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Humic substances and their effects on the micropropagation of banana

Substâncias húmicas e seus efeitos na micropropagação da banana Prata-Anã

Sustancias húmicas y sus efectos en la micropropagación del plátamo

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Resumo

A crescente preocupação em promover uma agricultura sustentável, buscando a preservação do meio ambiente e, ao mesmo tempo, o aumento da produtividade resultou na busca por tecnologias para a implantação de sistemas de produção agrícola, com abordagens ecológicas e uso responsável dos recursos naturais, nos âmbitos econômico, político e contextos sociais em cada região. Uma das alternativas potenciais para o alcance desses resultados seria o uso de substâncias húmicas, considerando seus mecanismos diretos e indiretos voltados ao aumento da produção agrícola. Assim, o objetivo deste trabalho foi avaliar a bioatividade dos ácidos húmico e fúlvico, bem como promover melhorias nos atributos fitotécnicos da bananeira cv. Prata-Anã micropropagado. Os tratamentos consistiram em cinco doses de ácido húmico e ácido fúlvico (2; 5; 10; 20 e 40 mg L-1) e uma testemunha sem substâncias húmicas. Maiores valores de comprimento da parte aérea e maior raiz foram obtidos com o uso de ácido fúlvico 40 mg L-1. Os resultados comprovam a capacidade do ácido fúlvico em melhorar o desenvolvimento in vitro de explantes de bananeira.

Palavras-chave: Bioatividade; Ácido húmico; Ácido fúlvico; Prata-Anã**.**

Abstract

The growing concern to promote sustainable agriculture, seeking environmental preservation and, at the same time, increased productivity resulted in the search for technologies for the implementation of agricultural production systems, with ecological approaches and responsible use of natural resources, in the economic, political and social contexts in each region. One of the potential alternatives to achieve these results would be the use of humic substances, considering their direct and indirect mechanisms aimed at increasing agricultural production. Thus, the objective of this work was to evaluate the bioactivity of humic and fulvic acids, as well as to promote improvements in the phytotechnical attributes of banana cv. Prata-Anã micropropagated. The treatments consisted of five doses of humic acid and fulvic acid (2; 5; 10; 20 and 40 mg L^{-1}) and a control without humic substances. Higher values for the length of the aerial part and the larger root were obtained with the use of 40 mg L^{-1} fulvic acid. The results prove the ability of fulvic acid to improve the *in vitro* development of banana explants.

Keywords: Bioactivity; Humic acid; Fulvic acid; Prata-Anã.

Resumen
La creciente preocupación por promover la agricultura sostenible, buscando la preservación del medio ambiente y, al mismo tiempo, el aumento de la productividad derivó en la búsqueda de tecnologías para la implementación de sistemas de producción agrícola, con enfoques ecológicos y uso responsable de los recursos naturales, en el ámbito económico, político y contextos sociales en cada región. Una de las posibles alternativas para lograr estos resultados sería el uso de sustancias húmicas, considerando sus mecanismos directos e indirectos dirigidos a incrementar la producción agrícola. Así, el objetivo de este trabajo fue evaluar la bioactividad de los ácidos húmicos y fúlvicos, así como promover mejoras en los atributos fitotécnicos del banano cv. Prata-Anã micropropagado. Los tratamientos consistieron en cinco dosis de ácido húmico y ácido fúlvico (2; 5; 10; 20 y 40 mg L-1) y un control sin sustancias húmicas. Se obtuvieron valores más altos para el largo de la parte aérea y la raíz más grande con el uso de 40 mg L-1 de ácido fúlvico. Los resultados demuestran la capacidad del ácido fúlvico para mejorar el desarrollo in vitro de explantes de plátamo.
Palabras clave: Bioactividad; Ácido húmico; Ácido fúlvico; Prata-Anã.

