and seaweeds present a close structural similarity regarding their  $\beta$ -(1→3)-glucan composition that allows to induce plant defense mechanisms (Paris *et al.*, 2019). A recent study showed that the elicitation of defense mechanisms against *P. viticola* in ‘Marselan’ grapevines using a laminarin extract was more associated to the direct antimicrobial activity related to the degree of acylation of the solution than to the elicitation performed by the bioactive compounds of the extract (Paris *et al.*, 2019). The hydrophobic balance of seaweed bioactive compounds and its toxicity against plant diseases is addressed through partial masking of hydroxyl functions by acylation with long chain aliphatic groups (Paris *et al.*, 2019). Nevertheless, the introduction of aliphatic groups can result in better interactions with the lipophilic cuticle and surfactant used as formulating agent, thus favoring penetration of the bioactive carbohydrate into plant leaves, and then increasing its bioavailability (Paris *et al.*, 2019). Based upon these findings, it is important to develop optimized seaweed formulations with appropriate dosages and properties to improve their solubilization and their retention on the leaf surface for better effectiveness of the seaweed elicitor treatments. As was explained in this section, plants develop acquired systemic resistance (SAR) in subsequent pathogen attacks when they have previously survived to a pathogen affection and can develop a medium-long term protection against a wide range of pathogenic species (Malik *et al.*, 2020). Therefore, continued exogenous application of the same elicitor throughout the growing season to induce the activation of defense mechanisms in plants may result in a certain degree of tolerance but it could not generate the same effects when a single application is used, particularly at the beginning of the season.

#### Uptake of macro and micro elements by grapevine leaves

Inorganic components of seaweed extracts may enter the plant through the leaves and stuck to reserve organs (Gutiérrez-Gamboa *et al.*, 2020c). The processes by which a nutrient solution applied to the leaves is assimilated by plants include contact, leaf adsorption, cuticular or stomatal penetration, uptake and absorption into metabolically active cell compartments in the leaf,

and finally, the translocation and utilization of the nutrients absorbed by the plant (Fernández *et al.*, 2013). Nutrient absorption by plant surfaces occurs via cuticle, cuticular stacks and imperfections, stomata, trichomes and lenticels (Fernández *et al.*, 2013). Epidermal structures such as stomata and lenticels are permeable to foliar fertilizer solutions, while lipophilic solutions may cross cuticles via a solution-diffusion process (Fernández *et al.*, 2013). To enter the leaf, molecules must diffuse across an air boundary layer at the leaf surface or move from a region of high to low concentration with a magnitude that is proportional to the concentration gradient (Nobel, 2009; Fernández *et al.*, 2013). This energy balance is dominated by the first Fick's Law of diffusion (Figure 1).



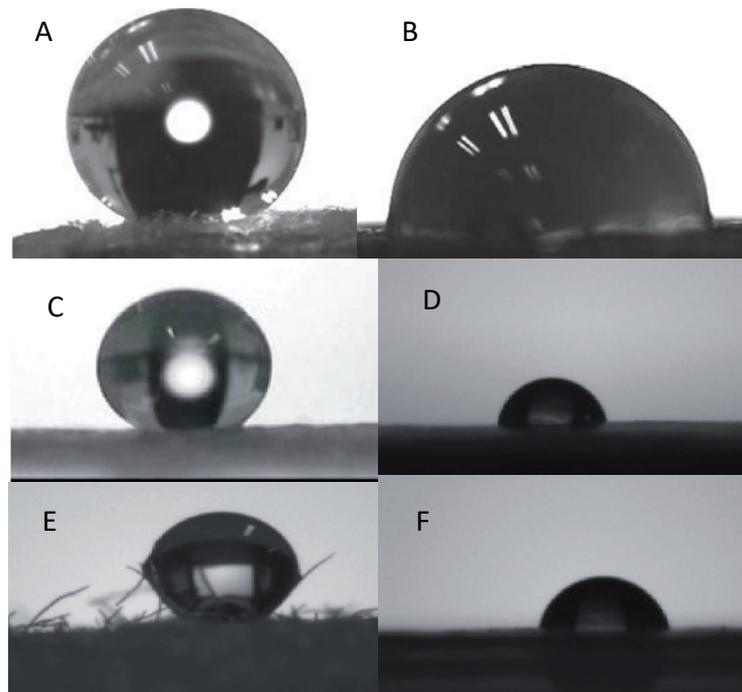
**Figure 1.** The effect of unstirred layers (dashed lines) on the concentration of species *j* near a membrane across which the solute is diffusing.

