Journal of Academia and Industrial Research (JAIR)
Volume 2, Issue 6 November 2013

ISSN: 2278-5213

REVIEW ARTICLE

Humic Acids and its Role in Plant Tissue Culture at Low Nutrient Level

S. Dhanapal[1*] and D. Sathish Sekar[2]
[1,2]Dept. of Biotechnology, Arignar Anna College (Arts and Science), Krishnagiri-635001, TN
[1]Dept. of Biotechnology, St. Peter's University, Avadi, Chennai-54, TN
dhanapalu@gmail.com*; +91 9659024262

Abstract

Role of humic acids (HA) in plant tissue culture as plant growth regulators has been reviewed. Humic acid is a principal component of humic substances, which are the most important organic constituents of the soil (humus). Humus is defined as the organic matter in soil, a mixture of partially and totally humified substances. Leonardite is the best source for humic acid and it is extracted by using alkali solutions like NaOH/KOH. Humic acid plays a vital role in the plant tissue culture as growth hormone for *in vitro* propagation of many plant seedlings. This review provides the role of HA in plant tissue culture, field trails and other uses in agriculture. This practice reduces the usage of growth regulators with 2/4 and 1/4 strength MS culture media. This review also helps researchers to carry out experiments with various economically important crops in cost effective manner.

Keywords: Humic acid, plant tissue culture, *in vitro* propagation, growth regulator, leonardite.

Introduction

Humic substances and non-humic substances are present in soil organic matter. Normally humic substances are high molecular weight, brown to black colored substance formed by secondary synthesis reactions. The HAs are soluble at higher pH values but insoluble in water under acidic conditions (pH<2) (Anonymous, 2010). Humic acids (HAs) play an important role in morphological and physiological effects on higher plants (Nardi *et al*., 2002; Eyheraguibel *et al.*, 2008). Soil productivity is regulated by dynamic component of soils that influences the many chemical, physical and biological properties. The main objective of using humic substances is to balance vegetative and reproductive growth as well as to improve herbage and protein yield in plant (Saruhan *et al*., 2011). The parts of decaying plant materials are organic matter, compost, humus, humates, humic acid and fulvic acid. These organic materials are source for minerals, energy and water. Certain organisms can also grow on organic substances which act as growth medium (Zimmer, 2004). Leonardite is otherwise called as soft brown coal and these are the best source of humic acids, found in layers of sedimentation. Leonardite not yet reached the state of coal and differs from soft brown coal by its degree of oxidation. Humic and fulvic acids are having positive impact on plant growth. By the way of increasing surface water penetration, infiltration and soil water-holding capacity, humic acids enhance the soil to provide an effective growing environment for turf. Apart from that, humic acids also raise the availability of micronutrients, phosphorus and potassium and also enhance germination rates and support better fibrous root growth (Gallant, 2004).

Humic acids also have antimicrobial activity by inhibiting the bacterial and fungal growth, thus decreases levels of mycotoxins in feed (Islam *et al.,* 2005). Earthworms (*Eisenia foetida)* produce humic substances that can influence plant growth by unknown mechanisms. These humic acids increase the root growth of maize (*Zea mays)* seedlings and also induce the plasma membrane H^+-ATPase activity (Facanha *et al*., 2002). Humic substances are composed of complicated organic mixtures and the humic materials are very large and complex molecules extracted from organic matter. The extracted humic materials have been used in several ways for plant production (Mikkelsen, 2005). With the help of plant cell and tissue culture techniques, the rapid multiplication of elite varieties can be achieved by employing shoot tips or floral apices (Cronauer and Krikorian, 1986).

The 'black gold' of agriculture

The coal lignite can be the excellent source of humic acid and humic acid can increase the crop growth (Sao *et al.,* 2010). Basically humic substances are not conventional fertilizers. Carbon, hydrogen, oxygen, nitrogen, phosphorus and sulfur account for nearly 100% of the composition of humic substances on a dust free basis (Steelink, 1985). Humates play a direct role in determining the potential of a soil and increase the productivity of the soil. Nutritionally, humates are the source of nitrogen, phosphorus and sulfur for both plants and microorganisms (Obreza, 1989). FTIR spectroscopy studies of humic acid from coal reveals that the participation of COOH and OH groups in binding to the trivalent and divalent metal ions (Erdogan *et al.*, 2007).

