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Full Length Research Paper

Humic acids of vermicompost as an ecological pathway to increase resistance of rice seedlings to water stress

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This paper discussed the potential role of humic acids (HA) in preventing oxidative stress in rice plants submitted to water stress. The rice seedlings (*Oryza sativa* L. cv. IACUB-30) was grown in nutrient solution and HA were extracted from vermicompost and analysed using Fourier-transform infrared (FTIR) spectroscopic and chemical methods. Changes in plant anatomy and morphology before and after stress initiation were employed as biomarkers of plant response. The growth rate over time, water content, dry-mass content, leaf carbohydrates, protein content and amino-acid content were among the parameters evaluated. Peroxidases (POX) activity, proline content, H_2O_2 content and membrane permeability were also studied. The results show that HA induced POX activity leading to a reduction in H_2O_2 content and greater conservation of membrane permeability. These results indicate that the HA play an important role as ecological and safety alternative to prevent oxidative stress in plant caused by drought stress. Our findings provide novel evidence for the protective action of HA against oxidative stress caused by water deficits.

Key words: Peroxidases, oxidative stress, humic acid.

INTRODUCTION

Abiotic and biotic stresses cause extensive damage to agriculture yield (Mittler, 2006; Falcón et al., 2010), for example drought stress, which promotes oxidative stress and formation of reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), superoxide radical (O_2^-) and hydroxyl radical (OH^-), thus leading in some cases to cell death, through lipid peroxidation of membranes and denaturation of nucleic acids and proteins (Agrawal et al., 2002). When these stresses are present, enzymes such as peroxidases (POX) act as antioxidative defence mechanisms by converting ROS into harmless molecular species (Narendra et al., 2006).

Natural humic acids (HA) can be an ecological alternative to increase tolerance of plants to drought, precisely because they have been shown to stimulate protein synthesis in various plant organs and enzyme synthesis and/or activity (Muscolo et al., 2007). HA play this role through a phytohormonal mechanism and many studies have found that it can dramatically stimulate H^+-ATPase in plants (Canellas et al., 2009; Dobbs et al., 2010). These versatile compounds also induce the activity of phenylalanine (tyrosine) ammonia-lyase (PAL/TAL) (EC 4.3.1.5). Recently studies reported the action of HA to stimulate the activity of catalase (CAT) and the generation of ROS, while stimulant plant compound by humic substances (SH), stimulate the activity of superoxide dismutase (SOD) and ascorbate peroxidase (APX) in plants under water stress conditions (Cordeiro et al., 2011; Schiavon et al., 2010; Vasconcelos et al., 2009). Thus, HA could create an appropriate hormones balance in the amount of equivalent fragments

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within the plants, thereby causing recognition by the hormone receptors and triggering the synthesis of metabolites with protective functions against oxidative stress.

The aim of this work was to determine if HA could protect rice plants against water stress by promoting and/or contributing to the stimulation of various effects on enzyme activity, and to verify this action by assessing the integrity of cell membranes and various indicators of plant growth and development.

MATERIALS AND METHODS

Isolation, purification and characterisation of HA

HA were obtained from cattle-manure vermicompost after 70 days of ripening. The procedure provided by the International Humic Substances Society (IHSS) (Swift, 1996) was followed, with some modifications described in a technical bulletin of the Brazilian Ministry of Agriculture, Livestock and Commodities (Benites et al., 2003). Total acidity was determined by titrating the HA solution with excess $Ba(OH)_2$ under an N_2 atmosphere. The HA solution was then back-titrated with HCl (0.1 M). Carboxyl groups was determined by chemical titration, adding an excess amount of $Ca(CH_3COO)_2$ in the prepared solution of AH, this solution was then stirred for 24 h and CH_3COOH released was titrated with NaOH solution (0.1 M) (Schnitzer and Gupta, 1965). The content of phenolic groups was determined by the mathematical difference between the total acid group content (total acidity) and the content of carboxylic acid groups (carboxylic acid).

