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Root Growth of *Arabidopsis thaliana* (L.) Heynh. treated with humic acids isolated from typical soils of Rio de Janeiro State, Brazil

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ABSTRACT

Humic substances isolated from soil organic matter had been used as stimulators of plant metabolism. *Arabidopsis thaliana* (L.) Heynh. with only five chromosomes, short cycle and size, is an important model to evaluate the physiological effects of these substances, which are qualitatively and quantitatively influenced by morphogenesis, mineralogy and chemistry of soils. The objective of this study was to evaluate the ambience effects on bioactivity of humic acids. A and B horizons of four typical soils of the North Fluminense were sampled. After isolation and purification, humic acids were applied to plants in increasing concentrations. The number and length of lateral roots and main root length were evaluated and, subsequently, the concentrations of maximum stimulation were determined by dose-response curves and regression equations. The results showed that more stable humic acids isolated from soil in less advanced stages of weathering, high activity clay and high base saturation resulted in better physiological stimulants for *Arabidopsis*.

Key words: Soil genesis, chemistry and fertility, humic substances, bioactivity, *Arabidopsis thaliana.*

RESUMO

Crescimento radicular de plantas de *Arabidopsis thalaiana* tratadas com ácidos húmicos isolados de diferentes solos típicos do Estado do Rio de Janeiro

As substâncias húmicas isoladas da matéria orgânica do solo têm sido usadas como estimuladores do metabolismo vegetal. As plantas de *Arabidopsis thaliana* (L.) Heynh. com apenas cinco cromossomos, ciclo curto e tamanho reduzido, aparecem como modelo para avaliar os efeitos fisiológicos dessas substâncias, as quais são qualitativa e quantivamente influenciadas pela morfogênese, mineralogia e química dos solos. Avaliar os efeitos dessas ambiências na bioatividade de ácidos húmicos foi o objetivo principal do presente trabalho. Para isso, foram amostrados os horizontes A e B de quatro solos típicos da Região Norte Fluminense. Após o isolamento e purificação, ácidos húmicos foram usados em concentrações crescentes nas plantas. O número e comprimento de raízes laterais e o comprimento da raiz principal foram avaliados e, posteriormente, determinadas as concentrações de máxima estimulação, obtidas por meio das curvas de dose resposta e das equações de regressão ajustadas. Os resultados mostraram que ácidos húmicos mais estáveis, isolados de solos em estádio de intemperismo menos avançado, com argila de alta atividade e alta saturação por bases resultaram em melhores estimulantes fisiológicos das plantas de *Arabidopsis*.

Palavras-chave: Gênse, química e fertilidade do solo, substâncias húmicas, bioatividade, *Arabidopsis thaliana*.

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INTRODUCTION

In the soil, the chemical stability of organic matter occurs with the formation of humic substances via humification. These substances are considered to be supra molecular aggregates organized in groups of various low molecular weigh organic compounds, containing predominantly hydrophilic (fulvic acid) or hydrophobic-hydrophilic (humic acids) domains (Piccolo, 2001). In natural systems, there is a mixture of these domains. These aggregates are maintained in solution by hydrogen bonds and hydrophobic interactions which, alone, are weak, but, together, provide structure to these substances and result in apparent high molecular weight. When, operationally, ionization is promoted using alkali extractants, both groups are dissolved, whereas acidification provides precipitation of humic acids only, which are less polar and more hydrophobic than fulvic acids, therefore more stable in the environment (Sposito, 2008). Thus, humic acids, as an intermediate fraction between the fulvic acids, more hydrophilic and soluble, and humin, more hydrophobic and insoluble, are listed as indicators of organic matter stability in the soil (Baldotto *et al.*, 2010). Because their conformation and structure vary with environmental conditions, e.g., ionic strength and pH, they have the properties of biomolecules from which they are derived (Sposito, 2008) in their supra molecular arrangement, which stimulate plant growth (Façanha *et al.*, 2002).