1. Introduction

The growing concern to promote sustainable agriculture, seeking environmental preservation and at the same time increasing productivity, has resulted in the search for technologies for the implantation of agricultural production systems, with ecological approaches and responsible use of natural resources, in the economic, political and social contexts in each region. One of the potential alternatives to achieve this objective is the use of humic substances (HS), considering their direct and indirect mechanisms for increasing agricultural production.

The most reported effects of HS on plants are related to a higher growth of the root system, as they stimulate the formation of lateral roots, adventitious roots, cellular elongation and formation of root hairs (Baldotto et al., 2011). In addition, these substances are also able to increase the efficiency of the use of nutrients, decrease the incidence of diseases, helping to reduce the applications of fertilizers and pesticides and stimulating root growth (Canellas et al., 2002).

Such morphological changes can also induce physiological changes, such as higher efficiency in the absorption of nutrients, leading to a higher production of biomass in reduced nutritional conditions in the culture medium and greater tolerance to nutritional stresses (Pinton et al., 1999).

In addition to the effects of humic acids on rooting, there are also stimulating effects on the shoot, such as accumulation of leaf nutrients (Jannin et al., 2012), chlorophyll biosynthesis (Jannin et al., 2012; Ahmad et al., 2013), carotenoid biosynthesis (Baldotto et al., 2009), presence of chloroplasts (Jannin et al., 2012) and the photosynthetic process (Jannin et al., 2012). Thus, they can cause increases in plant biomass (Baldotto & Baldotto, 2015), flower production (Baldotto & Baldotto, 2015), fruits (Lima et al., 2011) and seeds (Baldotto & Baldotto, 2014).

Thus, the objective of the work was to evaluate the bioactivity of humic acids (HA) and fulvic acids (FA), as well as promoting improvements in the phytotechnical attributes of banana cv. Prata-Anã micropropagated.

2. Material and Methods

The experiment was conducted at the Tissue Culture Laboratory of the Department of Agriculture (DAG) of the Federal University of Lavras (UFLA), Lavras-MG.

The explants used in the experiment came from banana plants cv. Prata-Anã already established *in vitro*. Plants (1 cm long) were grown in test tubes containing 15 mL of semi-solid MS (MURASHIGE & SKOOG, 1962) medium, plus 25 g L^{-1} sucrose and 6 g L^{-1} agar. The pH of the medium was then adjusted to 5.7 ± 0.1 before autoclaving at 121ºC and 1 atm of pressure for 20 min.

The treatments consisted of five doses of humic acid (HA) and fulvic acid (FA) (2; 5; 10; 20 and 40 mg L^{-1}), resulting in 10 treatments and a control without humic substances, replicates per treatment and each repetition containing 10 tubes. Subsequently, the tubes were kept in a growth room with a temperature of 25 ± 1 °C and lighting provided by white LED lamps for 30 days.

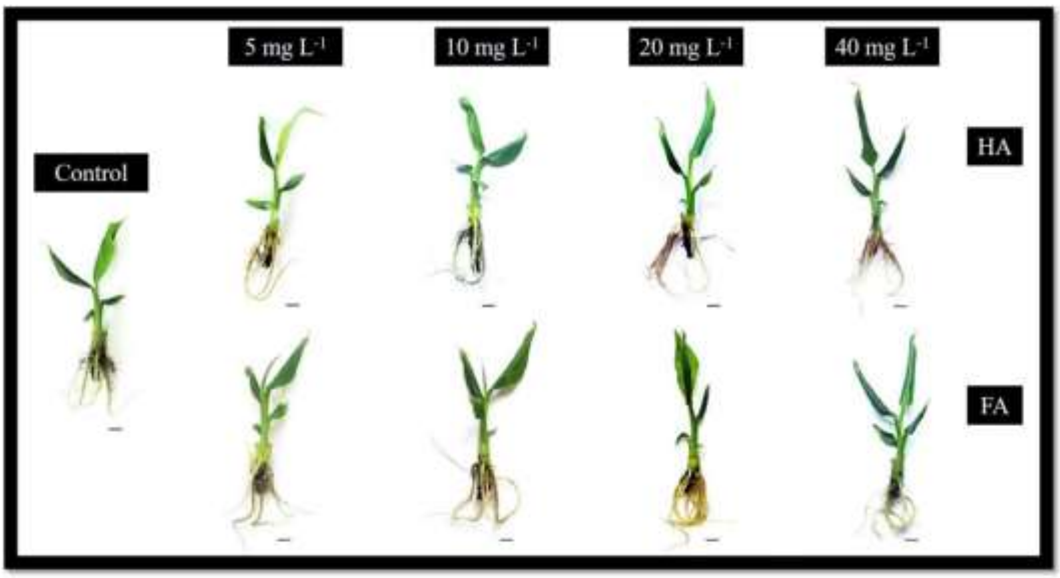
The acids used were commercial products extracted from Leonardite, following the methods of extraction and purification proposed by Swift (1996). The main properties of the humic materials used were: HA sample - electrical conductivity (EC): 37,7 dS m^{-1}, pH: 9,7, ratio E4 / E6: 4,84, C: 38%, N: 0,9%; FA sample - CE: 19,1 dS m^{-1}, pH: 5,6, ratio E4 / E6: 7,35, C: 39%, N: 0,35%.