The E_4/E_6 ratio was determined by dissolving 1 mg of HA in 5 ml of $NaHCO_3$ (0.05 $mol.L^{-1}$) and adjusting the pH to 8.3 with NaOH. The absorbance was measured at 465 and 665 nm (RayLight Spectrophotometer UV2100). The ratio of the two absorbance values corresponded to the E_4/E_6 ratio. The elemental composition of the HA (C, H, N, S) was determined using a LECO CHNS-932 elemental analyser, while oxygen content was determined by subtraction. The Fourier-transform infrared (FTIR) spectra of the HA were analysed in the range of 700 to 4000 cm^{-1} using tablets made up of 1 mg of HA in 100 mg of KBr (Nicolet Magna IR 550).

Plant materials and experiments

Experiments were conducted under controlled light and humidity conditions (light cycle of 12/12 h (light/dark), a influx of photosynthetic photons of 250 $\mu M.mol.m^{-2} s^{-1}$, relative humidity of 70% and temperatures of 28/24°C (day/night)) using rice seeds of the variety IACUB-30. The experimental setup consisted of plastic trays 17 cm long by 12.5 cm wide by 2 cm deep. A total of 30 trays were prepared: 10 trays for each of three treatments. The treatments consisted of a control and two concentrations of HA: 34 and 46 mg HA L^{-1} (T1 and T2, respectively). Hoagland solution containing the appropriate concentration of HA was prepared for each treatment, and an equal volume was added to each tray. A total of 15 seeds were then transferred to each tray, so that each treatment contained a total of 150 seeds. Water stress was induced by allowing the nutrient solution to evaporate naturally and monitoring of water loss in tissues plants was evaluated following gravimetric methods (Bian and Jiang, 2009; Fu and Huang, 2001).

Anatomical and morphological evaluations

The growth dynamics of the rice seedlings were recorded by marking each stem by hand and measuring the length of the stem from the edge of the seed and/or root development up to the mark throughout the course of seedling growth. The plants were removed from the containers at 25 days after germination (DAG) - this time was determined from results obtained in previous experiments under the same conditions. The plants were divided into leaves and roots to determine dry mass. The plant material was dried at 105°C for 5 h and then weighed. This procedure was repeated until the dried material reached a constant mass.

Biochemical evaluations

For determination of photosynthetic pigment, 5.0 mg of leaves tissue were placed in contact for 48 h in Acetone 80% (v:v). Supernatant was filtered and the absorbance was measured at 648.6, 664.2 and 470 nm. Equations used to calculate chlorophyll a (Chl a), chlorophyll b (Chl b) and carotenoids (Car) were described by Lichtenthaler (1987). Other biochemical evaluations were determined as follows.

Determination of protein content

Fresh leaves (0.1 g) were homogenized in a chilled (4°C) mortar using a buffer solution Tris-HCl at pH 7. The mixture was centrifuged at 12000 g for 1 h at 4°C. Supernatant was filtered, decanted and transferred to Eppendorf tubes. The total protein content was determined by the absorbance in a spectrophotometer at 500 nm using bovine serum albumin (BSA) as standard, according to Lowry et al. (1951).

Amino acid determination

Fresh tissues (0.5 g samples) were homogenized in 70% ethanol in a pestle and mortar. The mixture was centrifuged at 4400 g for 10 min and 10 ml of this extract was evaporated. The residue was dissolved in 5 ml of 0.2 M citrate buffer pH 5.0 and to 2 ml of this solution was added 1 ml of ninhydrin reagent. The absorbance was measured at 570 nm. Total free amino acid was determined from a standard curve prepared against glycine, according to Moore and Stein (1944).

Determination of reducing carbohydrates

2.0 g of fresh plant tissues was macerated in a mortar with 20 ml of hot distilled water. The material was separated by decantation, filtered under vacuum and the final volume was measured. The absorbance was measured in spectrophotometer at 530 nm using acid 3,5-dinitrosalicylic (DNS) and standard curve prepared with glucose as standard (Noelting and Bernfeld, 1948).

