Changes in root development of *Arabidopsis* promoted by organic matter from oxisols


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Keywords

Auxin-like activity; biological activity; humic substances; physiological effects; tropical soils.

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Abstract

The presence of ramified and abundant lateral roots is fundamental to plant growth on highly weathered soils. In this work, the effects of humic acid (HA), fulvic acid and hexanic–methanolic (HM) extract, after alkaline extraction from topsoil of seven different oxisols, on the development of roots of *Arabidopsis thaliana* was evaluated. Furthermore, we used another emergent plant model, that is, micro-tom (MT) tomatoes with size similar to that of *Arabidopsis* to test the effects of HA. It was observed that both humic fractions and the HM extract were able to change the root development, improving the number of lateral roots and their development in comparison to control plants. The promotion of root growth by the three organic matter fractions was higher than that observed by 10^{-6} mol L^{-1} indole acetic acid. The treatment of MT tomato mutant, less sensitive to auxin, with HA did not promote the emergence of lateral roots, being an additional indication of auxin-like activities of HA. However, some organic matter fractions exhibited, besides promotion of lateral roots number, increase in the length of principal root, which is not a typical auxin effect, indicating that these substances could contain other physiologically active substances.

Introduction

The activity of root systems is essential for plant environmental adaptation. Changes in the root architecture and physiology affect water and nutrient absorption, especially in oxisols with low cation exchange capacity (CEC) (Hodge, 2004). Humic substances (HS) are the major component of soil organic matter in high weathering soils, and their importance has been largely recognised as it improves physical, chemical and biological properties of soil (Whitehead, 1963; Nardi et al., 2004; Arancon et al., 2005). The tropical agriculture is widely dependent on the organic matter level in soils, and its decline can result in low productivity and environmental degradation (Singh, 2000). In addition, organic fertilisation could reduce environmental pollution and increase quality of agricultural products (Atiyeh et al., 2002; del Amor, 2006). Furthermore, HS can directly influence plant physiology, mainly root systems (Vaughan & Malcolm, 1985; Chen & Aviad, 1990; Nardi et al., 2002). Several works have been carried out to study the effects of HS on root growth (O’Donnell, 1973; Nardi et al., 1988, 1997, 2002b; Canellas et al., 2002), using water-soluble or low molecular weight HS that can form soluble complexes with metal ions (Pinton et al., 1999; Chen et al., 2003; Garcia-Mina et al., 2004) or, alternatively, show hormone-like activity (O’Donnell, 1973; Pizzeghello et al., 2001; Canellas et al., 2002; Quaggiotti et al., 2004; Zandonadi et al., 2007) including expression induction of plasma membrane H^{+}-ATPase in plants (Canellas et al., 2002).
Soil organic matter affects *Arabidopsis* roots

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2002; Quaggiotti et al., 2004). However, there are relatively few studies that deal with biological stimulation produced by tropical soil organic matter (Canellas & Façanha, 2004).

The small crucifer *Arabidopsis thaliana* (L.) Heynh is a model plant widely used in genetic and physiology studies as it has a relatively small and simple genome with five chromosomes and a short life cycle lasting approximately 6 weeks (Estelle & Somerville, 1986). Curiously, it is not frequently used to study physiological plants. Tomato is an economically relevant crop worldwide and is a plant model for physiological and genetic studies (Tanksley wide and is a plant model for physiological and genetic studies. In the present work, the HS effects were studied in *Arabidopsis* and tomato (*Solanum lycopersicum* L.) plants. Tomato is an economically relevant crop worldwide and is a plant model for physiological and genetic studies (Tanksley et al., 1992). Furthermore, the morphogenetic pattern different from that of *Arabidopsis* qualifies tomato plant as an additional model for comparative studies (Pratt et al., 1997). The aim of this work was to evaluate the effect of humic acid (HA), fulvic acid (FA) and hexanic–methanolic (HM) extracts after alkaline organic matter extraction from topsoil of seven different Brazilian oxisols on *Arabidopsis* root development. To investigate whether auxin-signalling mechanisms are involved in HS effects on lateral root development, we investigated the HS biological activity on the diageotropa (*dgt*) mutant of a tomato miniature cultivar [micro-tom (MT)].