The use of humic acids in promoting plant growth and development is well documented (Baldotto *et al.*, 2009). These substances favor growth (Canellas *et al.*, 2002, 2004, 2006; Rodda *et al.* 2006; Zandonadi *et al.* 2007; Dobbss *et al.* 2007; Canellas *et al.*, 2008a) and nutrient accumulation in several plants of agronomic interest (Vaughan & Malcolm, 1985; Chen & Avaide, 1990; Chen *et al.*, 2004). These effects reflect in the increase of root growth rate, increases in plant biomass and root architectural changes (root hairs and thin lateral roots), resulting in increased surface area and/or in root length (Canellas *et al.*, 2006). Leaf application of humic acids has been reported to promote growth in rice (Tejada & Gonzalez, 2004), wheat (Delfine *et al.*, 2005) and vine (Ferrara & Brunetti, 2008). In pineapple, the use of humic acids proved to be a practical in the acclimatization of plants propagated *in vitro* (Baldotto *et al.*, 2009).

Besides growth and nutritional aspects, possible physiological effects were shown by the quantification of photosynthetic pigments in the presence of humic acids by Baldotto *et al.* (2009). Treatment with humic acids provided higher levels of the photosynthetic pigments and significant increase in the ratio of chlorophyll *a* and *b*, as compared with the control. These results indicated that application of humic acid increases plant growth rate during acclimatization of plants propagated *in vitro*, favoring the establishment of plants in environment *ex vitro* and the subsequent establishment in the field. This can be an important alternative for reducing production costs, based on more efficient nutrient absorption and growth.

Most biostimulant effects of humic acids have been attributed to their activity similar to the plant hormone auxin, i.e. they can promote plant growth in relatively small concentrations. Some mechanisms of action have been proposed to explain the induction of root growth by humic acids, such as the formation of soluble complexes with cations in the rhizosphere (Chen & Avaide, 1990) and a presumed increased permeability of the plasma membrane by the surfactant action of humic acids (Canellas *et al.*, 2006). If, on the one hand, change in selective permeability of the plasmalemma, in theory, can increase the ion entry, on the other hand, it may also favor the exit, since the surfactant action does not preserve membrane selectivity (Nardi *et al.*, 2002).

Canellas *et al.* (2008a, b) found that corn seedlings treated with humic acids change their growth profile and root exudation of organic acids, significantly increasing the extrusion of citric and oxalic acids. When in contact with the acidic rhizosphere environment, super-structural particles of humic acids can break and generate subunits that can potentially change the cellular metabolism through activation of H^+-ATPases of the plasma membrane in root cells (Piccolo, 2001; Façanha *et al.*, 2002). These mechanisms lead to apoplast acidification and activation of cell wall-degrading exoenzymes, making the wall more susceptible to the action of vacuolar turgor pressure. This causes cell expansion and, consequently, root tissue expansion (Hager *et al.* 1991; Frias *et al.*, 1996).

Arabidopsis thaliana is an important model system for studying plant physiological processes, because it offers many advantages such as small size, short life cycle and small genome (Sommerville & Meyerowitz, 2002). This study aimed to quantify the stimulating action of humic acids isolated from typical soils of the State of Rio de Janeiro, Brazil, with different morphogenetic characteristics, mineralogical and chemical characteristics influencing the *Arabidopsis* root system.

MATERIALS AND METHODS

Soil samples

Soils from different classes, under common grass/pasture use, in the State of Rio de Janeiro were selected for this study. Samples were collected from surface and subsurface layers (horizons A and B). The samples consisted of the following soils' classes (Embrapa, 2006): Argilluvic Orthic Vertic Chernosol (Chernosol), Chromic

Palic Abruptic Luvisol (Luvisol), Dystrophic Red Yellow Acrisol (Acrisol), Typical Cohesive Yellow Latosol (Latosol), representing a typical sequence of the North Fluminense landscape.

The soils were sampled along the highway Itaperuna-Campos dos Goytacazes and described during the First Meeting of Classification and Correlation of Soils (Embrapa, 1980). Table 1 shows some characteristics of the soils. Aspects of genesis and chemistry of these soils and their relation to organic carbon stocks of are described in Baldotto *et al.* (2010).

