"MAIN MECHANISMS INVOLVED IN THE EFFECTS OF HUMIC SUBSTANCES ON SOIL-PLANT SYSTEMS"

Garcia-Mina, JM., Mora,V., Olaetxea,M., Baigorri, R., Fuentes, M., Garnica, M., San Francisco, S., Erro, J.,, Urrutia, O., Casanova , E., Lemenager,D., Yvin, JC.

R&D TImac Agro International (CRIAS and CIPAV Laboratories). Roullier Group
Department of Agricultural Chemistry. University of Navarra.

A number of studies have shown the ability of natural organic matter (NOM) in general and humic substances (HS) in particular, to affect the development of plants and microorganisms in many different natural ecosystems and agro-ecosystems. Regarding plants, these NOM and HS effects were expressed in both root growth and architecture, and shoot growth. However, these effects were different in intensity and quality depending on several intrinsic and extrinsic factors associated with HS structure and concentration, plant species and soil properties. Two main mechanisms have been proposed to explain the beneficial action of NOM and HS on plant growth. An indirect effect expressed through the improvement of plant nutrition by increasing soil nutrient availability, principally some micronutrients (mostly P and Fe); and a possible direct action affecting the transcriptional and post-transcriptional regulation of several enzymes and molecular transporters in the root. These biological effects within the plant seem to be associated with both nutrient root uptake ability and the efficient use of the nutrient in plant leaves.
In this communication, the relationships between the effects of HS on root development, shoot development, plant nutrition, and soil properties; are discussed. This study is developed in the context of the links existing between the signal role of some nutrients and the hormonal balance in both root and shoot.

Introduction

Many studies have demonstrated the ability of humic substances (HS) to affect the development of diverse plant species cultivated in several soil types, inert substrates and hydroponics (Chen et al., 2004b; Trevisan et al., 2010). However, the main mechanisms responsible for these effects of HS on plant development remain unclear and under discussion. In general two main hypothesis have been proposed to explain these HS actions on plant development: (i) the "nutritional hypothesis", which defends that the action of HS is mainly indirect, by acting on soil properties and the pool of plant-available nutrients in the soil (principally micronutrients, such as Fe and Zn)(Chen et al., 2004a,b); and (ii) the Hormone-like hypothesis which defends that, besides an action on soil, HS can directly act on plant metabolism through the interaction with root cells (Trevisan et al., 2010). Recently some studies have reported that both theories might be compatible. Thus, Aguirre et al. (2009) reported that HS activated the main physiological root responses involved in Fe acquisition under Fe starvation, even under Fe sufficient conditions. These action was expressed both at transcriptional and post-transcriptional levels (Aguirre et al., 2009). In these sense, another complementary work showed that the action of HS on the shoot growth is probably mediated by an effect improving nitrate root uptake and further root to shoot translocation (Mora et al., 2010). This effect is related to a promotion of cytokinin root to shoot translocation that would be responsible for the biostimulation of shoot metabolism and growth (Mora et al., 2010). These results have been confirmed by other study in rapeseed, including both microarray and physiological approaches (Jannin et al., 2012). All these results show that the two main hypotheses proposed to explain HS effects on plant development are, probably, interconnected to some extend.
In this communication we will try to both analyze and interconnect the two hypotheses stressing the most important experimental data that support each of them.

Discussion

The nutritional hypothesis
This theory is mainly based on the ability of HS to complex metals due to the presence of functional groups with chelating activity in their structure (Stevenson,

1994). In this context, diverse studies have shown that the treatment of several plant species with HS-metal complexes increases plant growth under condition of limitation in the availability of these micronutrients (Fe, Zn and Cu) (Chen et al., 2004a,b; Garcia-Mina et al., 2004). This effect was ascribed to the complex and its effect improving micronutrient root uptake, since HS applied alone did not present any effect (Chen et al., 2004b; Garcia-Mina et al., 2004). However when the concentration of HS was higher (about 150 mg L-1 instead of 50 mg L-1)the treatment with HS alone was accompanied by increases in plant growth.

The Hormone-like hypothesis

Several studies have shown that certain types of HS (mainly those obtained from vermicompost) are able to affect plant grown in hydroponics and without nutrient limitations (Trevisan et al., 2010; Mora et al., 2010, 2012). However, these effects were expressed for specific ranges of concentration normally higher than those involves in humic-micronutrient complex action. In general, the mechanisms proposed to explain these effects are based on the potential presence of auxin or auxin-like compounds imbibed in HS-supramolecular-aggregated structure (Trevisan et al., 2010). However, other studies employing purified sedimentary humic substances (HA), without detectable concentrations of the main phytoregulators in their composition, showed that HA is able to affect root and shoot functionality, both at transcriptional and post-transcriptional levels (Aguirre et al., 2009., Mora et al., 2010, 2012, Jannin et al., 2012). In this sense, our results indicate that HA probably affects shoot growth by improving nitrate root uptake and root to shoot translocation (Mora et al., 2010; Jannin et al., 2012). This action promotes the translocation of active cytokinins from the root to the shoot, thus enhancing shoot nutrient status and development (Mora et al., 2010). These functional relationships between nitrate root uptake and cytokinin-functionality in shoot involved in humic action on shoot development was supported by transcriptional and physiological studies carried out in rapeseed (Jannin et al., 2012).