Humic and fulvic acids are popular in organic farming. Leonardite is organic matter, which differs from soft brown coal by its high oxidation degree and it not reached the state of coal. The biological activity of humic acid from leonardite is said to be about five times stronger than the humic matter from other sources because of its molecular structure (Anonymous, 2010). Biostimulant is a substance that is neither a plant nutrient nor a pesticide, but it has an affirmative impact on plant health. A small quantity of biostimulant enhances plant growth and development such that the response cannot be attributed to application of traditional plant nutrients. It can be influence numerous metabolic processes such as respiration, photosynthesis, nucleic acid synthesis and ion uptake. Sometimes they can improve nutrient availability, water-holding capacity, increase antioxidants, improve metabolism and raise chlorophyll production (Gallant, 2004).

Humic acid is a vital factor for maintenance of soil fertility and plant growth because it possesses strange chemical and physical characteristics by means of which it interacts with various soil components and it is a main portion of soil organic matter (Deiana *et al*., 1990). Humic substances have numerous components and humic acid is one of the major components. During humification process, the plant and animal matter converted to humic matter through the biological activities of microorganisms (Anonymous, 2010). Humus is a combination of incompletely and completely humified substances in soil. Decaying is the natural process by which most humic substances formed from plant matter. About 80% of dark soils organic matter is made up of humic substances and are the long-lasting significant components of natural soil systems, persisting for hundreds or even thousands of years although they can be smashed in less than 50 years by some agricultural practices. Besides soils, humic substance can also be present in different sources like rivers, lakes, oceans, compost, sediments, soils, peat bogs and soft coal in varying concentrations. Humic substances cover 80% of total weight of the converted coal (Mayhew, 2004). Nearly 60 to 70% of the total organic matter often constitutes by humic substances (Schnitzer and Khan, 1972).

Humic acid as growth regulator in plant tissue culture

Extracts from leonardite have exposed positive yield and hormone like responses on a number of proscribed environment and field grown crops. Field trials on tomatoes increase 10.5% of yield over untreated controls (Brownell *et al.,* 1987). Humic substance influences plant growth depending upon the source, concentration and molecular weight. A low molecular size (LMS<3500 Da) fraction easily reaches the plasmalemma of higher plant cells but high molecular size fraction (HMS>3500 Da) is not absorbed and can interact only with the cell wall. Therefore, a LMS fraction is the major candidate for determining the affirmative effects of HS on plant growth (Nardi *et al*., 2002). The second metabolism of plants can also be activated by humic substances to increase CO_2 uptake, synthesis of ATP and respiration of mitochondria and to influence photosynthesis (Anonymous, 2010). Humic acids activate some ion uptakes along with stimulating the lateral roots at effective concentration of micronutrients. However, ion adsorption by plant roots is not easily explainable due to the varieties of HAs that differ from origins. At the concentration of 25 and 50 ppm, HAs could improve the growth of egg plant seedlings in tissue cultures at low nutrient level (1/4 MS) (Obsuwan *et al*., 2011). Based on their differences in origin, nature and concentration, HAs can able to stimulate or inhibit plant growth. Enhanced significant growth responses can be showed in the application of humic substances to nutrient solution, to soil or sand (Lulakis and Petsas, 1995). Humic substances improved the growth by increasing the uptake of micronutrient (Lee and Bartlett, 1976). Various plants such as tomato, cotton and grape growth can be promoted by foliar sprays of HAs (Brownell *et al*., 1987).