Materials and methods

Soil samples and chemical characterisation

Soil samples were collected from the superficial horizon of seven different profiles of oxisols [previously described by EMBRAPA (1980; 1999)] from different regions of Brazil: Xanthic Hapludox (P1) – Campos dos Goytacazes, Rio de Janeiro State, geographical coordinates: 21°45’S and 41°19’ W Greenich (Gr); Haploperox (P2) – Vacaria, Rio Grande do Sul State, geographical coordinates: 28°30’S and 50°56’W Gr; Rhodic Humic Hapludox (P3) – Nova Lima, Minas Gerais State, geographical coordinates: 20°04’S and 43°58’W Gr; Sombrihumox (P4) – Nova Friburgo, Rio de Janeiro State, geographical coordinates: 22°16’S and 42°31’W Gr; Rhodustall (P5) – Santo Angelo, Rio Grande do Sul State, geographical coordinates: 28°17’S and 54°15’W Gr; Hapludox (P6) – Mendes, Rio de Janeiro State, geographical coordinates: 22°31’S and 43°43’ W Gr; Rhodic Hapludox (P7) – Brasilia, Federal District, geographical coordinates: 15°47’S and 47°55’W Gr.

The main soil characteristics were determined according to the EMBRAPA (1979) soil handbook, and the results are shown in Table 1. The localisation of soils and the schematic representation of area under oxisols in Brazil are shown in Fig. 1.

Table 1 Some pertinent characteristics of topsoil from oxisols used to isolate organic matter fractions

<table>
<thead>
<tr>
<th>Oxisols</th>
<th>pH</th>
<th>Capacity (cmol kg⁻¹) Sand (g kg⁻¹)</th>
<th>Lime (g kg⁻¹)</th>
<th>Clay (g kg⁻¹)</th>
<th>Kiα</th>
<th>C (g kg⁻¹)</th>
<th>CFA (g kg⁻¹)</th>
<th>CIA (g kg⁻¹)</th>
<th>CHM (g kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>5.1</td>
<td>3.24</td>
<td>600</td>
<td>20</td>
<td>380</td>
<td>2.05</td>
<td>8.64</td>
<td>0.13</td>
<td>0.09</td>
</tr>
<tr>
<td>P2</td>
<td>5.1</td>
<td>8.56</td>
<td>50</td>
<td>230</td>
<td>720</td>
<td>1.77</td>
<td>43.00</td>
<td>0.61</td>
<td>0.05</td>
</tr>
<tr>
<td>P3</td>
<td>5.8</td>
<td>2.13</td>
<td>430</td>
<td>290</td>
<td>280</td>
<td>0.11</td>
<td>12.84</td>
<td>0.10</td>
<td>0.02</td>
</tr>
<tr>
<td>P4</td>
<td>4.8</td>
<td>3.03</td>
<td>140</td>
<td>110</td>
<td>750</td>
<td>1.62</td>
<td>29.49</td>
<td>0.32</td>
<td>0.08</td>
</tr>
<tr>
<td>P5</td>
<td>6.8</td>
<td>7.43</td>
<td>130</td>
<td>250</td>
<td>620</td>
<td>1.97</td>
<td>10.70</td>
<td>0.28</td>
<td>0.06</td>
</tr>
<tr>
<td>P6</td>
<td>5.3</td>
<td>6.50</td>
<td>400</td>
<td>110</td>
<td>490</td>
<td>1.53</td>
<td>12.50</td>
<td>0.16</td>
<td>0.06</td>
</tr>
<tr>
<td>P7</td>
<td>5.1</td>
<td>5.19</td>
<td>350</td>
<td>160</td>
<td>490</td>
<td>0.72</td>
<td>25.42</td>
<td>0.40</td>
<td>0.07</td>
</tr>
</tbody>
</table>

C, total organic carbon; CFA, organic carbon as fulvic acids; CIA, organic carbon as humic acids; CHM, organic carbon as hexanic–methanolic extract; P1, Xanthic Hapludox; P2, Haploperox; P3, Rhodic Humic Hapludox; P4, Sombrihumox; P5, Rhodustall; P6, Hapludox; P7, Rhodic Hapludox.