After 30 days of the explants inoculation, the following attributes were evaluated: plant height; leaf number; number of shoots; pseudostem diameter (cm); root length (cm); number of roots, total fresh and dry mass of plants and roots (g) and photosynthetic pigments (chlorophyll *a*, chlorophyll *b* and carotenoids). For the evaluation of the dry mass of the plant material, the material was dried in an oven at 60ºC for 72 h or until constant mass.

3. Results and Discussion

Longer shoots (11.5 cm) were obtained using 40 mg L^{-1} fulvic acid (Figures 1 and 2A). Morphological changes induce physiological changes, such as higher efficiency in the absorption of nutrients that leads to a higher biomass production capacity in limited nutritional conditions of the culture medium, giving a higher tolerance to nutritional stresses (Pinton et al., 1999). Yildirim (2007) and Baldotto et al. (2009) worked with the substances and observed an increase in plant growth. Sharif et al. (2002) observed a significant increase of approximately 32% in dry mass of corn when compared to the control. In addition, the pH values decreased by only 0.2 and 0.3 units in treatments that received humic acid, where the buffer effect kept the pH almost constant. Da Silva et al. (2015) observed that these acids were able to increase the growth of plants, and defined them as efficient stimulants aimed at the production of dragon fruit seedlings.

Figure 1. Banana plants cv. Prata-Anã grown under five doses of humic acid (HA) and fulvic acid (FA) (0; 2; 5; 10 e 20 and 40 mg L^{-1}). Bar = 1 cm.