Extraction and purification of humic acids

Humic acids were isolated from soils according to the recommendations of the International Humic Substance Society (IHSS, 2009), using NaOH 0.1 mol L^{-1} under N_2 atmosphere, in the soil:extractor ratio 1:10 (mass:volume). The material was stirred for 24 h and centrifuged at 5,000 g for 30 min. The supernatant was collected and the pH of the extract was immediately adjusted to 1.5 with HCl 6 mol L^{-1}. After 18 h, the fulvic acid fraction was siphoned and discarded. The remaining material (precipitated humic acids) was dissolved in NaOH 0.1 mol L^{-1} and centrifuged at 5,000 g for 10 min and the precipitate (clay) discarded. Solubilization and reprecipitation of humic acids was repeated twice. The precipitate of humic acids was dissolved and remained in 5% HF+HCl for 48 h, to remove residues of clay minerals and paramagnetic ions, and then centrifuged at 5,000 g. Humic acids were washed with 200 mL of HCl 0.01 mol L^{-1} and centrifuged at 5,000 g. The precipitate was washed with distilled water until a negative test for Cl^-, using 0.1 mol L^{-1} $AgNO_3$, and transferred to 10 mL dialysis membranes (cut-off 14 KDa, Thomas Sci.) to reach electrical conductivity of distilled water (1.0 µS cm^{-1}). After dialysis, humic acids were freeze-dried and stored in desiccators.

Bioactivity of humic acids

Evaluation of humic acids bioactivity followed was according to the protocol by Dobbss *et al.* (2007). Seeds of *Arabidopsis thaliana*, ecotype Columbia 4 (co4), were sterilized in 95% ethanol (v/v) for 5 min, in 20% NaClO for 7 min, and washed five times with distilled water. Seeds were sown in plastic plates in the vertical position lined with one layer of filter paper and one layer of synthetic fine mesh fabric (poliprint). Both the top and the bottom of this structure remained open and covered with black plastic to prevent roots from being exposed to light. After sowing, the plates were incubated at 8°C in the dark, for 24 h, and transferred to growth room at 25 °C, 90 µmol m^{-2} s^{-1} photosynthetic photon flux density and a 14 h photoperiod.

The cultivation system consisted of immerging the plates containing pre-germinated seedlings in a water tank in the first five days. Five days after germination, the seedlings were thinned to four plants per plate, and Hoogland solution modified to a final concentration of 1 mmol L^{-1} N was applied (Dobbss *et al.*, 2007). Four mL of solution containing 0, 20, 40 and 80 mg L^{-1} of humic acids were added to each plate and 4 mL of deionized water only to the control treatment. After the treatments, the plates were incubated into the water reservoir for 48 h and, then, the nutrient solution was added. Two weeks later, the plates were stained with toluidine blue (0.05%) and scanned (600 dpi) for root analysis through an image electronic processing. Two plants corresponding to the central position of each plate were used for the analysis. Evaluation of change in root architecture considered the number of lateral roots, lateral root length and main root length.

Data analysis

Means of the analysis performed in duplicate and standard errors of means were estimated for each experimen-

Table 1. Chemical analysis of soils of the State of Rio de Janeiro

Soil class	Corg	pH	P	K	Ca^{2+}	Mg^{2+}	Na^+	Al^{3+}	H+Al	SB	T	t	m	V
	g kg^{-1}		mg dm^{-3}		$cmol_c$ dm^{-3}									%
A Horizon														
Chernosol	17.2	6.6	7	83	18.2	0.4	0.10	0.0	2.8	18.9	21.7	18.9	0	87
Luvisol	16.2	6.6	7	38	9.9	0.2	0.06	0.0	2.4	10.3	12.7	10.3	0	81
Acrisol	13.6	6.4	5	60	4.7	2.6	0.09	0.0	3.3	7.5	10.8	7.5	0	70
Latosol	13.1	5.5	5	74	1.8	1.9	0.09	0.1	4.6	4.0	8.6	4.1	2	46
B Horizon														
Chernosol	7.9	7.0	2	36	16.9	0.5	0.09	0.0	1.4	17.6	19.0	17.6	0	93
Luvisol	5.1	6.4	5	14	3.0	0.5	0.08	0.0	1.3	3.6	4.9	3.6	0	74
Acrisol	3.0	5.9	2	17	3.0	1.9	0.07	0.0	1.9	5.0	6.9	5.0	0	73
Latosol	3.0	4.7	1	7	0.3	1.0	0.09	0.4	2.9	1.4	4.3	1.8	22	33