Organic matter extraction

Alkaline-soluble HS were extracted with 0.1 mol L⁻¹ NaOH, 1:10 (v/v), under N₂ atmosphere for 4 h followed by centrifugation (3000 g). The extraction procedure was repeated until the extracts presented zero absorbance at 280 and 465 nm. The separation of HA from the alkaline extract was obtained by acidification to pH 1 with 6 mol L⁻¹ HCl. Dissolution and precipitation was repeated three times. HA were treated for 8 h with 200 mL of a mixture of HF and HCl (5 mL conc. HCl plus 5 mL conc. HF in 1 L of water). After centrifugation, HA were washed with water until a negative test against AgNO₃ and were dialysed (cut-off 14 kDa, Thomas Sc., Rancho Domingues, CA, USA) and freeze-dried. FA were obtained with XAD-8 resin (36 g mL⁻¹ of FA solution). The column was washed with water (two volumes), and the retained FA were eluted with 0.01 mol L⁻¹ NaOH. The salt content was decreased with the use of Amberlite IRA 120 H⁺ resin (Rohm and Haas Company, Philadelphia, PA, USA). The FA were dialysed against ultra-pure water.

\[ K_i = \frac{[AgNO_3]}{[HA]} \]
in a mini-hydroponic system that we have developed, in which the roots grow between layers of different materials measuring 6 x 5 cm. A layer of thin white synthetic fabric is placed on a layer of germinating paper. These two layers are wet with distilled water, and 10 seeds are placed along the 5-cm margin on the synthetic one, then a layer of transparent plastic is placed on the seeds. A black plastic cover, previously folded, is placed around the three layers, leaving the 5-cm borders opened. This plate of layers is left overnight at 10°C, and then, each plate is placed vertically in one cell of an ice tray with distilled water (Fig. 2). These ice trays are placed in a plant growth cabinet with a photoperiod of 14 h of light and 8 h of darkness, a light intensity of 90 μmol m\(^{-2}\) s\(^{-1}\) and temperature of 22–24°C. After 5 days in the cabinet, the five best plants are left in each plate and the germinating papers are rinsed four times with 1 mL of solutions containing organic matter fractions. A minimum of distilled water is left in each ice tray cell to irrigate the plates by capillarity. After 3 days of treatment, the germinating papers are rinsed four times with 1 mL of a modified Hoagland & Arnon (1950) solution with 1 mmol L\(^{-1}\) of N, as ammonium nitrate, described by Medici et al. (2004). This low N level is used to avoid the root branching by nitrate (Zhang et al., 1999; Lea & Azevedo, 2006). Three days later, this rinsing with nutrient solution was repeated. After 2 weeks of growth, the black covers and the germinating papers were taken off the plates and the roots, kept between the two other layers, were stained with toluidine blue (0.05%) and the images were digitalised by scanner (300 d.p.i.) for the root analysis by the Delta-T scan software (Delta-T Devices, Cambridge, UK). Two plants in the central position of the plates were chosen for the evaluation of number of lateral roots, length of principal root and density and length of lateral roots. Two experiments were carried out with this system. In the first, the treatments given were HA, FA and HM extract isolated from one representative oxisol (Sombrihumox) at 0, 3, 30 and 300 mg L\(^{-1}\) (pH 5.8). In this experiment, there was also a treatment with indole acetic acid (IAA; Sigma Co., St Louis, MO, USA) at 10\(^{-6}\) mol L\(^{-1}\), which is frequently used in Arabidopsis assays (Parry & Estelle, 2006). In the second experiment, the treatments given were the organic matter fractions from the seven oxisols at 40, 100 and 100 mg L\(^{-1}\) for HA, FA and HM, respectively. These concentrations were expected to be enough to stimulate changes in plant roots and to avoid inhibition effects. To analyse the influences of HS and HM extract, the treatments were randomised with eight repetitions. The data from each organic matter fraction (HA, FA and HM) were analysed by the statistical procedure analysis of variance from SAEG software systems from Universidade Federal de Vigneda, and the means were tested by least significant
difference (LSD), $P < 0.05$ test. The means were normalised with respect to control plants (control = 100%) to compare with IAA results.

