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Review
HUMIC SUBSTANCES AS PLANTS BIOSTIMULANTS UNDER ENVIRONMENTAL STRESS CONDITIONS

Revisión bibliográfica
Las sustancias húmicas como bioestimulantes de plantas bajo condiciones de estrés ambiental

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ABSTRACT. Plants are frequently subjected to unfavorable situations for their optimal development and operation caused by alterations in the environment. This set of unfavorable situations is known as environmental stress. Biostimulants are substances that by their action can stimulate the plant growth, improve the absorption of nutrients and increase yields under environmental stress conditions regardless of whether they contain nutrients in their composition. There are several categories of specific biostimulants, among them protein hydrolysates, algae extracts, chitosan, humic and fulvic acids, mycorrhizal fungi and growth promoting bacteria. The objective of this review is to inform the biostimulating effect of the application of humic substances in plants under stress conditions. It also synthesizes aspects related to humic substances such as their structural characteristics and classification. Results are shown using humic substances as biostimulants where their potential to stimulate different metabolic and physiological processes in abiotic stress conditions is demonstrated. It concludes that humic substances present a complex structure, a multiplicity of functional groups and small molecules that interact by weak junctions, which makes them exhibit a great variety of beneficial functions among which is their potential to increase the yields and attenuate the effects of abiotic stresses. Therefore they constitute a viable alternative to avoid the consequences of climate change and use natural and environmentally friendly products.

Key words: humus, climate change, physiological effects, abiotic stress, yield

RESUMEN. Las plantas están sometidas frecuentemente a situaciones desfavorables para su desarrollo y funcionamiento óptimos, ocasionadas por alteraciones en el medio ambiente. Este conjunto de situaciones desfavorables se conoce con el nombre de estrés medio ambiental. Los bioestimulantes constituyen sustancias, que por su acción pueden estimular el crecimiento de la planta, mejorar la absorción de nutrientes e incrementar los rendimientos en condiciones de estrés ambiental, independientemente de que contengan elementos nutrientes en su composición. Existen diversas categorías de bioestimulantes específicos, entre ellos, los hidrolizados de proteínas, extractos de algas, quitosana, ácidos húmicos y fúlvicos, hongos micorrízicos y bacterias promotoras del crecimiento. El objetivo de esta revisión es mostrar el efecto bioestimulante de la aplicación de sustancias húmicas en plantas, bajo condiciones de estrés. También se sintetizan aspectos relacionados con las sustancias húmicas como son sus características estructurales y su clasificación. Se informan resultados empleando sustancias húmicas como bioestimulantes, donde se comprueba su potencial para estimular diferentes procesos metabólicos y fisiológicos en condiciones de estrés ambiental. Se concluye que las sustancias húmicas presentan una estructura compleja variable, una multiplicidad de grupos funcionales y pequeñas moléculas heterogéneas que interactúan mediante uniones débiles, lo cual hace que exhiban una gran variedad de funciones beneficiosas entre las que se encuentra su potencial para incrementar los rendimientos y atenuar los efectos de estreses abióticos. Por tanto, constituyen una alternativa viable para evadir las consecuencias del cambio climático y emplear productos naturales y amigables con el medio ambiente.

Palabras clave: humus, cambio climático, efectos fisiológicos, estrés abiótico, rendimiento

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INTRODUCTION

The plants are frequently subjected to unfavorable situations for their optimal development and operation, caused by alterations in the environment. This set of unfavorable situations is known as environmental stress (1).

The factors external to the plant that constitute stress conditions can be of two types: biotic and abiotic (physical, chemical and physico-chemical.) Abiotic cover a wide range of environmental factors, among which are: temperature, water, radiations, chemical substances, etc. Abiotic stresses are the main cause of crop losses in the world and cause a decrease in the yield of more than 50 % of most crops (2).

To increase agricultural productivity, it is necessary to increase the search for cultivars that develop with greater tolerance to abiotic stresses (3). The scientific contributions made in this regard, until recently, were aimed at adapting the environment for a better development of the plants, applying a large amount of chemical products, such as herbicides and insecticides, depleting the resources water and nutrients necessary for the plant tolerate stressful conditions.

However, currently there is a new conception that is to adapt the plant to this changing environment, without exhausting resources or using chemical-synthetic products, only achieving greater efficiency in the use of these resources and increased production, with the use of the same strategies that perhaps contributed to the survival of these living beings during their evolution in even more stressful conditions.

Among the products that have been used to combat the effects of stress and raise the yields of plants, are the biostimulant products (4). These substances and materials, when applied to plants or culture media, have shown potential to modify the physiology of plants, promote their growth and improve their response to stress; its action differs from that of nutrients and pesticides (5).