Determination of POX activity

POX (EC 1.11.1.7) activity was determined from the enzymatic extract using guaiacol as substrate. The reaction consisted of 0.5 ml of enzyme extract, 0.5 ml of sodium acetate buffer 50 mM pH 5.6, 0.5 ml of 20 mM guaiacol and 0.5 ml of H_2O_2 60 mM. Mixtures were incubated at 30°C for 4 min. Enzyme activity was calculated from the change in absorbance and was expressed as mg $protein^{-1}$ min^{-1}, molar extinction coefficient (26·6 mM^{-1} cm^{-1}) (Maehly and Chance, 1967).

Determination of H_2O_2 content

For the H_2O_2 content, 1 g of leaves tissues was grinded with N_2 (l)

Table 1. Elemental composition, atomic ratios, E_4/E_6 ratio and acidic functional group contents of the HA.

% (m:m)					H/C	O/C	C/N	mol.kg^{-1} (c)			E_4/E_6
C	H	O	N	S				Carboxyl	Phenols	Total acids	
56.7	4.84	34.6	3.07	0.72	0.08	0.61	18.4	9.24	2.03	11.27	4.22

and 10 ml of cooled acetone was added at 10°C. The solution was filtered and 4 ml of titanium reagent added to precipitate the titanium-hydro peroxide complex. This reaction was centrifuged at 10 000 g for 10 min and the precipitate dissolved in 10 ml of H_2SO_4 (2 M) and centrifuged again. The absorbance was measured at 415 nm. Hydrogen peroxide contents were calculated by comparing with a standard curve drawn with known hydrogen peroxide concentrations (Rao et al., 1997).

Determination of membrane permeability

Membrane permeability was determined by electrical conductivity methods. Fresh leaves from treated plants were sliced into small discs (1 cm diameter, 0.5 g), and placed in flasks containing 30 ml deionized water. The flasks were shaken at 25°C and the electrical conductivity of the solution measured at intervals of 3 min for 30 min with the conductivity meter (Gui-Lian et al., 2009).

Determination of proline content

The content of free proline was determined as described by Bates et al., 1973. Leaves tissues were homogenized in 3% (w/v) sulfosalicylic acid and centrifuged at 14000 g for 10 min. The reaction contained 2 ml of glacial acetic acid, 2 ml of ninhydrin reagent (2.50% w/v ninhydrin in 60% v/v 6 M phosphoric acid) and 2 ml of supernatant. The incubation lasted for 1 h at 90°C. The upper toluene phase was decanted into glass cuvette and absorbance was measured at 520 nm. The concentration was assayed using proline as the calibration standard.

Statistical analyses and data processing

The statistical analyses consisted of a simple ANOVA (analysis of variance). Tukey's multiple-comparison test was performed when a studied factor showed significance. For these tests, the statistical program STATGRAPH (v. 5.1 plus) was used. The data were processed and the results were graphed using Microsoft Office Excel 2003 for Windows XP.

RESULTS AND DISCUSSION

Characterisation of HA

Table 1 shows the elemental composition and some chemical properties of the HA obtained. The amounts of the various elements and the relative percentages of carboxylic and phenolic acids are consistent with the range of HA standards reported in the literature by the IHSS (Ritchie and Perdue, 2003). The values of the E_4/E_6 ratio (Table 1) showed a high degree of structural condensation (Kononova, 1966). The values of this ratio (E_4/E_6) were inversely proportional to the degree of aromatization (condensation of aromatic structure) where for the AH, values are between 3.0 and 5.0. Several authors have found similar (Campitelli and Ceppi, 2008; Fukushima et al., 2009).