Diageotropic ($dgt$) assay

The $dgt$ tomato mutant Solanum lycopersicum L. is characterised by horizontal shoot and root growth, thin stems, hyponastic leaves and lack of lateral roots and is insensitive to exogenous IAA (Kelly & Bradford, 1986). The mutation is in a single gene and was introgressed into dwarf tomato (MT). Thus, MT-$dgt$ is a model to test if a given substance acts like auxin. Seeds of MT-$dgt$ were surface sterilised with 95% (v/v) ethanol for 5 min and with 0.7% (w/v) NaClO for 15 min. These seeds were washed and inoculated into Murashige–Skoog medium (Murashige & Skoog, 1962) with half of macronutrient concentration, 0.3% saccharose and 0.8% agar. HA from P1, P7 and P4 were used at 50 mg L$^{-1}$, and the plates were incubated for 10 days at 25°C and 16 h of photoperiod. After incubation, the number of emerged lateral roots was evaluated. The experiment was entirely randomised, with 10 repetitions for each treatment. The MT tomato without the $dgt$ mutation was also used as a control and is referred in the text as wild type.

Results

Organic matter was extracted from oxisols with different chemical characteristics (Table 1). The Ki index (i.e. the ratio between Si and Al on molar basis) ranged from 0.11 to 2.05, an indicative of high weathering degree of these oxisols. The clay content also varied widely (280–750 g kg$^{-1}$), but because of their low activity, the CEC is very low (2.13–8.56 cmol C kg$^{-1}$) and pH values are found in an acidic range (from 4.8 to 6.8). The surface chemical properties of these soil samples are affected by C content that varied widely (8.64–43.0 g C kg$^{-1}$). Despite the relative high C content of soil samples, the soluble humified organic matter comprises less than 10% C (Table 1).

Soil organic matter from these oxisols is characterised by low HA/FA ratio, that is, the major alkaline-soluble humic fraction is FA. However, the highest content of soluble C was found in HM extract (Table 1). Chemical characterisation of HA and FA fractions is shown in Table 2. HA present higher C content, lower $E_4/E_6$ ratio and higher intensity of fluorescence at 456 nm than FA. The HM extract has low C content but high N content, affording low C/N ratios (Table 3). Despite the accentuated chemical differences among HA, FA and HM extract isolated from different oxisols (Tables 2 and 3), all organic fractions promote changes in Arabidopsis root development.

The increase in root growth is one of the major effects of HS, and it also depends on plant species and HS source and concentration (Vaughan & Malcolm, 1985). To find an optimal humic concentration, a dose–response curve of HA, FA and HM extract versus number of lateral roots emerged was designed for HS from Sombrihumox (Fig. 3). A quadratic model describes HA and FA effects.
but not the HM extract effects. The maximum effects of the three organic matter fractions obtained by first derivative of these quadratic equations were higher than the effect of IAA at 10^{-2} mol L^{-1} (Fig. 4).

The biological activity of soil organic matter fractions, such as the HS, may be evaluated by plant growth response, as a result of direct effects of HS on plant metabolism (Vaughan & Malcolm, 1985; Nannipieri et al., 1993). In this work, we found that humic fractions extracted from soils from the same soil group have similar bioactivity as shown by the plant root development (Figs 5–8). Nevertheless, HS can influence plant growth by mechanisms not yet very clear. Here, the different HS effects can be explained by the high chemical variability among the humic fractions of the oxisols (Table 1).