The definition of biostimulants includes organic materials and microorganisms that are applied to crops to improve the absorption of nutrients, stimulate growth, improve tolerance to stress and their quality (5). According to the author there are several categories of specific biostimulants, including protein hydrolysates (6), algae extracts (7), chitosan (8), humic and fulvic acids (9), mycorrhizal fungi (10) and bacteria growth promoters (11). Biostimulants are classified into three large groups based on the source and content. Humic substances have been recognized for their biostimulant action, these constitute one of the three groups, which also include different products, containing hormones and those that have amino acids in their formulation (4).

Humic substances have a direct impact on the physiology of the plant. By direct effects it is understood that they are not mediated by soil characteristics or nutrient availability, but involve the regulation of cellular activity, metabolic changes, alter gene expression and have hormonal action (5).

The objective of this review is to show the biostimulant effect of the application of humic substances in plants under conditions of abiotic stress.

Some aspects are also synthesized, such as the structural characteristics and classification of humic substances. Results are shown using biostimulants based on humic substances, where their potential to stimulate different metabolic and physiological processes under stress conditions is verified.

Humic substances

Humic substances, (SH), are defined as the most widely distributed organic biosynthesis products on the surface of the earth (12), which exceed the amount of carbon contained in all living organisms by approximately one order of magnitude (13) . Regarding the origin and formation of humic substances, it is suggested that these organic materials are the result of concerted reactions of several biotic and abiotic processes (14), which result from the decomposition of plant, animal and microbial residues but also come from the activity metabolism of soil microorganisms using these substrates (5).

The SH constitute more than 80% of the soil organic matter (MOS) (15), although they can be present in aquatic environments and in the atmosphere (16). These can be found, in various concentrations, in different sources such as: rivers, lakes, oceans, organic materials, minerals such as leonardite, sediments, among others (17).

Because they represent the largest component of the mixture of materials comprising the MOS (14) is very important to study their structure and properties and how these contribute to soil fertility, acting on physical, physical-chemical properties, chemical and biological soil (5). Although the structural elucidation of these substances, given their characteristics, is still quite complex, investigations have been carried out throughout history in search of a structural model that is in correspondence with the characteristics of SH (18).

Estructure and classification

There are several conceptions about the structure of SH, among these conceptions the most accepted is that they constitute macromolecules of a polyelectrolyte that have a variable conformation, depending on the conditions of the soil solution (pH, ionic strength). That is to say, they constitute organic colloids that present molecular mass, density of electric charge and acidity. This model allows to explain the main interactions of the SH, such as: the interaction with minerals of the soil, the capacity of adsorption and complexation of ions; that is, reactions of agronomic-productive interest.

In other models, it is suggested that SHs have a micellar structure, with a hydrophobic part and a hydrophilic part (18). It is currently accepted that humus constitutes a group formed by supramolecular associations of organic molecules, relatively small and heterogeneous (9), basically assembled by weak interactions (9,19). The sequential molecular fractionation of this supramolecular structure is based on the binding forces of the organic substances in the humic matrix (20).

It is accepted that there are three fractions within the humic substances that are classified according to their solubility according to the pH: humina (H), humic acid (AH) and fulvic acid (FA); where humina constitutes the fraction insoluble in water at any pH value, humic acids are soluble in a basic medium and insoluble in an acidic environment, while fulvic acids are the soluble fraction at any pH value (21).

In the light of more modern studies, fulvic acids are redefined as a result of associations of small hydrophilic molecules, in which there are sufficient functional groups to maintain the dispersed fulvic aggregate in solution at any pH value, while the humic acids are formed by associations of predominantly hydrophobic compounds (fatty acids, steroidal compounds, chains of methylene groups), which are stabilized at neutral pH by hydrophobic dispersive forces. These conformations grow in size when intermolecular hydrogen bridges increase at low pH values, which causes these humic materials to flocculate (22).

Although the structural elucidation of these substances, given their characteristics, is still quite complex because it is influenced to a large extent by its source, quantitative and qualitative information has been found on the functional groups present in AH and AF (23). According to the spectroscopic studies, the SH generally have aromatic structures (benzenes and polysubstituted phenols), as well as $^{-}$OH phenolic and alcohols, $^{-}$COOH carboxylic acids, esters, quinones, among others (21).

At present, it is argued that these substances structurally possess a hydrophobic and a hydrophilic domain and a certain relationship between them is the cause of the biological effects of stimulation found in plants already mentioned by different authors (24,25). Experimental evidences have been presented (26) showing that the hydrophobicity and the amount of acid functional groups of AH are necessary in the stimulation of the bioactivity of these substances. According to the studies introduced in “Humeomics” (20,27), SHs have a supramolecular structural organization with large hydrophobic structures and other small hydrophilic ones. The hydrophobic fractions are basically composed of humic fractions of linear aliphatic chains and condensed aromatic rings, while the hydrophilic fractions are composed of irregular humic fractions.