The FTIR spectrum of the HA obtained in this experiment was similar to those reported in the literature for HA from both soil and vermicompost materials (Figure 1) (Amir et al., 2010; Calderín et al., 2007; Droussi et al., 2009). A broad, intense band was present at 3437.04 cm^{-1}, corresponding to OH stretching (carboxylic, phenolic and alcoholic) associated with inter- and intra-molecular hydrogen bonding. Amide-NH stretching also contributes to this peak. The bands at 2927.85 and 2850.7 cm^{-1} are associated with symmetric and asymmetric aliphatic CH stretching, while the band at 1714.66 cm^{-1} can be assigned to C=O stretching of aldehydes or ketones. The band at 1649.09 cm^{-1} is also due to C=O stretching, but of quinones and amides. Bands characteristic of C=C and C=N stretching of aromatic structures and amides and CN deformation are present at 1510.22 and 1462 cm^{-1}. At 1421.49 cm^{-1}, there was a CH_2-deformation zone corresponding to OH deformation of carbonyl groups and CO stretching of phenols. More also, the bands at 1222.83 and 1126.39 cm^{-1} can be attributed to CO stretching, OH deformation of carboxylic acids and CO stretching of phenols and esters. The bands observed at 1091.68 and 1035.74 cm^{-1} can be assigned to CO stretching of ester groups and the remains of polysaccharides.

According to the spectroscopic characteristic on the HA, they have the principal properties for produce equivalents effects to actions mode reported by literature. Root applications of HA purified with similar spectroscopic characteristic caused a significant increase of roots length and H^+ ATPase activity (Mora et al., 2010) and another authors, working with HA transformed by synthesis reactions, activated the auxin synthetic reporter DR5::GUS (Canellas et al., 2010).

Anatomical and morphological evaluations

Dynamics of plant growth, water content of plants and leaf and root dry mass

From the total water content in plants shown in Figure 2, result indicated that from day 10, DAG showed a decrease in water content in plant organs in contact with the control treatment. It is clear that after this time, the water deficit started and hence the intensification of

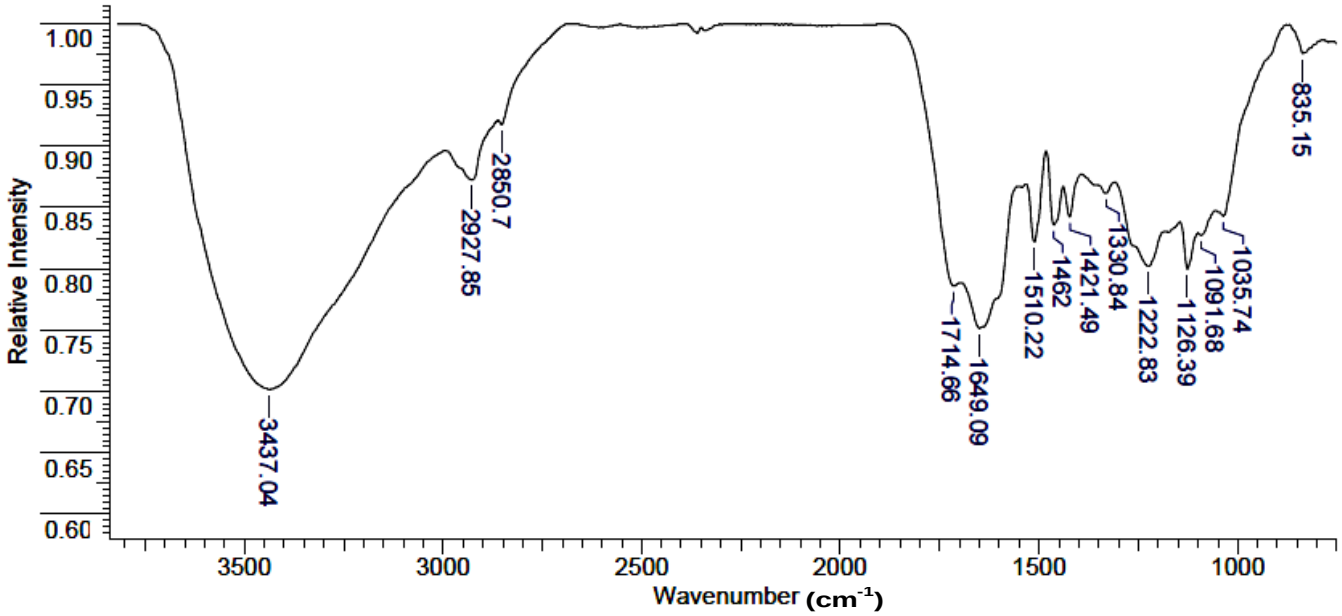

Figure 1. DRIFT spectrum of the HA obtained. Bands assignments were according to Francioso et al. (2007), Piccolo (2002) and Schiavon et al. (2010).