The visual aspects of root systems treated with HA, FA and HM extract from different oxisols are shown in Fig. 5, and their results are shown in Figs 6–8. The HA1 yields the highest number of lateral roots and HA5 the lowest, but both exhibited statistically higher values than control plants. Curiously, HA5 promotes the largest enhancement on lateral root length (Fig. 6B). Smaller lateral root lengths were observed for plants treated with HA1, HA2, HA3 and HA4, but this effect was not observed when HA from P5, P6 and P7 were used. Lateral root density (i.e. number of lateral roots per millimetre of principal root) was affected by HA treatment (Fig. 6D). The HA isolated from P1 affords the highest number of lateral roots by principal root length unit.

The FA treatments also affect the number of lateral roots and root length (Fig. 7), with the largest effects exhibited by FA2, FA5 and FA7. Primary root growth was strongly inhibited by FA isolated from P3 and P6. This effect was not observed with FA from P1, P2 and P7. An increase of two to three times of lateral root density was observed with the FA and HA treatments. FA extract from P2 promotes the highest stimulation on number of lateral roots followed by FA from P5 and P7. The smallest stimulation was observed with FA6 and FA3, which were nevertheless larger than that observed in control plants. The major effects of FA were on length of lateral roots, with FA4 and FA5 promoting an 11-fold stimulation with respect to control plants (Fig. 7B), while the smaller stimulation (twofold) was found in FA2 and FA7.

### Table 2: Elemental composition, E4/E6 ratio and intensity of fluorescence of humic and fulvic fractions isolated from different oxisols

<table>
<thead>
<tr>
<th>Oxisols</th>
<th>C (g kg^{-1})</th>
<th>H (g kg^{-1})</th>
<th>N (g kg^{-1})</th>
<th>O (g kg^{-1})</th>
<th>Ash (g kg^{-1})</th>
<th>E4/E6 Ratio</th>
<th>Intensity of Fluorescence</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Humic acids</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1</td>
<td>562.5</td>
<td>64.0</td>
<td>52.5</td>
<td>536.7</td>
<td>106.0</td>
<td>7.2</td>
<td>107</td>
</tr>
<tr>
<td>P2</td>
<td>542.0</td>
<td>64.1</td>
<td>58.9</td>
<td>524.2</td>
<td>166.0</td>
<td>6.9</td>
<td>158</td>
</tr>
<tr>
<td>P3</td>
<td>503.1</td>
<td>51.1</td>
<td>49.9</td>
<td>605.8</td>
<td>116.0</td>
<td>6.7</td>
<td>81</td>
</tr>
<tr>
<td>P4</td>
<td>432.0</td>
<td>56.6</td>
<td>57.8</td>
<td>653.7</td>
<td>86.0</td>
<td>4.0</td>
<td>150</td>
</tr>
<tr>
<td>P5</td>
<td>468.0</td>
<td>60.9</td>
<td>46.6</td>
<td>608.7</td>
<td>66.0</td>
<td>6.4</td>
<td>82</td>
</tr>
<tr>
<td>P6</td>
<td>504.8</td>
<td>61.1</td>
<td>68.5</td>
<td>526.8</td>
<td>38.0</td>
<td>5.4</td>
<td>115</td>
</tr>
<tr>
<td>P7</td>
<td>536.5</td>
<td>62.7</td>
<td>52.9</td>
<td>540.1</td>
<td>82.0</td>
<td>8.9</td>
<td>114</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>507 (45)</td>
<td>60 (5)</td>
<td>55 (7)</td>
<td>571 (51)</td>
<td>80 (26)</td>
<td>6.5 (2)</td>
<td>115 (30)</td>
</tr>
<tr>
<td><strong>Fulvic acids</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1</td>
<td>535.3</td>
<td>66.5</td>
<td>86.0</td>
<td>509.8</td>
<td>170</td>
<td>9.5</td>
<td>28</td>
</tr>
<tr>
<td>P2</td>
<td>286.7</td>
<td>52.4</td>
<td>60.9</td>
<td>797.6</td>
<td>160</td>
<td>13.2</td>
<td>16</td>
</tr>
<tr>
<td>P3</td>
<td>271.0</td>
<td>57.7</td>
<td>89.6</td>
<td>779.3</td>
<td>100</td>
<td>11.8</td>
<td>36</td>
</tr>
<tr>
<td>P4</td>
<td>498.1</td>
<td>60.1</td>
<td>78.0</td>
<td>561.4</td>
<td>240</td>
<td>9.3</td>
<td>31</td>
</tr>
<tr>
<td>P5</td>
<td>253.4</td>
<td>44.7</td>
<td>48.5</td>
<td>851.0</td>
<td>130</td>
<td>10.8</td>
<td>40</td>
</tr>
<tr>
<td>P6</td>
<td>328.4</td>
<td>44.6</td>
<td>60.7</td>
<td>764.0</td>
<td>180</td>
<td>9.3</td>
<td>42</td>
</tr>
<tr>
<td>P7</td>
<td>462.7</td>
<td>63.6</td>
<td>82.6</td>
<td>588.7</td>
<td>150</td>
<td>10.0</td>
<td>19</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>376 (78)</td>
<td>55.7 (9)</td>
<td>72 (16)</td>
<td>693 (135)</td>
<td>164 (44)</td>
<td>10.6 (1)</td>
<td>30 (10)</td>
</tr>
</tbody>
</table>

