Humic substances: a valuable agronomic tool for improving crop adaptation to saline water irrigation

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ABSTRACT

Availability of fresh water for crop irrigation is becoming scarce and rather expensive. In this context, the research about the potential reutilization of non-conventional water sources becomes highly relevant, principally in arid and semi-arid areas. On many occasions, these new water resources involve water with a moderate concentration of salt, making it necessary to improve plant growth under moderate saline conditions. Besides plant breeding techniques, the use of molecules able to improve plant adaptation to saline conditions has great interest. Between these molecules, humic substances (HS) have proven to be efficient as stress-protectors under specific conditions of stress intensity and moment of application. The HS are main components of the soil organic matter and dissolved organic matter resulting from the biotic and abiotic transformation of fresh organic matter in natural ecosystems. Although knowledge about their structure is still under open debate, HS contain aromatic and aliphatic domains presenting O-, N- and S-containing functional groups with high biological and chemical activities. The aim of this presentation is to summarize the main effects of humic acids (HA) applied either on the root or on the shoot, on the metabolism and hormonal balance of plants cultivated under normal and stressing conditions, from studies carried out for our group and collaborations during the last years. Summarizing, all these results show HA as a promising tool for improving crop adaptation to irrigation using moderate saline water sources.

Key words | abiotic stress, crop irrigation, humic substances, mineral plant nutrition, reclaimed water, saline stress

INTRODUCTION

Many factors affecting climate have negative consequences, such as lower rainfall and higher temperatures, influencing the availability of freshwater for crop irrigation (NRC 2012). This fact demands the development of intensive research in order to reutilize alternative water resources in agriculture (Tran et al. 2016). This research involves the integration of several alternative strategies in a whole programme for crop management. These strategies include, and in most cases require, the possibility of re-utilizing partially purified residual waters (treated wastewater) for crop irrigation, mainly in semiarid and arid areas (Tran et al. 2016).

Though previously treated, these recycled waters have some degree of salinity (Pedrero & Alarcón 2009; Morgan 2011). This fact demands the use of plant varieties with a certain resistance to salinity. These plant varieties may be obtained from traditional plant breeding or genetic modification, but also by applying compounds with the ability to enhance plant adaptation to osmotic stress (García-Mina & Hadavi 2016; Van Oosten et al. 2017).

A family of natural compounds that has shown high ability to enhance the growth of plants cultivated under salinity is humic substances (HS) (Du Jardin 2015; Van Oosten et al. 2017). This family can be defined as a...
system of natural biomolecules resulting from the gradual transformation of fresh organic matter derived from animal and plant decay, by the action of abiotic (oxidation, reduction, polymerization etc.) and biotic (microorganisms) processes. As a function of their solubility in water solutions with different pH, HS are classified in humic acids (HA), which are soluble at basic pH but insoluble at acidic pH; fulvic acids (FA) that are soluble at basic and acidic pH; and humin, which is insoluble independently of the pH (Olaetxea et al. 2018). Although HS structural features are not well known and are still the object of an open and intense debate (Olaetxea et al. 2018), recent studies have demonstrated that HS have singular structural features that are not present in natural, well-characterized biomolecules (Cao & Schmidt-Rorh 2018). In solution, these molecules tends to form stable molecular aggregates (self-assemblies) that provide them with new chemical properties probably affecting their biological activity (Olaetxea et al. 2018). This fact allows us to consider HS as a new family of natural supramolecules (Piccolo 2002). Likewise, other studies have demonstrated that although HS present high polydispersity, they have some singular features that are principally associated with the HS fraction more than with the origin (Ateia et al. 2017; Derrien et al. 2017). These results support the presence of singular chemical features defining HS but also underline the dependence of HS effects on plant growth on the HS type.

Many studies have shown that HS are able to improve plant adaptation to saline stress (Rose et al. 2014). Although the mechanisms behind this ability are not known in detail, many studies suggest that they are supported by an action of HS on signaling pathways regulated by plant hormones and molecular second messengers, such as reactive oxygen species (ROS) and calcium (Berbara & Garcia 2014; Olaetxea et al. 2018).