Stomata may represent the main pathway for N absorption since they do not present a waxy layer and its waxy cuticle has different degrees of polarity resulting in an efficacious N transmission compared to leaf cuticle (Norms and Bukovac, 1968; Leece, 1976; Cheng *et al.*, 2020). Grapevines leaves have a greater number of stomata on their abaxial side than on the adaxial one (Rogiers *et al.*, 2011). Therefore, grapevine is considered as a hypostomatic species and stomata density of its leaves varies greatly in response to soil temperature and atmospheric CO<sub>2</sub> concentration (Rogiers *et al.*, 2011). Nevertheless, the stomatal pathway contribution to the foliar uptake process should be further elucidated as well as the role of other epidermal structures such as trichomes and lenticels (Fernández *et al.*, 2013). In this sense, a reinterpretation of the plant cuticle as a lipidized epidermal cell wall region and, consequently, its contribution to nutrient uptake has been recently proposed (Fernández *et al.*, 2016). Some studies on the penetration of substances through the cuticles

indicate that the process is only passive and not mediated by energy, since they are devoid of living cells (Fernández *et al.*, 2016). Therefore, the cuticle should be considered as a composite material made of lipophilic components (apolar compounds) such as waxes and cutin, and hydrophilic components (polar compounds), such as polysaccharides (Fernández *et al.*, 2016, 2017).

Ultrastructure and chemical features of the leaf render it difficult the wetting and the permeability of polar nutrient solution foliar applications (Fernández and Eichert, 2009). In this sense, erect and prostrate trichomes are the most characteristic

features present on the epidermal surfaces of grapevine leaves (Konlechner and Sauer, 2016). Contact angles are hugely affected by these hairs, resulting in individual cases in values  $>150^\circ$  (high hydrophobicity) in the presence of a high reclining of hair density (Konlechner and Sauer, 2016) (Figure 2). ‘Grüner Veltliner’, ‘Welschriesling’, ‘Blauer Wildbacher’ and ‘Sauvignon Blanc’ grapevine varieties presented remarkably hydrophobic abaxial leaves ( $> 140\text{-}152^\circ$ ), while ‘Pinot Noir’ and ‘Portugais Bleu’ varieties, which present very low or absent hair growth, exhibited low hydrophobicity on both leaf sides ( $> 95\text{-}105$ ) (Konlechner and Sauer, 2016).



**Figure 2.** Contact angles with water drops of the abaxial surface of (A) ‘Grüner Veltliner’ (*Vitis vinifera* subsp. *vinifera* L.) leaf ( $> 152^\circ$ ) and (B) adaxial side of ‘Saint Laurent’ (*Vitis vinifera* subsp. *vinifera* L.) leaf ( $< 90^\circ$ ); (C) adaxial *Eucalyptus globulus* ( $> 140^\circ$ ), and (D) *Ficus elastica* leaves ( $> 83^\circ$ ); (E) Peach ( $> 130^\circ$ ) and (F) Apple ( $> 84^\circ$ ) fruit surfaces. Obtained from Konlechner and Sauer (2016), and Fernández *et al.* (2013).

The most important environmental factors that directly affect the uptake of nutrients by leaves are temperature and relative humidity (Fernández *et al.*, 2013). High temperatures may increase the solute diffusion of the active ingredients and adjuvants, but may decrease solution viscosity, surface tension and the point of deliquescence (Ramsey *et al.*, 2005; Fernández *et al.*, 2013). Relative humidity may improve uptake efficacy by increasing cuticle hydration and delaying droplet drying (Ramsey *et al.*, 2005). For these reasons, humectants are used successfully in warm and dry areas to increase uptake efficacy of the foliar applications (Ramsey *et al.*, 2005). Thus, penetration of hydrophilic substances may be enhanced by hydration of the

cuticle, while transcuticular transport of non-polar solutes is increased by factors which reduce wax viscosity (Kirkwood, 1999).