P1, Xanthic Hapludox; P2, Haploperox; P3, Rhodic Humic Hapludox; P4, Sombrihumox; P5, Rhodustalf; P6, Hapludox; P7, Rhodic Hapludox; SD, standard deviation.

### Table 3: Elemental composition and ash content of hexanic–methanolic extracts isolated from different oxisols

<table>
<thead>
<tr>
<th>Oxisols</th>
<th>C (g kg^{-1})</th>
<th>H (g kg^{-1})</th>
<th>N (g kg^{-1})</th>
<th>Ash (g kg^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>199</td>
<td>45</td>
<td>48</td>
<td>27</td>
</tr>
<tr>
<td>P2</td>
<td>79</td>
<td>8</td>
<td>7</td>
<td>14</td>
</tr>
<tr>
<td>P3</td>
<td>168</td>
<td>31</td>
<td>48</td>
<td>26</td>
</tr>
<tr>
<td>P4</td>
<td>185</td>
<td>39</td>
<td>42</td>
<td>31</td>
</tr>
<tr>
<td>P5</td>
<td>123</td>
<td>17</td>
<td>12</td>
<td>25</td>
</tr>
<tr>
<td>P6</td>
<td>99</td>
<td>16</td>
<td>8</td>
<td>17</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>135 (46)</td>
<td>24 (14)</td>
<td>25 (20)</td>
<td>22 (6)</td>
</tr>
</tbody>
</table>

P1, Xanthic Hapludox; P2, Haploperox; P3, Rhodic Humic Hapludox; P4, Sombrihumox; P5, Rhodustalf; P6, Hapludox; P7, Rhodic Hapludox; SD, standard deviation.
The HM extract obtained from oxisols after alkaline extraction also promotes Arabidopsis root growth, with enhancements from 150% to 264% for number of lateral roots and from 405% to 2280% for length of lateral roots (Fig. 8A and Fig. 8B). This dramatic effect on root growth is observed mainly for the HM3 extract followed by HM4 and HM5 extracts. However, the effects on length of principal roots are dependent on the source. For example, HM2, HM3 and HM7 extracts enhance the length of the principal root, while HM1, HM4 and HM5 extracts shorten the principal root length.

The HA isolated from P1, P7 and P4 do not have the ability to induce lateral root formation in dagt mutant, which have a defective gene for auxin response, while the wild-type tomato exhibited lateral root development after treatment with these HA isolates (Fig. 9).