Corg = organic carbon (Walkley & Black); pH in water = 1: 2.5 ratio, P and K = Mehlich extractor^{-1}; Ca^{2+}, Mg^{2+} and Al^{3+} = 1 mol L^{-1} KCl extractor, H+Al = 0.5 mol L^{-1} CaOAc extractor at pH 7.0; SB = K^+ + Ca^{2+} + Mg^{2+} + Na^+; T and t = effective cation exchange capacity and at pH 7.0, respectively, m = Al^{3+} saturation; V = base saturation.

tal variable, as well as Pearson's linear correlations for some variables. Correlation estimates were subjected to F test with 1 and 5% probability levels (Steel & Torrie, 1960). Bioactivity of *Arabidopsis* as a function of humic acids rates was examined by the regression analysis, and the models were chosen based on the response shapes described by Baldotto *et al.* (2009), significance of regression coefficients and coefficient of determination. Growth rates and concentrations of humic acids correponding to the points of maximum in the response curves were estimated based on the regression equations. The increase rates as a function of humic acids concentrations were estimated by the slope of the curves.

RESULTS

All samples of humic acids isolated from typical soils of the North Fluminense showed the capacity to stimulate root growth in Arabidopsis higher than the control (Table 2), for example, in Figure 1, humic acids isolated from

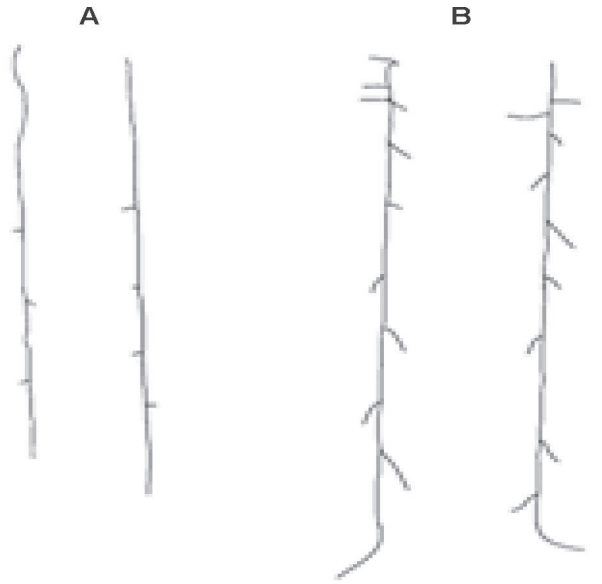

Figure 1. Root growth in *Arabidopsis thaliana.* Control sample (A) and plant treated with humic acids (B) isolated from the A horizon of a typical Chernosol of the State of Rio de Janeiro.

Table 2. Root growth of *Arabidopsis thaliana* treated with humic acids (HA) isolated from typical soils of the State of Rio de Janeiro

Soil class	Horizon	HA	NLR(1)		LLR(2)		LMR(3)	
			Mean	SEM	Mean	SEM	Mean	SEM
		— mg L^{-1} —			%			
Control	-	0	3.2	0.3	100	0	100	0
Chernosol	A	20	6.0	0.0	231	2	113	3
		40	9.5	0.5	254	20	134	5
		80	9.0	0.0	200	22	104	3
	B	20	13.0	1.0	204	19	112	3
		40	9.5	0.5	276	6	122	3
		80	8.0	0.0	296	4	115	2
Luvisol	A	20	9.0	0.0	307	33	95	6
		40	8.5	0.5	207	33	115	1
		80	10.0	1.0	341	22	112	1
	B	20	6.0	1.0	211	4	100	1
		40	10.5	1.5	446	2	130	4
		80	9.5	1.5	291	91	132	4
Acrisol	A	20	4.5	0.5	120	2	148	3
		40	4.5	0.5	159	19	153	4
		80	5.5	1.5	146	6	139	3
	B	20	7.5	0.5	163	19	162	1
		40	5.0	0.0	93	4	121	5
		80	4.5	0.5	135	6	165	2
Latosol	A	20	7.5	0.5	180	6	130	6
		40	8.0	0.0	154	9	140	0
		80	6.0	1.0	172	17	91	4
	B	20	10.0	1.0	78	4	119	1
		40	9.0	0.0	119	4	125	0
		80	10.0	1.0	109	20	135	0

(1) NLR = number of lateral roots, (2) LLR = length of lateral roots, (3) LMR = length of main root; Means are followed by their respective standard errors (SEM).