The purpose of this mini-review is to briefly discuss the main potential mechanisms by which HS improve the growth of plants cultivated in saline soils or irrigated with saline water. We differentiate the HS root application from HS foliar application, since two different mechanisms are probably involved in the beneficial action of HS when applied on the leaf or to the root. We focus our review on the action of HA.

THE PLANT GROWTH PROMOTION OF HA APPLIED TO PLANT ROOTS IS REGULATED BY HORMONAL-SIGNALING PATHWAYS AND MOLECULAR SECOND MESSENGERS ALSO INVOLVED IN PLANT STRESS RESISTANCE

The very relevant role of some phytoregulators in the ability of plants to grow under high salinity conditions has been well established (Verma et al. 2016; Kaur & Pati 2017). Among them, abscisic acid (ABA), auxin (indoleacetic acid, IAA) and nitric oxide (NO) seem to be the most relevant (Verma et al. 2016; Kaur & Pati 2017). Recently, brassinosteroids and strigolactones have also presented a protective role (Kaur & Pati 2017), as well as salicylic acid (SA) (Kaur & Pati 2017). Finally, the role of cytokinins is not clear since there are studies showing a beneficial action promoting stress adaptation while other studies pointed out a decrease in stress adaptation upon cytokinin exogenous application (Kaur & Pati 2017).

Thus, ABA concentrations in shoot and root increase with saline stress (Verma et al. 2016; Kaur & Pati 2017). This increase is directly linked to the control of stomata closure, thus controlling water transpiration and maintaining water homeostasis (Verma et al. 2016; Kaur & Pati 2017). Besides that, ABA also promotes the up-regulation of a complex network of genes that codify proteins involved in plant adaptation to stress. In the root, ABA seems to work together with IAA in the regulation of root growth and architecture in order to achieve more resistance against osmotic stress (Verma et al. 2016; Kaur & Pati 2017).

In this context, it is noteworthy that when HS are applied to plant roots they promote an increase in both IAA and ABA in the root (Olaetxea et al. 2018). These HA-mediated effects are crucial for the shoot and root growth promoting action of HS (Olaetxea et al. 2018). Thus the HA action on IAA signaling pathways regulates root plasma membrane H⁺-ATP-ase activity as well as the proliferation of lateral and adventitious roots (Zandonadi et al. 2007; Mora et al. 2012). Likewise, the use of inhibitors of IAA perception showed that this phyto-regulator is directly involved in the shoot-promoting action of HS (Mora et al. 2014a).

As for ABA, the action of HS in enhancing ABA concentration in roots regulates the root hydraulic conductivity
and root aquaporin activity, and is also crucial for the shoot promoting action of HA. In fact, IAA and ABA seem to be interconnected to each other in the whole action of HA in the root (Olaetxea et al. 2015) (Figure 1).

It is therefore very plausible that the protective action of HS for plants from osmotic stress is related to these major effects of HA on IAA and ABA signaling pathways. In this sense, it is very interesting that the principal event that arises from the interaction of HA with cell walls at the root surface probably causes a transient mild stress that might trigger all the biochemical and molecular events involved in the beneficial action of HA on plant growth (Olaetxea et al. 2018). This type of mild stress, also known as eustress, could act as an enhancer of plant adaptation to abiotic stress conditions (Figure 1).

Another very relevant actor is NO. Several studies have shown that NO is involved in the plant response to different types of stresses, either nutritional or osmotic (Mora et al. 2014b). This effect of NO seems to be mediated by ROS signaling pathways (Mora et al. 2014b). The involvement of NO in the beneficial effects of HA on plant growth has been reported by various studies (Zandonadi et al. 2010; Mora et al. 2012, 2014b). This action is also probably involved in the whole action of HS on root growth, and can also have implications in the improvement of root nutrient uptake caused by HA (Mora et al. 2014b). As mentioned before, it might also trigger secondary effects on ROS metabolism.