### Discussion

Root architecture changes with hormonal and environment stimulations, and the amount of lateral roots is influenced by a diverse and very complex series of endogenous and exogenous signals (Torrey, 1986; López-Bucio et al., 2003; Sorin et al., 2005; Zandonadi et al., 2007). For a very long time, substances with physiological activity have been found in the humified organic matter, which are able to improve root growth (Bottomley, 1917; O’Donnell, 1973). The physiological activity of organic matter from a predominant Brazilian soil class was studied in the present work.

The process of oxisols formation is characterised by intense transformation of parental matter by chemical weathering and consequently intense removal of SiO$_4$. Thus, Moniz (1972) indicated that SiO$_2$/Al$_2$O$_3$ molar ratio (Ki) is a good index to evaluate weathering degree, and according to this index, the studied oxisols have the following weathering sequence: P1<P5<P2<P4<P6<P7<P3. The lower $E_{4}/E_{6}$ ratio and higher intensity of fluorescence at 456 nm exhibited in HA than in FA is in accordance with the general theory of humification processes that suggests that HA is a more condensed fraction in comparison with FA (Stevenson, 1994).
Overall, the effects on Arabidopsis indicate the presence of hormone-like substances in the organic matter from oxisols. The increase in number of lateral root, exhibited mainly by HA1, HA2, HA3, HA6, FA2, FA3, FA7, HM1, HM3, HM4, HM6 and HM7, indicates the presence of auxin-like substances (Dong et al., 2006). The inhibition of principal root length, exhibited mainly by HA2, FA3, FA6 and HM5, is also a typical behaviour of exogenous addition of higher IAA concentration. The observed results from dgt tomato mutant also support the putative presence of auxin-like substances in HA isolated from P1, P7 and P4. Some authors employing different methods determined the presence of structural fragments similar to auxins in HS (Muscolo et al., 1998; Canellas et al., 2002). Thereafter, Canellas et al. (2002) and Zandonadi et al. (2007) found that HA interacts with cell membranes similarly to auxin. However, the presence of atypical auxin responses such as stimulation of growth of principal roots observed with HA2, FA3, FA6 and HM5 was previously reported by Zandonadi et al. (2007) who also observed that exogenous IAA application at very low concentration (10^{-15} M) induced H+ pump activation and elongations of principal root axis in initial stage of root development. However, it is possible that the presence of other physiologically active substances, such as alkamides, enhances principal root growth, but the ability of the root system to respond to it was found to be independent of auxin signalling (Ramírez-Chavez et al., 2004).

It is interesting that Arabidopsis treated with some organic matter fractions exhibited a kind of compensation between number and length of lateral root, as these organic fractions increase the number of lateral root and decrease the lateral root length, as observed in HA1, HA2, HA3, FA2, FA7, HM6 and HM7. As the plants were grown under low nitrate level, this compensation between number and length of lateral roots is in agreement with the model presented by Zhang et al. (1999).

The effect on root development is obtained with low concentration of organic matter, and, in general, when the concentration is high, an inhibition effect is observed. The HS dose–response curve is bell shaped, typical of hormone assays (Vaughan & Malcolm, 1985). The maximum effects

![Figure 5](image-url)
of organic substances from soil Sombrihumox (P4) on the number of lateral roots were higher than the effect of 10^{-6} M IAA. *Arabidopsis* plants treated with this IAA level exhibited higher number of lateral roots than the control plants, which is in agreement with the report of Dong *et al.* (2006).

*Arabidopsis* is a well-studied plant, and a genetic investigation identified genes with auxin-signalling function, and two large protein families were extensively identified: auxin response factor (22 identified genes) and Aux/IAA (29 identified genes) (Parry & Estelle, 2006). Gray *et al.* (2001) have shown that auxin could act by promoting the degradation of the Aux/IAA transcriptional repressors through the action of the ubiquitin protein ligase SCF^{TIR1}. Recently it was reported that TIR1 is an auxin receptor that mediates Aux/IAA degradation.