Chernosol. Stimuli was dependent on the concentrations applied in the treatments. In general, curvilinear increases were observed in growth rates of Arabidopsis' root as a function of the concentrations of humic acids isolated from soils (Table 3). The response curves to the application of increasing concentrations of humic acids showed quadratic and square root variation, being evidence for the dose-dependent effect of humic acids in plant development. These response curves resulted in a significant increase over the control to a peak and then decrease as higher concentrations of humic acids were applied. The increase in *Arabidopsis'* root growth rate with the concentrations of humic acids was different in each evaluated soil class and horizons.

Table 4 shows the results of growth rates for *Arabidoposis'* root system as a function of humic acids concentrations. The number of lateral roots, maximum lengths of main and lateral roots and the humic acids concentrations varied with the origin of humic acids.

At the point of maximum, on average, the number of lateral roots in plants treated with humic acids increased by 148% compared with the control, with responses in the following order: Acrisol B < Acrisol A = Latosol A < Latosol B = Luvisol A = Luvisol B < Chernosol A ≅ Chernosol B.

The length of these lateral roots, in general, in plants treated with humic acids, was on average 104% higher than the control plants, in the following order: Acrisol B < Latosol B < Latosol B Acrisol A < Chernosol A < Chernosol B < Luvisol B < Luvisol A. The length of the main root was the variable that showed the least change, with a mean increase of 26% in treatments with humic acid compared with the control, with the means following the order: Luvisol A <Chernosol A ≅ Luvisol B < Latosol B ≅ Latosol A ≅ Chernosol B < Acrisol A ≅ Acrisol B.

Table 3. Regression equations for root growth of *Arabidopsis thaliana* treated with humic acids (HA) isolated from typical soils of the State of Rio de Janeiro

Soil class[(1)]		**Regression equation**	**R^2**
Sample	**Horizon**		
		Number of lateral roots	
Chernosol	A	$\hat{y} = 2.907 + 0.226^{**} x - 0.001^{**} x^2$	0.968
	B	$\hat{y} = 4.407 + 0.339^{*} x - 0.003^{*} x^2$	0.623
Luvisol	A	$\hat{y} = 3.753 + 0.220^{*} x - 0.001^{*} x^2$	0.850
	B	$\hat{y} = 2.757 + 0.260^{**} x - 0.002^{**} x^2$	0.939
Acrisol	A	$\hat{y} = 3.500 + 0.026^{o} x$	0.873
	B	$\hat{y} = 3.312 + 1.334^{**} x^{0.5} - 0.139^{**} x$	0.738
Latosol	A	$\hat{y} = 3.398 + 0.221^{**} x - 0.002^{**} x^2$	0.953
	B	$\hat{y} = 3.889 + 0.254^{**} x - 0.002^{**} x^2$	0.802
		Length of lateral roots (% in relation to control)	
Chernosol	A	$\hat{y} = 106.20 + 6.85^{**} x - 0.071^{**} x^2$	0.965
	B	$\hat{y} = 99.19 + 6.290^{**} x - 0.047^{*} x^2$	0.999
Luvisol	A	$\hat{y} = 134.20 + 6.640^{o} x - 0.027^{o} x^2$	0.594
	B	$\hat{y} = 72.77 + 13.11^{**} x - 0.128^{**} x^2$	0.857
Acrisol	A	$\hat{y} = 96.01 + 2.12^{(0.31)} x - 0.018^{(0.18)} x^2$	0.907
	B	$w = \bar{y} = 102$	
Latosol	A	$\hat{y} = 110.50 + 2.49^{(0.26)} x - 0.022^{(0.12)} x^2$	0.648
	B	$\hat{y} = \bar{y} = 123$	
		Length of main root (%in relation to control)	
Chernosol	A	$\hat{y} = 97.38 + 1.456^{**} x - 0.017^{**} x^2$	0.876
	B	$\hat{y} = 99.40 + 0.857^{**} x - 0.008^{**} x^2$	0.982
Luvisol	A	$\hat{y} = 98.47 + 0.203^{o} x$	0.542
	B	$\hat{y} = 99.55 + 0.455^{**} x$	0.760
Acrisol	A	$\hat{y} = 102.70 + 2.380^{**} x - 0.024^{**} x^2$	0.945
	B	$\hat{y} = 103.35 + 10.10^{o} x^{0.5} - 0.046 x$	0.548
Latosol	A	$\hat{y} = 99.79 + 2.090^{**} x - 0.027^{**} x^2$	0.999
	B	$\hat{y} = 100.90 + 0.871^{**} x - 0.005^{**} x^2$	0.984