It is concluded that the structural supramolecular order of SH is the result of heterogeneous humic molecules of non-uniform relationship that interact according to their size, shape, chemical affinity and hydrophobicity (20).

Among the physical-chemical techniques for the study of the structural characteristics of SH from different sources of origin have been used Infrared Spectroscopy by Fourier Transform (FT-IR), Spectroscopy (UV-vis) and Carbon-13 Nuclear Magnetic Resonance (^{13}C-NMR) and Chromatography. These techniques are even more powerful when they are coupled together or to other methods such as high performance liquid chromatography (HPLC) and pyrolysis (Py) (28). Through these techniques it has been possible to verify that SH of wastewater have an aliphatic character, with structures that belong to proteins and polysaccharides and a high presence of functional groups of acids and high aromaticity (29).

The variability of SH structure during vermicomposting has been studied. In these studies more than 300 compounds were identified, mainly those derived from lignins, carbohydrates, proteins, alcohols and fatty acids, terpene compounds and hydrocarbons, whose relative abundances vary according to the progress of the stabilization of organic matter (29).

Biostimulating actions of humic substancies

The indirect effects of SH in plants include the improvement of the chemical, physical-chemical and biological characteristics of the soil, through an increase in water and nutrient retention, influence on the diversity of beneficial microorganisms and the formation of complexes with ions, mainly micronutrients such as Fe and Zn. These effects are widely accepted as contributors to soil fertility and the mechanisms of indirect action elucidated and widely accepted.

The bioestimulantes effects of the SH on the growth and development of the plants have been extensively studied, finding increase in the length of the stem, root, leaves, fresh and dry mass, size and quality of the fruits; as well as the increase in crop yields (30). The promotion of plant growth by SH, defined here as biostimulation, is well documented in the literature (15,31-33). In support of this, a previous study showed that the dry mass of shoots and roots of herbaceous plants increased by about 22 % in response to the exogenous application of SH (34).

The Research Group on Organic Matter and Biostimulants (MOBI) of the Department of Chemistry of the Agrarian University of Havana has obtained a new aqueous extract of SH from vermicompost of cow dung (35). The biostimulation of different doses of the vermicompost extract has been proven in crops of agronomic interest such as corn (*Zea mays* L.) (36), lettuce (*Lactuca sativa* L.) (37), tomato (*Solanum lycopersicum* L.) (38), and beans (*Phaseolus vulgaris* L.) (39). The foliar application of these extracts in tomato plants (40) promoted the biological development of the plants, as well as the agricultural productivity in indicators such as the mass of the fruit and the yield during two consecutive years. The physical and chemical characterization of these extracts has shown the presence of humic substances such as humic and fulvic acids, phytohormones, beneficial microorganisms, amino acids, and essential elements (21) that could contribute in their biostimulant action, not only focused on the presence of humic substances.

Among the metabolic processes that contribute to promote the growth and development of plants is the stimulation of the activity of key enzymes in the metabolism of C and N by SH. Enzymes related to nitrogen metabolism such as nitrate reductase, glutamate dehydrogenase and glutamine synthetase were stimulated by SH in different experimental conditions (19,41). The positive effect of AH at different doses on the main enzymes involved in the reduction and assimilation of inorganic nitrogen was described (41).

Another enzyme whose activity is increased by SH is the H^+ -ATPase of the plasma membrane (42,43), also called proton pump because it is involved in the primary transport of said ions, stimulating a gradient that provides energy for the transportation of other ions and that contributes to cellular elongation.

The stimulation of this enzyme in the roots was related to the promotion in the secondary transport of ions and the absorption of nutrients (19). In other investigations it is found that the modification in the development of the root system, its architecture and the emergence of lateral roots (15) increases the efficiency in the absorption of nutrients and their use by plants.

In summary, the effects of SH on the growth and development of plants, point to the positive influence on ion transport facilitating the absorption, direct action on metabolic processes such as: respiration, photosynthesis and protein synthesis, by increasing or decrease in the activity of various enzymes, the content of metabolites and the hormonal activity of these substances (44,45).

These clear modifications in primary metabolism induced by SH have been confirmed by molecular biology techniques (46), which shows that humic substances exert their effects on the physiology of the plant through complex transcriptional networks and mechanisms of action of multiple facets, partially connected to its proven auxinic activity but involving independent pathways of indoleacetic acid (IAA) (47). These mechanisms are still widely studied and discussed.