**Figure 6** Effect of humic acids (HA; 40 mg L^{-1}), isolated from different Brazilian oxisols, on *Arabidopsis thaliana* root development. (A) Number of lateral roots, (B) lateral root length, (C) principal root length and (D) lateral root density are shown. Data represent means of eight plants ± standard deviation. Lateral root length and principal axis length are normalised in relation to control (100%). Means followed by different letters are statistically different by the least significant difference *P* < 0.05 test. C, control; 1, Xanthic Hapludox; 2, Haploperox; 3, Rhodic Humin Hapludox; 4, Sombrihumox; 5, Rhodustalf; 6, Hapludox; 7, Rhodic Hapludox.
and auxin-regulated transcription (Dharmasiri et al., 2005; Kepinski & Leyser, 2005).

Despite the increasing physiological and molecular information on lateral root formation, the hormonal mechanism that controls lateral root initiation and orientation remains poorly understood (Aloni et al., 2006; Parry & Estelle, 2006). According to Aloni et al. (2006), the primary signal for cell differentiation and formation of lateral roots is promoted by auxin, which induces differentiation by a descending movement from shoot to root apex through the vascular cylinder (pericycle or differentiated xylem). Cytokinin inhibits lateral root emission on the root apex, and ethylene (C2H4) is locally produced in the cells of the protoxylem, which determines

Figure 7 Effect of fulvic acids (FA; 100 mg L\(^{-1}\)), isolated from different Brazilian oxisols, on Arabidopsis thaliana root development. (A) Number of lateral roots, (B) lateral root length, (C) principal root length and (D) lateral root density are shown. Data represent means of eight plants ± standard deviation. Lateral root length and principal axis length are normalised in relation to control (100%). Means followed by different letters are statistically different by the least significant difference \(P < 0.05\) test. C, control; 1, Xanthic Hapludox; 2, Haploperox; 3, Rhodic Humic Hapludox; 4, Sombrihuixox; 5, Rhodustalf; 6, Hapludox; 7, Rhodic Hapludox.
the exact location of mitosis sites for cell initiation. Ethylene production is a result of a high concentration of auxin in the protoxylem cells.

Probably, the complex, heterogeneous, natural, humified organic matter may act as a buffer, either absorbing or liberating signalling molecules according to modifications in the rhizosphere, such as the acidification brought about by the activity of plasma membrane H⁺-ATPase (Canellas et al., 2002; Zandonadi et al., 2007) or exudation of organic acids (Façanha et al., 2002), thus behaving as a regulator of hormonal balance with respect to lateral root emergence.

In this work, we show that relatively high (HA > 14 kDa) and low (FA < 700 Da) molecular weight HS can induce changes in Arabidopsis root development, and according to Muscolo et al. (2006), HS have very
complex structures and it is very difficult to identify the relationship between the single compounds of HS and their biological activity. Recently, Sutton & Sposito (2005) reviewed the concept of structural features of HS and found no reasons to exclude biopolymers or any substances with well-defined biochemical structures as part of organic matter. Piccolo et al. (1996) provide evidence that the apparent size of humic fragments changes drastically with addition of simple organic acids. The effect of addition of carboxylic acid to humic fractions indicates that aggregate disruption is greatest when more hydrophobic humic materials are combined with organic molecules containing both hydrophobic and hydrophilic segments. This result, in turn, suggests that humic materials are held together by hydrophobic interactions, which are easily disrupted when simple organic molecules penetrate large hydrophobically bonded associations and separate them into smaller, higher energy H-bonded associations (Piccolo, 2002). Simpson (2002) examined HS solutions and observed both aggregation and disaggregation behaviour consistent with that described by Piccolo (2002). Changes brought about by organic acid exudation from plant roots or by \( H^+ \) pumping by plasma membrane ATPase may liberate substances from the HS matrix, which promote clear changes in root development, as those observed in this study.

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**References**