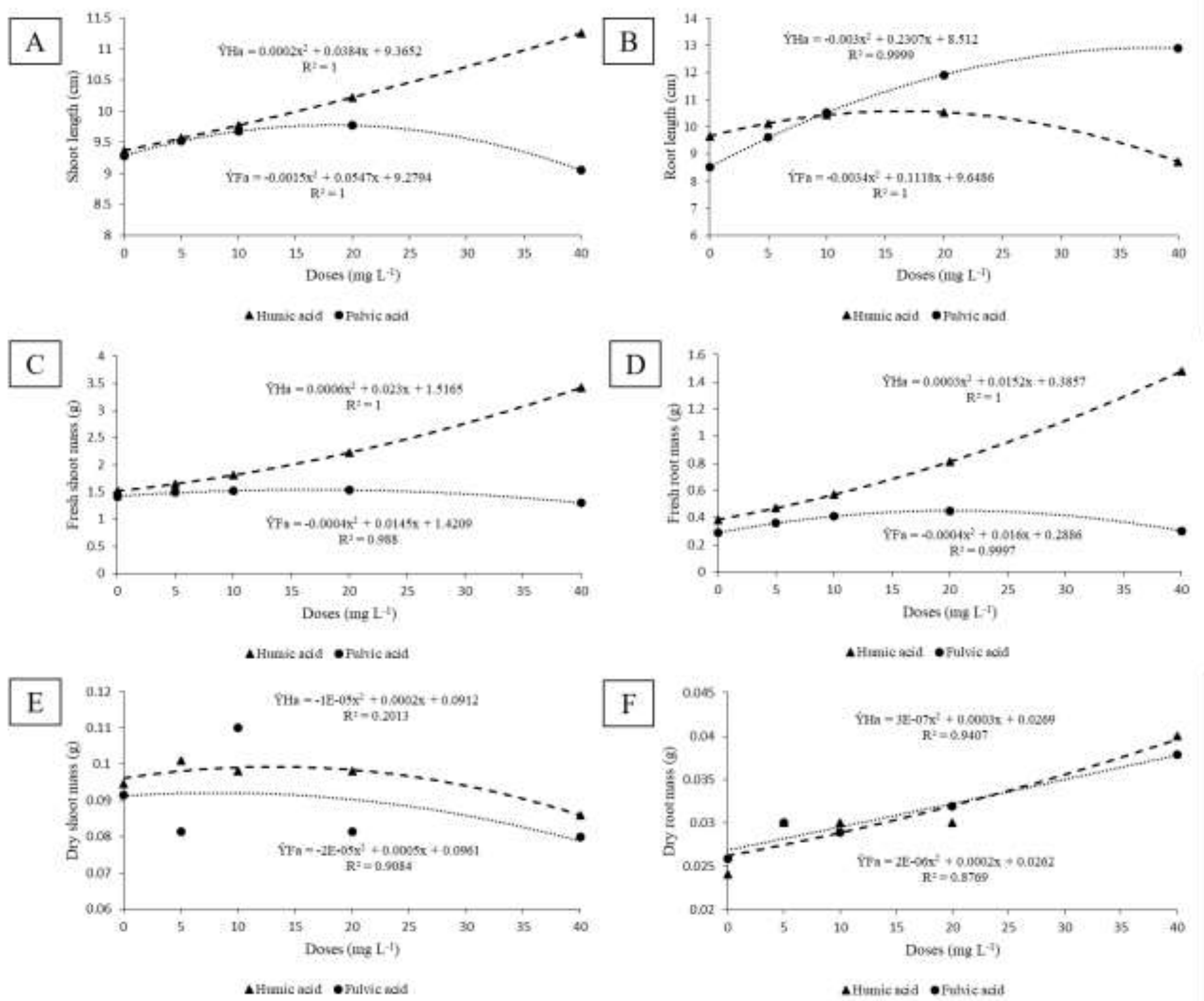
Source: Authors.

Best results for the root length (13 cm) were obtained using 40 mg L^{-1} fulvic acid, but this same dose of humic acid reduced the length of the roots (Figure 2B). The most reported effects of HS on plants are related to the greater growth of the root system, as they stimulate the formation of lateral roots, adventitious roots, cellular elongation and formation of root hairs (Baldotto et al., 2011). Silva et al. (2001) found that humic acids (HA) showed greater capacity to induce lateral roots in the initial stage of development with the lowest concentration tested (160 mg L^{-1}).

According to Façanha et al. (2002), the increase in the root system is related to stimulating the activity of H+-ATPase. This increase in enzymatic activity would cause an increase in the activity of the proton pump and, consequently, acidification of the apoplast. Thus, it would favor the increase of plasticity, promote the elongation of the cell wall, and root growth.

However, both for the fresh mass of root and plant height, as for the dry mass, better results were obtained with the use of 20 mg L^{-1} fulvic acid. (Figure 2).