Regarding HA effects on the root, which might be behind the above-mentioned effects on the shoot, our studies showed that HA is able to effect the concentration in the root of ethylene, indole-acetic acid (IAA), abscisic acid (ABA) and nitric oxide (NO) (Mora et al., 2012). However, this study also showed that the macro-morphological action of HA on root architecture was not dependent on HA-effects on the hormonal balance in the root. All these findings are presented in Figure 1.

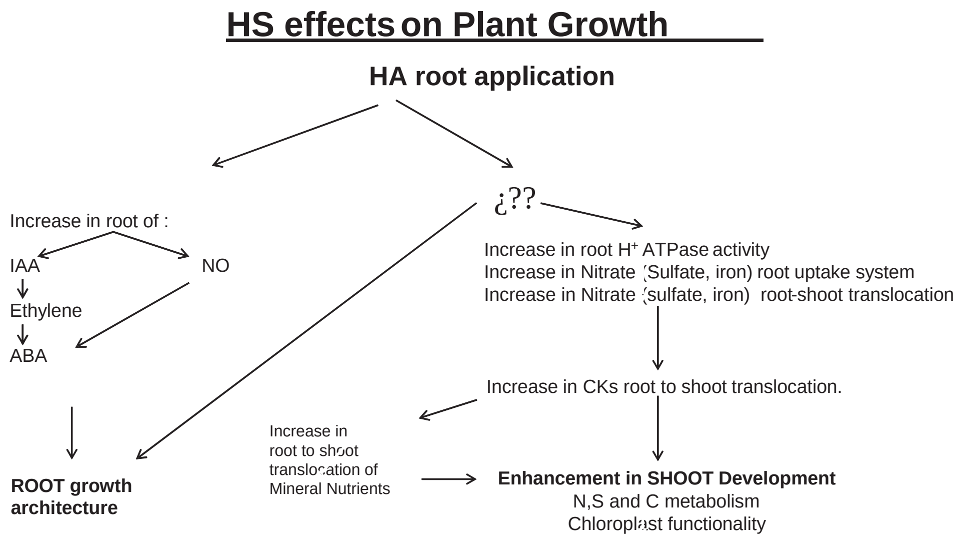

Figure 1. Hypothetical mechanism of HA action on plant Growth

Conclusion

All these findings, taken together, indicate that the involvement of the mechanisms included in each hypothesis may play an important role in the effect of HS on plant growth depending on the concentration of active-HS (HS in soil solution) that are present in rhizosphere. Thus, when the concentration of active-HS (HS in soil solution) is too low the effect of HS can be ascribed to a nutritional effect by improving the root uptake of nutrients, mainly micronutrients. When the active concentration of HS is higher a direct action on plant metabolism may be present, complementary to the nutritional action.

References

Aguirre E, Leménager D, Bacaicoa E, Fuentes M, Baigorri R, Zamarreño A M, and García-Mina J M 2009 The root application of a purified leonarditehumic acid modifies the transcriptional regulation of the main physiological root responses to Fe deficiency in Fe sufficient cucumber plants. Plant Physiol. Biochem. 47: 215-223.

Chen Y, Clapp C, and Magen H 2004a Mechanism of plant growth stimulation by humic substances: The role of organo-iron complexes. Soil Sci. Plant Nutr. 50: 1089-1095.

Chen Y, De Nobili M, and Aviad T 2004b Stimulatory effects of humic substances on plant growth.In F Magdoff, R Weil eds. Soil Organic Matter in Sustainable Agriculture. CRC Press, Boca Raton, Florida, pp 103-129

García-Mina, JM., Antolín, M.C., Sanchez-Diaz, M. 2004 Metal-humic complexes and plant micronutrient uptake: a study based on different plant species cultivated in diverse soil types. Plant and Soil. 258, 57-68.

Jannin L, Arkoun M, Ourry A, Laîné P, Goux D, Garnica M, Fuentes M, San Francisco S, Baigorri R, Cruz F, Houdusse F, Garcia-Mina JM, Yvin JC, and Etienne P 2012 Microarray analysis of humic acid effects on Brassica napus growth: Involvement of N, C and S metabolisms. Plant Soil DOI 10.1007/s11104-012-1191-x

Mora V, Bacaicoa E, Zamarreño AM, Aguirre E, Garnica M, Fuentes M, and García-Mina JM 2010 Action of humic acid on promotion of cucumber shoot growth involves nitrate-related changes associated with the root-to-shoot distribution of cytokinins,-polyamines and mineral nutrients. J. Plant Physiol 167: 633-642

Mora V, Baigorri R, Bacaicoa E, Zamarreño AM, and García-Mina JM 2012 The humic acid-induced changes in the root concentration of nitric oxide, IAA and ethylene do not explain the changes in root architecture caused by humic acid in cucumber. Environ. Exp. Bot. 76: 24– 32

Stevenson, F.J. (1994). Humus Chemistry. Genesis, Composition, Reactions. Jhon Wiley & Sons, Inc., New York, pp. 496.

Trevisan S, Francioso O, Quaggiotti S, and Nardi S 2010 Humic substances biological activity at the plant-soil interface: from environmental aspects to molecular factors. Plant Signal Behav. 5 : 635-643.