## SEAWEED BIOSTIMULATION IN VITICULTURE

During the past two decades, world main winegrowing regions are facing unpredictable climatic conditions and a rise in temperature, mostly during the summer period, which have strongly affected grape ripening dynamics and quality (Jones and Davis, 2000; Tomasi *et al.*, 2011; Keller, 2020). Seaweed-based biostimulants may provide vineyard protection against abiotic and biotic factors, improving grapevine productivity

(Salvi *et al.*, 2019) and play a key role in the development of environmentally friendly viticulture strategies, mitigating the negative effects of climate change (Sabir *et al.*, 2014; Frioni *et al.*, 2018). Most of the reports related to the effects of foliar application of seaweeds in viticulture have been

focused on their impact in mitigating the negative effects of abiotic and biotic stress, grapevine productivity and grape berry physicochemical components (Table I), which are discussed in the following sections.

**Table I**

Selected recent scientific reports regarding the effects of different seaweeds foliar application in grapevine

Reference	Algae	Effects
Khan <i>et al.</i> (2012)	Amino acid mixture + <i>Ascophyllum nodosum</i>	Increased leaf size and chlorophyll content, cluster number per shoot, stalk length, berry weight and soluble solids.
Strydom (2014)	Biocel+K+leonardite	Increased berry soluble solids, decreased total acidity and anthocyanin content.
Sabir <i>et al.</i> (2014)	<i>A. nodosum</i>	Did not affect the studied parameters.
Sabir (2015)	Calcite + <i>A. nodosum</i>	Improved pollen viability.
Zermeño-González <i>et al.</i> (2015)	<i>Sargassum spp.</i>	Increased CO <sub>2</sub> assimilation rate, productivity and soluble solids.
Gutiérrez-Gamboa <i>et al.</i> (2019)	<i>A. nodosum</i> + microelements	Increased fruit set and productivity in Carmenère grapevines.
Frioni <i>et al.</i> (2018, 2019)	<i>A. nodosum</i>	Increased anthocyanin accumulation in Sangiovese and Pinot Noir, avoiding the affection of grapes by <i>Botrytis cinerea</i> .
Gutiérrez-Gamboa <i>et al.</i> (2017b,c, 2018)	Amino acid mixture + <i>Durvillaea antarctica</i>	Increased amino acids content in grapes and the concentration of higher alcohols and esters in wines.
Salvi <i>et al.</i> (2019)	<i>A. nodosum</i>	Increased gas exchange and avoided photoinhibition of leaves. Delayed ripening and promoted the synthesis of phenolic compounds in leaves and berries.
Taskos <i>et al.</i> (2019)	<i>A. nodosum</i> + N to soil	Increased the productivity and the anthocyanins extractability from berry skins.

### Seaweed effects on grapevine productivity

The application of a commercial extract of the seaweed *Ascophyllum nodosum* (ACADIAN®) at a dosage of 2.35 L/ha produced an increase in yield, cluster and berry weight, cluster size and firmness in ‘Thompson Seedless’ table grape variety, but had a negative effect on the accumulation of soluble solids in the berries (Norrie *et al.*, 2002). Copper and other micronutrients absorption in one-year-old grapevines of the ‘Karaerik’ variety was improved after the application of three commercial extracts of seaweeds (Maxicrop®, Proton® and Algipower®) at a dosage of 1.0 to 2.0 g/L (Turan and Köse, 2004). Probably, seaweed extracts may increase membrane permeability of roots, leaves and stomata cells, enhancing copper uptake by plants (Verkleij, 1992). In another study, the foliar application to grapevines of an *A. nodosum* commercial extract (Alga Special®) at a dose of 170 mL/hL increased vegetative growth, shoot length and diameter and leaf area in ‘Feteasca Alba’ grapevine variety (Popescu and Popescu, 2014). *A. nodosum* brown alga is very effective at