[(1)] A and B refer to horizons dominated by characteristics obtained from the environment and the genetic characteristics, respectively, [(P)], [o], * and ** = significant at P, 10, 5 and 1% probability respectively.

DISCUSSION

Humic acids isolated from typical soils of the North Fluminense region promoted distinct physiological changes in *Arabidopsis* compared with the control.

Humic acids, despite their size, have a relatively high mass, which would limit its entry into the plant cell. One hypothesis for the beneficial effect generated by these humified fractions of soil organic matter on plant growth is their role in plant metabolism through molecular signaling processes analogous to those of hormones. Canellas *et al.* (2002) found that sub-structural units of humic acids were able to access receptors on the surface or inside the plasma membrane of root cells, stimulating development.

In this study, the maximum increase in formation and length of lateral roots, with the simultaneous lower increase in the main roots of plants treated with humic acids, as compared with the control, were, in general, in the following order: Luvisol > Chernosol> Acrisol ~ Latosol. For comparison of the effects of humic acids with effects of growth regulators, we determined the correlation between the length of lateral roots and length of main root, which was negative and significant ($R = - 0.73^{P<0.05}$). The same behavior, i.e., decrease in the main root and increase in lateral root formation, is reported by studies using auxins as biostimulants (Façanha *et al.*, 2002).

Canellas *et al.* (2002) reported that the synthesis of plasma membrane enzymes, isolated from corn roots, was correlated with the presence of auxins in humic acids, changing the pattern of root development in seedlings. Zandonadi *et al.* (2007) and Dobbs *et al.* (2007) proved that different humic acids are not capable of promote lateral rooting in seedlings of tomato mutants defective for auxin signaling (dgt), making it even more evident the idea that humic acids act similarly to auxin.

The hormone-like effect, especially similar to that of auxins, as discussed by Canellas *et al.* (2002), Nardi *et al.* (2005), Zandonadi *et al.* (2007) and Dobbss *et al.* (2007), is based on the increase in formation and length of lateral roots in relation to the growth of the main root in plants treated with humic acids. For the Acrisol and Latosol, particularly in subsurface layers, the fertility is limited to the point that humic acids did not provide an effect different from the control. These results suggest the absence of minimum conditions for the formation of chemical-structural bioactive humic acids in these soils. Likewise, indirectly, it can be inferred that the humic precursors found no necessary ambience conducive to similar evolution of humic aggregates among the soils sampled. Soils with the most bioactive humic acids are the most fertile (Table 1).

Humic substances, designated as supramolecular by Piccolo (2001), have the properties of biomolecules from which they are derived (biopolymers), fragments that form an integral or labile part of the molecular architecture and thus control their conformation, chemical reactivity and bioactivity. Additionally, acidification of the humic acids' solution with organic acids of low molecular weight exudated by plant roots can influence the structure and conformation of the supramolecular arrangement of these humic substances with relative disintegration of these clusters, increasing their bioactivity (Piccolo, 2001; Sposito, 2008).

Canellas *et al.* (2008b) studied the exudation profile of plants treated with humic substances and found a remarkable change: the presence of citric, malic, tartaric and oxalic acids occurred, or was increased in the exudates of corn roots in response to application of humic acids. Chromatographic analysis of these solutions revealed only the presence of substances of low molecular

Table 4. Values of maximum ($w_{Máx}$) root growth of *Arabidopsis thaliana* treated with humic acids isolated from typical soils of the State of Rio de Janeiro and concentration for each condition