Many studies have shown that the deleterious effect of salt stress on plant development is related to an uncontrolled increase in ROS production, mainly H2O2 and the radical superoxide (O2·-) (Verma et al. 2016; Kaur & Pati 2017). This increase causes important oxidative damage to very relevant biomolecules like proteins and lipids (Verma et al. 2016; Kaur & Pati 2017). Although an adequate control of ROS levels is directly linked to the fine regulation of important physiological processes like root development and adaptive stress responses (Kaur & Pati 2017), the inability of the plant to modulate cellular ROS

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**Figure 1** | Effects of humic substances applied to the root on ABA-, IAA- and NO- signaling pathways in plants cultivated in no stressing conditions (ABA: abscisic acid; IAA: indoleacetic acid; NO: nitric oxide; ETH: ethylene).
concentration under severe stresses leads to plant death (Kaur & Pati 2017). Interestingly, HA has the ability to cause a controlled increase in ROS concentration accompanied by an efficient activation of the main enzymes involved in the maintenance of ROS homeostasis, like superoxide dismutase, catalase and ascorbate oxidase (García et al. 2012, 2016; Berbara & Garcia 2014). Several studies reported the ability of HA extracted from vermicompost to improve the growth of rice plants suffering osmotic stress (García et al. 2012, 2016). This action was linked to a concomitant action on ROS concentration in the root (García et al. 2016). These effects were confirmed in a later study that also showed the ability of HS to regulate the expression of genes codifying the main anti-oxidative enzymes and their activities (García et al. 2016). These results clearly show how a vermicompost-HA is able to both induce ROS production and regulate ROS concentration, thus suggesting that ROS may work as a signal for the control of root development and plant responses to stresses mediated by HS (Berbara & Garcia 2014 and references therein; García et al. 2012, 2016). (Figure 2).

**THE FOLIAR APPLICATION OF HA IS RELATED TO SIGNIFICANT VARIATIONS IN THE WHOLE HORMONAL BALANCE OF THE PLANT THAT COULD IMPROVE THE PLANT ADAPTATION TO ABIOTIC STRESSES**

Besides the beneficial action of root-applied HA, a number of studies have also shown that the application of HA-based formulations on plant leaves also enhances plant growth, especially under stress conditions (Van Oosten et al. 2017). However, these effects are less consistent than those observed when HA is applied on the root (De Hita et al. 2018, unpublished results).

In principle, the mechanisms responsible for the effects caused by foliar-applied HA do not have to be the same as those involved in the action of root-applied HA. However, there is no information about these mechanisms (Olaetxea et al. 2018).

Preliminary studies carried out in our laboratory explored the effect of the foliar application of a sedimentary HA on the leaves of cucumber plants growing in hydroponics showed that HA cause significant changes in leaf

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**Figure 2** | Potential mechanisms by which humic substances can enhance the plant adaptation to abiotic stress. Special role of reactive oxygen species (ROS) metabolism (SOD: superoxide dismutase; CAT: catalase; ABA: abscisic acid; IAA: indoleacetic acid; NO: nitric oxide; ETH: ethylene).
concentration of two relevant plant hormones related to stress: ABA and the conjugate of jasmonic acid (JA) with isoleucine (JA-Ile) (Figure 3) (De Hita et al. 2018, unpublished results). However, the increase in plant growth was transient, thus suggesting the need for several applications during the plant cycle. On the other hand, these increases in hormones involved in the plant responses to abiotic and biotic stresses suggest that HS foliar application might also have a positive effect by improving the plant’s adaptive response to several types of stresses. However, the effect caused by HS seems to be less consistent and relevant than that observed when HS are applied on the root, and probably it may be restricted to plants suffering some type of stress.

CONCLUSION: HA APPLICATION MIGHT BE A VERY USEFUL TOOL TO ENHANCE PLANT ADAPTATION TO MODERATE SALINE WATER IRRIGATION

In conclusion, the results presented above indicate that an adequate use of HA, integrated into the whole crop management plan, may be very interesting in order to maintain high yields in crops irrigated with reused water presenting moderate levels of salt.

This fact is very relevant, since moderate salinity is interesting in order to increase the nutraceutical value of fruits. Thus, some studies have demonstrated that moderate salinity provides the fruit with higher content of polyphenols and antioxidants as well as a more intense and pleasant flavor (Sgherri et al. 2008; Gómez-Caravaca et al. 2012).

The results, taken together, indicate that the application of HA to the root, for instance using fertigation, has more consistent effects that the HA application on the leaves. However, more studies are needed in order to better know the potential value of foliar-applied HA since their effects on JA-Ile, for instance, indicate that might help the plant to develop a more intense defensive response to pathogen attacks.

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