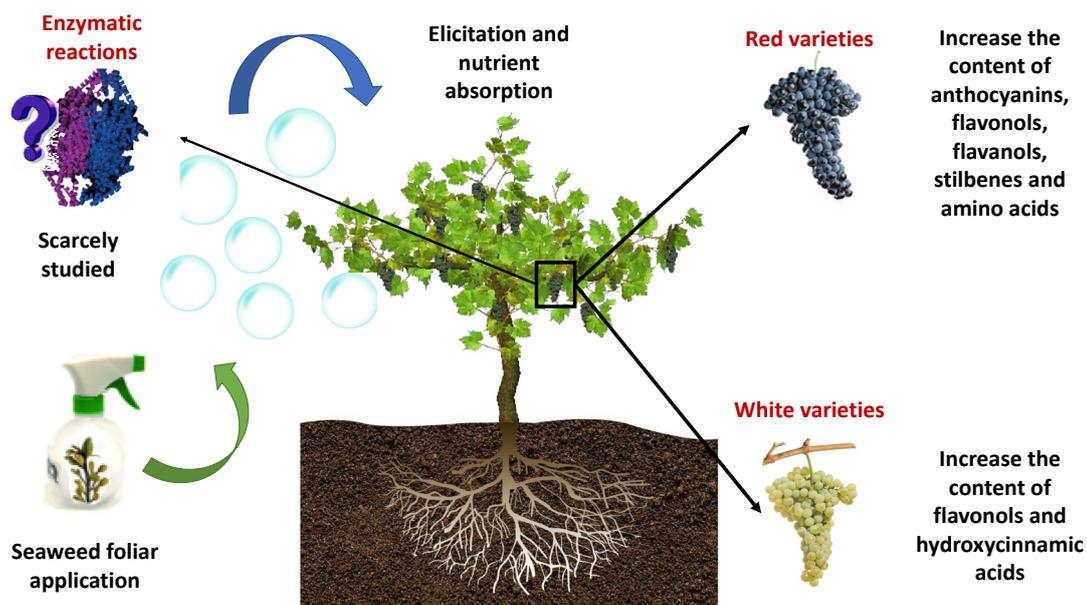
accumulating nutrients and minerals from the surrounding seawater and its harvested biomass is exploited as fertilizer in horticulture to improve physiological responses in plants when applied (Pereira *et al.*, 2020). A soluble extract of *A. nodosum* applied foliarly to grapevines at a concentration of 0.5 % (v/v) increased leaf dry weight, berry weight and volume, and Ca, Zn, S, B and chlorophyll leaf contents in ‘Narince’ grapevine variety (Sabir *et al.*, 2014). Fruit set and productivity in ‘Carmenère’ grapevines cultivated under cold and warm climates was improved after the foliar application of a commercial *A. nodosum* extract (BM86®) at a dose of 3 L/ha (Gutiérrez-Gamboa *et al.*, 2019b). Foliar application of a commercial fertilizer (Primo®) elaborated based on an *A. nodosum* algae sprayed in multiple applications of 0.1 mL/L increased leaf size and chlorophyll content, fruit set, number of clusters per shoot, rachis length, berry weight and size, soluble solids and pH values in musts, and reduced berry dehydration in ‘Perlette’ table grapevine variety (Khan *et al.*, 2012). Despite the high effectiveness

of seaweed foliar applications on productivity reported in the abovementioned works, some of these reports were limited to only one year of trials, living out of consideration that seasonal climate effects have an important influence in determining yield and their components in grapevines (Echeverría *et al.*, 2017). Grapevine clusters that make up for the current season yield, began to differentiate the preceding growing season in the compound bud (Vasconcelos *et al.*, 2009). Therefore, the maximum number of clusters per grapevine and by consequence, the potential grapevine yield, is determined in the previous

season and it is not likely affected by seaweed applications performed in the current season.