Lignocellulosic waste enhances the root elongation of maize seeds cultivated under hydroponic condition because of the presence of humic like substances (HLS). Positive effects were also observed on the whole plant growth as well as on root, shoot and leaf biomass. All of these effects can be related to high water and mineral utilization of plants undergoing this treatment. HLS can induce the flowering precocity and modified root development. So, it suggests possible response that indicates the interaction of HLS with development process (Eyheraguibel *et al*., 2008). According to their solubility in acid and alkaline solutions, humates (SH), humic (HA) and fulvic (FA) acid were extracted from vine-cane mature compost by using a solution of 0·1 M $Na_4P_2O_7$ plus 0·1 M NaOH. After that, it was purified and tested for their effects on growth of tomato seedlings and results shows that the humic substances were favorable to shoot and root growth at intermediate concentrations (100-300 ppm), but inhibitory at high concentrations (1000-2000 ppm) (Lulakis and Petsas, 1995). The sorption process of heavy metal is influenced by the presence of organic matter in soil system. Humic substance is environmentally significant in metal trapping and transport in the environment (Spark *et al*., 1997; Elkins and Nelson, 2002). Humic substances have a very profound influence on the growth of plant roots. Enhancement of root initiation and increased root growth may be observed during the application of humic acids and fulvic acids to the soil (Pettit, 2004). Enhanced uptake of macronutrients, such as nitrogen, phosphorus and sulfur is directly related to the stimulatory effects of humic substances (Chen and Aviad, 1990).

Micronutrients such as Fe, Zn, Cu and Mn uptake are also influenced by humic substances (Chen *et al.,* 1999). Humic substances influence plant growth directly and indirectly. The indirect effects of humic compounds on soil fertility include, (i) raise the soil microbial population including beneficial microorganisms, (ii) Better soil structure, (iii) Enhance the cation exchange capacity and the PH buffering capacity of the soil. Directly, humic acid compounds may have a variety of biochemical effects either at cell wall, membrane level or in the cytoplasm, including improved photosynthesis and respiration rates in plants, better protein synthesis and plant hormone like activity (Chen and Aviad, 1990). Microbiological activity can be stimulated by humic substances, by which it is possible to enhance the uptake of minerals (Mayhew, 2004). If the adequate amount of humic substances is present within the soil, then it is a fertile soil. So the requirement for nitrogen, phosphorus, potassium and fertilizer applications may be reduced (Pettit, 2004).

Conclusion

Humic acid has contributed a rich source to the growth of plants *in vitro*, especially HA from leonardite possessing activity as growth regulators. This review can help others to explore the high usage of HA in *in vitro* propagation. However, there have been limited reports on the effect of HA on plant growth and development of *in vitro* propagation, especially at low nutrient level in which the sources of plant food were limited. Since HAs were different in origin, the HA are derived from mix manures, leonardite and some agricultural wastes. Therefore, in upcoming days, many economically important plants can be propagated through *in vitro* propagation with optimal concentration of HA at the low concentration of Murashige and Skoog medium (MS) along with field trials. Field experiments to determine appropriate amounts among different types of HAs still pose challenge to the researchers.

Acknowledgements

Authors wish to acknowledge the Management and Dr. M. Subbiah, Principal of Arignar Anna College (Arts and Science), Krishnagiri, for their support and valuable suggestions to prepare this review article.