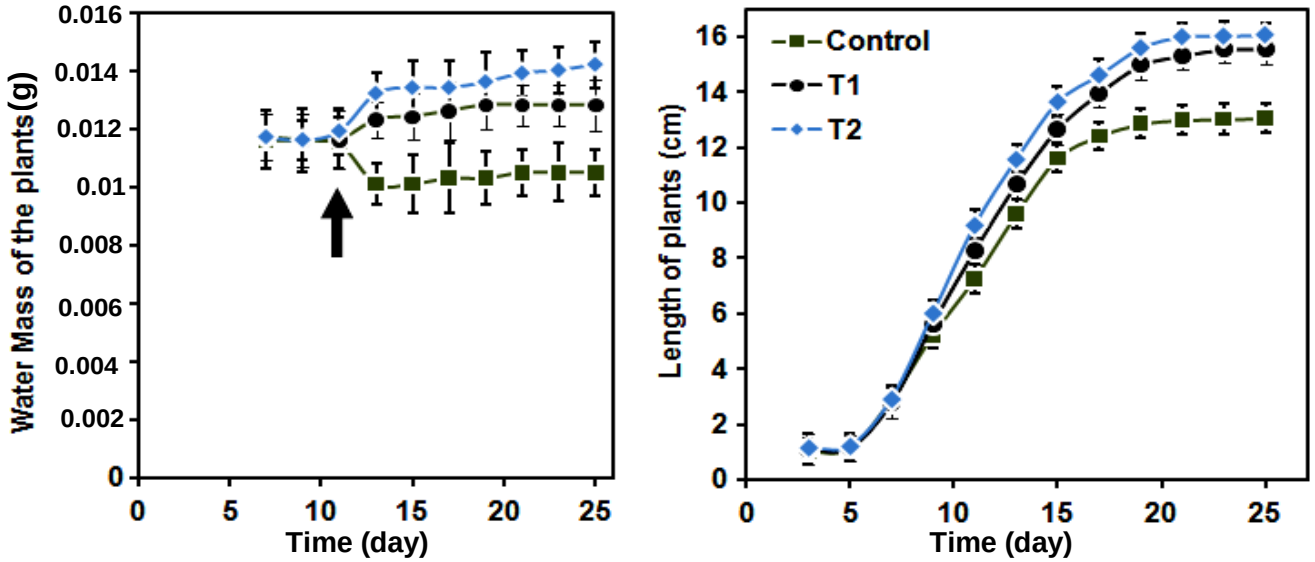

Figure 2. Behavior of plant growth and water content of plants from 3 to 25 DAG. Bars represent averages ± SE (standard error) from three replicate experiments (Tukey's test, $p \leq 0.05$).

stress. In the control treatment plants, the sharpest decline in water content seems to happen from 10 until 14 DAG, and then the plants seem to maintain a homeostasis when the water content in organs remained constant until 25 DAG. However, the behavior in the water content in plants treated with HA maintains higher hydration rate especially for T2. The available water content began to decrease after the tenth day of germination of plants, when it can be regarded as the beginning of water stress deficit.

HA are thought to promote plant growth through a hormone-like action (similar to that of auxin) that stimulates cell division (Dobbss et al., 2007). As shown in Figure 2, however, our data showed no positive effect of HA on the growth of seedlings up to 10 DAG. The first effects on growth performance were observed after this moment specifically. This is the exact moment at which the water content in the tissues begins to decrease

Table 2. Average content of leaf and root dry mass at 10 and 15 DAG.