### Seaweed effects on grape berry and wine composition

Currently, there is little information available in the scientific literature addressing the effects of foliar applications of seaweeds to grapevines on the content of amino acids, volatile and polyphenol compounds in grapes and wines - Figure 3 (Frioni *et al.*, 2019; Salvi *et al.*, 2019; Taskos *et al.*, 2019; Gutiérrez-Gamboa *et al.*, 2020c).



**Figure 3.** Summary of the effects of seaweeds foliar application to grapevines on grape composition.

A commercial nitrogen fertilizer (Basfoliar® Algae SL) based on the *Durvillaea antarctica* algae applied to ‘Cabernet Sauvignon’ grapevines at 2 kg N/ha in veraison increased the content of several amino acids in grapes and wines, which resulted in a higher content of some higher alcohols and ethyl esters in the elaborated wines, improving their fruity and floral aromas (Gutiérrez-Gamboa *et al.*, 2017b,c, 2018). Several works confirmed that the foliar application in the phenological state of veraison improves the nutrient absorption by grapevines compared to soil application (Lacroux *et al.*, 2008; Lasa *et al.*, 2012; Verdenal *et al.*, 2015; Dienes-Nagy *et al.*, 2020). Therefore, combined foliar applications of both nitrogen and seaweed extracts may stimulate grapevine nitrogen metabolism and therefore the synthesis of amino acids, leading to stored nitrogen in source organs. This nitrogen can be transported to sink organs, such as young leaves and grapes (Verdenal *et al.*, 2016; Gutiérrez-Gamboa *et al.*, 2017a; Verdenal *et*

*al.*, 2020). In other studies, Frioni *et al.* (2018) and Salvi *et al.* (2019) observed that foliar applications of *A. nodosum* extracts to grapevines at a dosage of 3g/L and 1.5 kg/ha, respectively, increased skin anthocyanin accumulation and total phenolic content in berries. Frioni *et al.* (2019) demonstrated that this increase was related to the specific modulation of genes involved in the activation of metabolic pathways of flavonoid synthesis. These authors also showed that grapevines treated with an *A. nodosum* fertilizer were less affected by gray rot (*Botrytis cinerea*) compared to untreated grapevines. On the other hand, Taskos *et al.* (2019) observed that foliar applications of an *A. nodosum* extract at a concentration of 1 % (v/v) to ‘Merlot’ grapevines increased yield, cluster berry number, anthocyanin extraction capacity from berry skins and seed tannins, while decreasing grape total polyphenol index. Gutiérrez-Gamboa (2020) reported that seaweed foliar applications to ‘Tempranillo Blanco’ grapevines at 0.25 % (v/v)

increased catechin and flavonol contents, regardless of the climatic conditions of the season, whereas the application of the seaweed at 0.50 % (v/v) to grapevines increased the concentration of several amino acids on a dry season compared to a wet one. Therefore, seaweeds can likely behave as elicitors and as such could trigger enzyme activity responsible for the synthesis of phenolic compounds in grapevine tissues in vineyards cultivated under different climatic conditions. On the other hand, Gutiérrez-Gamboa *et al.* (2020c) reported that a seaweed extract applied at 0.25 % (v/v) to ‘Tempranillo’ grapevines increased the content of several amino acids compared to the application at 0.50 % (v/v) of the same extract on a dry season. In this way, Paladines-Quezada *et al.* (2019) indicated that the application of methyl jasmonate (abiotic elicitor) to grapevines caused significant changes in structural components of grape skin cell walls, such as phenolic compounds, proteins and sugars. In their report ‘Monastrell’ and ‘Cabernet Sauvignon’ cultivars showed a positive effect of the application while ‘Merlot’ exhibited an opposite one. These results suggest an important varietal dependence when an elicitor is applied to grapevines, probably due to the great variability in the structural components of the cell walls of the grape berries.