Biostimulating effect in conditions of abiotic stress

In addition to the role of SH as regulators of primary and secondary metabolism, the possibility of using these substances to mitigate the effects of different abiotic stresses such as water stress, saline and high concentrations of heavy metals is discussed. These stresses induce the production of reactive oxygen species (ROS) that consequently cause oxidative stress, which results in serious yield losses in crops (48). In common beans the application of humic acids under conditions of high salinity (120 mM NaCl) increased the levels of endogenous proline and reduced the rupture of the membrane, which are indicators of adaptation to a saline environment (9).

An experiment was carried out with the foliar application of humic substances in tomato (*Solanum lycopersicum* L.) cultivation that was cultivated in a soil with natural levels of salinity. The plants that received the foliar applications of SH showed an improvement in the conditions and internal quality of the fruit (49,50).

The efficiency of vermicompost humates has been proven as mitigating the effect of salinity in the emergence and growth of basil (*Ocimum basilicum* L.) using two varieties of basil (Napoletano and Sweet Genovese). The percentage and emergence rate, radicle length, seedling height, fresh and dry radicle and aerial biomass were measured. The use of humates stimulated all the variables under salinity conditions, highlighting the Napoletano variety with application of the biostimulant as the treatment with the best results, allowing the tolerant variety to improve its emergence and growth and the sensitive variety increasing its tolerance to salt stress (51).

In rice (*Oryza sativa* L.) it was proved that the AH applied to the roots subject to water stress increased the peroxidase activity (POX), the proline content and reduced the H_2O_2 content (52). Recently, the stimulation of several enzymatic mechanisms associated with antioxidant defense systems was reported, as well as the genes for aquaporins, which are proteins associated with the transport of water and H_2O_2.

In rice plants subjected to water stress and root treated with nutrient solution of AH, changes in leaves and roots in the expression of aquaporins were reported, which translated into a greater permeability of the root membrane, attributing to these substances a protective effect against water stress (53). Aquaporins are known as the main intrinsic proteins (MIPs) that regulate the flow of transmembrane water and whose activity is regulated by independent and dependent pathways of abscisic acid (ABA) (53).

The application of AH to corn plants exerted an effect on the production of ERO and increased the activity of catalase (CAT) (54). The importance of enzymatic and non-enzymatic antioxidant defense has been demonstrated under conditions of water stress (55). The enzymatic defense is also stimulated by the presence of SH. The levels of superoxide dismutase (SOD) and ascorbate peroxidase (APX) improved with the application of a biostimulant based on SH and amino acids, although this improvement in the antioxidant system was not able to increase the tolerance of soybean plants (*Glycine max* L.) and maize (*Zea mays* L.) under conditions of water stress (55).

The foliar application of the same compounds to plants of common bean (*Phaseolus vulgaris* L.) grown in soils with high content of heavy metals showed protective effects, mediated by the activation of antioxidative defense mechanisms (56). The activity of the δ1 pyrrolin-5-carboxylate reductase (P5CR) and the phenylalanine ammonia lyase (PAL) was stimulated, resulting in increases in the content of proline and phenolic compounds (56). Humic acids of high molecular mass exerted effects on the secondary metabolism, associated with the synthesis of phenols (57).

Although the mechanism that explains the relationship between ERO and auxins in regulating the anti-stress response is still not well understood (58), it is known that compounds such as nitric oxide (NO) have an intermediate role in the action of SH in plants which, in addition, has antioxidant properties and acts as a signaling molecule in the synthesis of enzymes related to the ERO catalysis. This molecule intervenes in the resistance of plants to abiotic stresses. In maize plants treated with humic acid from vermicompost, a stimulation was reported in the biosynthesis of (NO), which can arise as a messenger in early stages of root development (59).

Future studies could be devoted to investigate the protective effects of vermicompost extracts rich in humic acids in plants, applied by foliar or in the culture medium, given the proven action of humic acids under stress conditions and the biostimulant effects of the extracts of vermicompost, containing organic substances and naturally enriched by humic substances.

CONCLUSIONS

- It is concluded that the humic substances are characterized by presenting a complex, variable structure and a multiplicity of functional groups and small heterogeneous molecules that interact through weak junctions, which makes them exhibit a great variety of beneficial functions, among which is their potential to increase yields and mitigate the effects of environmental stresses and whose sources of origin are in the organic matter of the soil, in minerals such as leonardite and organic waste of various origins, after a process of transformation by the microbial flora.

- ♦ Although its mode of action continues to be one of the most debated aspects, studies have been carried out regarding its structure, properties and function, which have allowed the establishment of possible mechanisms such as the activity of auxin-like to explain biostimulating effects. This, in addition, of the significant action on secondary metabolism with the stimulation of antioxidant compounds, allows us to conclude that humic substances and the great variety of products that contain them, could be a viable alternative to evade the consequences of climate change and use products natural and friendly with the environment.

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