References

1. Anonymous. 2010. Humic and fulvic acids: The black gold of agriculture. Retrieved from www.humintech.com/pdf/humicfulvic acids.pdf.
2. Brownell, J.R., Nordstrom, G., Marihart, J. and Jorgensen, G. 1987. Crop responses from two new leonardite extracts. *Sci. Total Environ*. 62: 491-499.
3. Chen, Y. and Aviad, T. 1990. Effects of humic substances on plant growth. In: McCarthy P, Calpp CE, Malcolm RL. Bloom, Readings. ASA and SSSA, Madison, WI. pp.161-186.
4. Chen, Y., Clapp, C.E., Magen, H. and Cline, V.W. 1999. Stimulation of plant growth by humic substances: Effects on iron availability. In: Ghabbour, EA, Davies G. (eds.), Understanding humic substances: Advanced methods, properties and applications. Royal Society of Chemistry, Cambridge, UK. pp.255-263.
5. Cronauer, S.S. and Krikorian, A.D. 1986. Biotechnology in Agriculture and Forestry (ed. Bajaj Y. P.S.). Springer-Verlag, Berlin. 1: 233-252.
6. Deiana, S.C., Gessa, B., Manunza, R., Rauza, K. and Seeber, R. 1990. Analytical and spectroscopic characterization of humic acids extracted from sewage sludge, manure and worm compost. *Soil Sci*. 150: 419-424.
7. Elkins, K.M. and Nelson, D.J. 2002. Spectroscopic approaches to the study of the interaction of aluminium with humic substances. *Coordin. Chem. Rev*. 228: 205.
8. Erdogan, S., Baysal, A., Akba, O. and Hamamci, C. 2007. Interaction of metals with humic acid isolated from oxidized coal. *Polish J. Environ. Stud*. 16(5): 671-675.
9. Eyheraguibel, B., Silvestre, J. and Morard, P. 2008. Effects of humic substances derived from organic waste enhancement on the growth and mineral nutrition of maize. *Biores. Technol.* 99(10): 4206-4212.
10. Facanha, A.R., Canellas, L.P., Olivares, F.L. and Anna, L.O. 2002. Humic acids isolated from earthworm compost enhance root elongation, lateral root emergence and plasma membrane H+-ATPase activity in maize roots. *Plant Physiol.* 130: 1951-1957.
11. Gallant, A. 2004. Biostimulants: What they are and how they work. TURF and Recreation. pp.1-4.
12. Islam, K.M.S., Schuhmacher, A. and Gropp, J.M. 2005. Humic acid substances in animal agriculture. *Pak. J. Nutrit*. 4(3): 126-134.
13. Lee, Y.S. and Bartlett, R.J. 1976. Stimulation of plant growth by humic Substances. *Soil Sci. Soc. Am. J*. 40: 876-879.
14. Lulakis, M.D**.** and Petsas, S.I. 1995. Effect of humic substances from vine-canes mature compost on tomato seedling growth. *Biores. Technol.* 54(2): 179-182.
15. Mayhew, L. 2004. Humic substances in biological agriculture. ACRES (USA) a voice for eco-agriculture. 34:1&2.
16. Mikkelson, R.L. 2005. Humic materials for agriculture. *Better Crop*. 89(3): 6-10.
17. Nardi, S., Pizzeghello, D., Muscolo, A. and Vianello, A. 2002. Physiological effects of humic substances on higher plants. *Soil Biol. Biochem*. 34(11): 1527-1536.
18. Obreza, T.A., Webb, R.G. and Biggs, R.H. 1989.Humate materials: Their effects and use as soil amendments. *Citrus Ind*. 70(10): 36-38.
19. Obsuwan, K., Namchote, S., Sanmanee, N., Panishkan, K. and Dharmvanij, S. 2011. Effect of various concentrations of humic acid on growth and development of eggplant seedlings in tissue cultures at low nutrient level. *World Acad. Sci. Engg. Technol.* 80: 276-278.
20. Pettit, R.E. 2004. Organic matter, humus, humate, humic acid, fulvic acid and humin: Their importance in soil fertility and plant health [Online]. Retrieved from www.humate.info/mainpage.htm.
21. Sao, y., Bhatt, V.R. and Swarnkar, P.K. 2010. Characterization of humic acids derived from lignite coal and FYM and effect of lignite, humic acid and FYM on yield of fodder Maize. *Int. J. Curr. Trend. Sci. Technol*. 1(2): 20-26.
22. Saruhan, V., Kuvuran, A. and Babat, S. 2011. The effect of different humic acid fertilization on yield and yield components performances of common millet (*Panicum miliaceum* L.). *Sci. Res. Essays*. 6(3): 663-669.
23. Schnitzer, M. and Khan, S.U. 1972. Humic substances in the environment. Dekker Publ. New York, NY. pp.9-23.
24. Spark, K.M., Wells, J.D. and Johnson, B.B. 1997. The interaction of a humic acid with heavy metals. *Aust. J. Soil Res.* 35: 89.
25. Steelink, C.A. 1985. In Implications of essential characteristic of humic substances. Humic substances in soil, sediment and water: Geochemistry, isolation and characterization. Wiley-Interscience, New York. pp.457-476.
26. Zimmer, G. 2004. Humates and humic Substances, Bio-Correct inputs for the eco-farmer. ACRES (USA) a voice for eco-agriculture. 34: 1.