#### **Enzyme metabolism activation**

Only one study reported the enzymatic reactions that occur after commercial seaweed extracts were applied to grapevines (Frioni *et al.*, 2019). They showed that the application of a seaweed extract did not affect the expression of flavonoid 3-*O*-glucosyltransferase (UFGT), flavonoid *O*-methyl transferase 2 (OMT2), leucocyanidin dioxygenase (LDOX), glutathione S-transferase (GST), flavonoid 3' hydroxylase (F3'H), flavonoid 3'5' hydroxylase (F3'5'H) or dihydroflavonol reductase (DFR) at full veraison stage. At the moment in which the grapes reached 19 °Brix, seaweed applications enhanced UFGT, LDOX, GST, F3'H, F3'5'H and DFR transcription levels. At technological maturity, relative expression of UFGT, OMT2, LDOX, GST and F3'5'H was higher in non-treated grapevines (Frioni *et al.*, 2019). Despite the interesting results shown by these authors, there was no clear relationship between the relative expression of genes and the accumulation of phenolic compounds or other secondary metabolites in grape berries. Therefore, the knowledge regarding the effects of algae foliar application to grapevines on the relative expression of genes involved in the synthesis of secondary metabolites in grapes is not yet fully understood.

#### **SHORT-TERM RESEARCH PERSPECTIVES**

Seaweed-based biostimulants may reduce the incidence and severity of grapevine diseases,

improve yield and change grape and wine composition, as described above. However, the mechanisms of action involved in these processes are not fully understood. The knowledge of leaf surface wetting properties must be combined with the advance in the insights on their interactions with the physico-chemical properties of seaweed foliar formulations, including surfactants, with the aim to promote an optimal and prolonged use of the biostimulants applied, especially under hot, dry and cold weather conditions (Konlechner and Sauer, 2016). The contribution of the stomatal pathway to the foliar nutrient uptake process should be further studied as well as the role of other epidermal structures such as trichomes and lenticels (Fernández *et al.*, 2014). Frioni *et al.* (2019) reported for the first time that seaweed applications to grapevines may modulate genes related to flavonoid pathways and the upregulation of some defense-related genes. The modulation of genes related to the accumulation of volatile compounds, such as terpenes, C<sub>13</sub> norisoprenoids, benzenes, alcohols and carbonyl compounds could be investigated to elucidate the upregulation of other defense-related genes in grapevines that have not yet been considered in research studies. Cell walls of seaweeds are rich in sulfated polysaccharides, including carrageenan in red algae, ulvan in green algae and fucoidan in brown algae (Cunha and Grenha, 2016). Most of the studies on the application of seaweeds in viticulture have been carried out using the brown algae *Ascophyllum nodosum*, so other brown, green, red algae could be studied. Biological activity of seaweed cell wall polysaccharides strongly depends on their structural properties, such as degree of substitution, molecular weight, type of sugar and functional groups (Synytsya *et al.*, 2010). Therefore, the knowledge of these functional and physicochemical characteristics is important to develop an optimized seaweed formulation in order to improve the solubilization and the retention of these products on the leaf surface.

#### **CONCLUDING REMARKS**

The use of seaweeds in viticulture has allowed to improve grapevine productivity and to enhance grape and wine quality, mostly in terms of their phenolic composition. Mechanisms of action of seaweed products applied to the grapevines have not been fully studied but this review provides an overview on their effects as elicitors and foliar fertilizers. Their effectiveness seems to be mainly associated to seasonal climatic conditions, to the species of seaweed applied, its structural properties, leaf surface wetting properties of the grapevine variety, the stress and resistance level of the grapevine prior to the application, dosage of the formulation and their implications on relative expression genes in leaves and berries. Upcoming

studies must be carried out to understand these conditions in order to obtain specific formulations for each grapevine variety. Finally, despite that *Vitis vinifera* grapevine varieties possess defense mechanisms against elicitors, the biosynthesis of defense-related compounds and other active and passive mechanisms is too slow, and it may take from few hours to a few days to get activated. In addition, the grapevine defense capacities depend on the organs elicited such as leaves, inflorescences, grapes, among others, and the phenological stages in which elicitation occurred. Therefore, the induction of the grapevine natural defense mechanisms through seaweed commercial products application must be considered in the context of an integrated vineyard management approach.

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