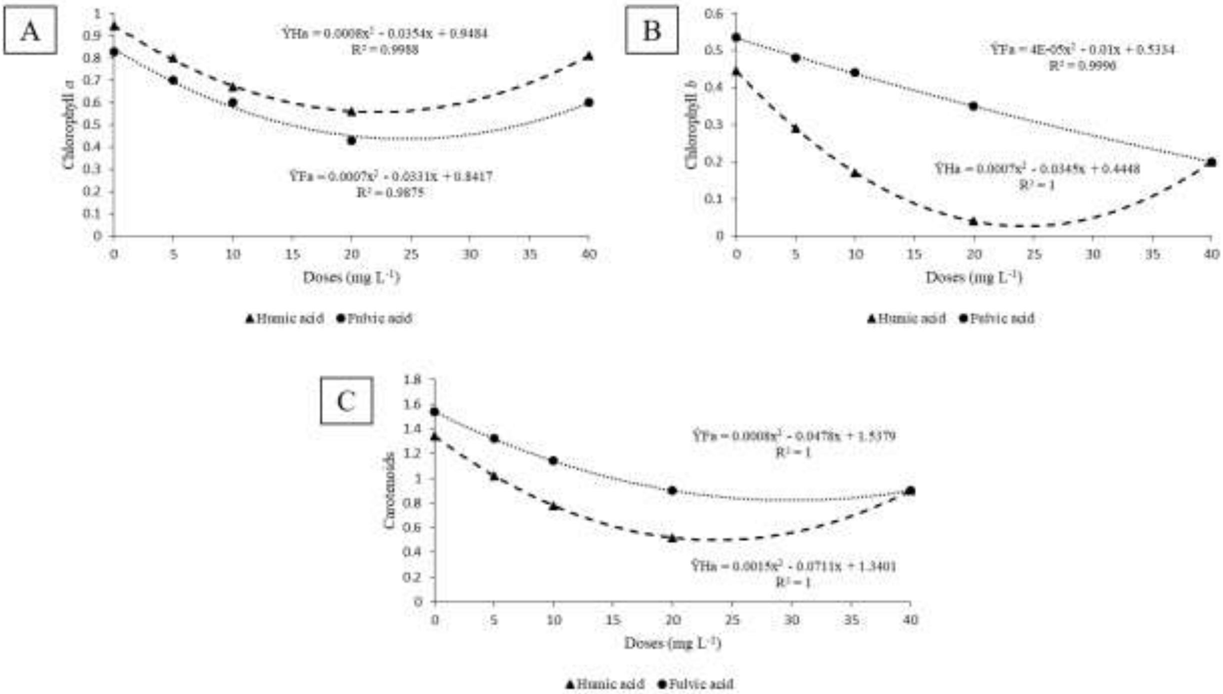
Figure 2. Phytotechnical variables analyzed in banana plants cv. Prata-Anã under different *in vitro* concentrations of humic and fulvic acid (0; 5; 10; 20 and 40 mg L^{-1}).



Source: Authors.

Ferrara and Brunetti (2008) found that the application of humic acids to grape leaves caused an increase in chlorophyll *a* and *b* and the chlorophyll *a* and *b* ratio. However, Sladky (1985) demonstrated that fulvic acids are more stimulating than the humic fraction in the respiration rate and density of chloroplasts of plants, more due to their structural characteristics. Baldotto (2014) tested the effects of humic substances on the acclimatization of pineapple seedlings cv. Vitória and observed that the levels of photosynthetic pigments, including chlorophyll *a*, chlorophyll *b* and carotenoids, varied little on average between treatments. However, in the present study, the influence that both acids had on photosynthetic pigments was negative (Figure 3). As humic or fulvic acid was added, the pigment concentration reduced.

Figure 3. Photosynthetic pigments analyzed in leaves of banana plants cv. Prata-Anã under different *in vitro* concentrations of humic and fulvic acid (0; 5; 10; 20 and 40 mg L^{-1}).



Source: Authors.

4. Conclusion

Higher values for the length of the aerial part and the larger root were obtained with the use of 40 mg L^{-1} fulvic acid.

Thus, the results allow proving the capacity of humic substances to improve the *in vitro* development of banana explants.

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