Soil class	Horizon	NLR(1)		LLR(2)		LMR(3)	
		$\hat{y}_{Máx}$	Concentration	$\hat{y}_{Máx}$	Concentration	$\hat{y}_{Máx}$	Concentration
			mg L^{-1}	%	mg L^{-1}	%	mg L^{-1}
Control	-	3.2	-	100	-	100	-
Chernosol	A	12	56.5	271	48.9	129	42.8
	B	13	20.0	308	66.9	145	53.6
Luvisol	A	10	61.0	492	80.0	112	80.0
	B	10	60.5	408	51.2	132	80.0
Acrisol	A	8	80.0	158	58.8	162	49.6
	B	6	23.0	nd	nd	165	80.0
Latosol	A	8	55.2	181	56.6	140	38.7
	B	10	63.5	nd	nd	139	80.0

(1) NLR = number of lateral roots, (2) LLR = length of lateral roots, (3) LMR = length of main root; nd = not determined due to lack of fit of regression equations: average of B Acrisol = 102% and B Latosol = 123%.

weight, indicating the division of humic acids aggregates initially applied, confirming the conceptual model presented by Piccolo (2001). In this context, similar to the results of root growth observed here, Nardi *et al.* (2002), Canellas *et al.* (2002, 2008a, b), Zandonadi *et al.* (2007) and Baldotto *et al.* (2009) observed biostimulant effects of humic acids, which can be explained by their nature as providers of polymers conceptualized by Sposito (2008). The release of these biostimulant substances by humic acids, in the rhizosphere, is associated with the response of treated plants in the form of exudation of organic acids. The humic acids isolated from the Acrisol and Latosol, whose growth responses did not differ from the control, do not contain such biostimulants. Considering that the same vegetation cover contributes similarly as a source of organic matter, it can be inferred that the different humic acids components of each soil do not allow similar storage of such bioactive substances in the supramolecule. The chemical structural aspects of the same humic acids were studied by Canellas *et al.* (2008a) and Baldotto *et al.* (2009). The results indicated that humic acids of the Chernosol and Luvisol are more humified, hydrophobic and stable than those of the Acrisol and Latosol.

According to Sposito (2008), the protection of organic matter by coating clay particles is one of the main mechanisms of stabilization and accumulation of humic substances in natural systems. Ion exchange reactions and interactions as hydrogen bonds, unstable water molecules forming Lewis acids (water bridging) and van der Waals forces occur between these mineral components and units of polar and nonpolar organic substances. Such mechanisms are consistent with the close relationship between the colloidal mineral activity, stability, and ability to stimulate plants as found in this study.

Two findings can be highlighted: i) the formation of a humic aggregate stable and bioactive are similar and require contact with clay silicates 2:1 of high ion-exchange capacity and eutrophic, and ii) recent studies on humic substances (Piccolo, 2001) indicate that despite they have high molecular weight as in polymers, their structure consists of complex associations of heterogeneous molecules, stabilized by hydrophobic interactions with apparent high molecular weight. Also, biopolymers, derived from plant residues and microbial activity, are incorporated into more stable fractions of the humified organic matter and it is related to the quality of its ambience (Canellas *et al.*, 2008a). Thus, we found that the hydrophobicity of humic acids, inversely correlated with the stage of soil weathering, was the mechanism responsible for the preservation of organic substances capable of positively stimulating root growth of *Arabidopsis*, activating H^+-ATPases, promoting plasma membrane depolarization and, thereby, activating secondary transporters responsible for the increase in absorption of macro and micronutrients (Canellas *et al.*, 2002, Sondergaard et al. 2004; Canellas et al., 2008a).

CONCLUSIONS

All humic acids studied stimulated the root growth of *Arabidopsis thaliana* in relation to control.

More stable humic acids isolated from soil in less advanced stages of weathering, with high-activity clays and high base saturation were the best physiological stimulants of *Arabidopsis*.

Increases in formation and length of lateral roots in relation to the main root length of plants treated with humic acids compared with the control were found in the following order: Luvisol $\geq$ Chernosol > Acrisol $\geq$ Latosol.

In addition to the dependence on the soil class, there were higher differences in *Arabidopsis'* physiological response as a function of concentrations of humic acids than in response to the horizon sampled within the soil class.

The mean concentration to achieve the maximum values for the variables number and length of lateral roots and length of the main root was approximately 60 mg L^{-1} of humic acids.

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