Treatment	Leaf dry mass (g)		Root dry mass (g)	
	10 DAG	15 DAG	10 DAG	15 DAG
Control	0.735 ± 0.065[b]	0.771 ± 0.102[b]	0.898 ± 0.078[b]	1.257 ± 0.095[c]
T1	0.850 ± 0.057[a]	0.883 ± 0.049[a]	1.033 ± 0.055[a]	1.481 ± 0.064[b]
T2	0.856 ± 0.058[a]	0.916 ± 0.046[a]	1.050 ± 0.053[a]	1.555 ± 0.060[a]

Different letters indicate significant differences according to Tukey's test, $p \leq 0.05$.

with greater intensity. From 10 DAG, the average growth of plants in contact with the control treat-ment was lower than the growth of plants under the treatments with HA, and also the plant growth rate decreased after 15 DAG, especially for the control plants. However, plants treated with HA continued to grow under water deficit conditions.These results are another proof to the fact that when plants are faced with stress, it at the same time affects the growth and development of them.

Dissolved HA in water can stimulate the emission of root hairs, increasing in number and in quantity, and the increase in the number of corticosteroid and epidermal cells (Zandonadi et al., 2010). Other studies show a stimulating action of the root length, plant growth and various physiological indicators, also due to hormone-like action of humic substances. Atiyeh et al. (2002) Nardi et al. (2002) showed evidence of possible penetration of structures of humic substances within the plants and as a consequence, bring about direct or indirect action of the metabolic processes of plant growth and development. Some of these proven effects may explain both the stimulatory effect on growing, such as increased water intake in the treated plants in our work with HA.

Table 2 shows the biomass content of plants at 10 DAG when the plants were growing rapidly and at 15 DAG when growth slowed, which coincides with the most extreme moments in the water intake by plants. Plants treated with HA, especially those in treatment T2, showed an increasing trend in biomass synthesis compared to control plants. This behaviour was observed in both radical and foliar organs. The difference between treat-ments in biomass production was unquestionably higher at 15 DAG than at 10 DAG for both organs. In treatment T2, leaf dry mass was stimulated by 18.7% at 15 DAG and by 16.5% at 10 DAG compared to the control. Similarly, root biomass was stimulated by 23.7 at 15 DAG and by 16.9% at 10 DAG. For plants under T1 treatment, stimulation in the dry mass of leaves was 15.5 and 14.5% for the 10 and 15 DAG, respectively. At the root, stimulation was 15.1 and 17.8% for the 10 and 15 DAG, respectively, both compared with respect to content control in plants. The results therefore indicated that treatment T2 had the greatest effect on biomass production in rice plants before and after the natural induction of stress. T2 treatment can stimulate both the processes of elongation and cell division, as the processes of production of metabolites in plants, greater degree than T1.

Biochemical evaluations

POX activity, foliar H_2O_2 content, membrane permeability and proline content

Peroxidases are protein/enzymes extracellular of class III, enzymes known to have great importance in the defense of plants to abiotic stresses (Agrawal et al., 2002; Hiraga et al., 2001; Rakwal et al., 2001). Relation-ship between the metabolic activity of these enzymes with an action of hormone activity and the possible hormone-mimetic action of HA with these hormones justified the kinetics activity study of these enzymes POX, seeking influence of HA in their response at the roots and leaves level. Figure 3 shows the kinetics of POX enzymes in each organ and illustrates the activity of enzymes related to plant defence against oxidative stress. POX activity increased at 17 DAG for both organs, indicating the onset of oxidative stress in the plants. Plants in both HA treatments exhibited higher POX activity levels than control plants from 17 DAG onward. The highest POX activity values for both HA treatments were however detected in the roots. Moreover, the leaves of HA-treated plants exhibited increased POX activity compared to those of control plants from 12 DAG onward, whereas the roots of HAs-treated plants exhibited increased POX activity only after 17 DAG.

These behaviours of POX enzymes in plants treated with HA can be explained by different modifications that can be produced in the complex mechanism of plant defense. A possible explanation could be by the similar action of abscisic acids (ABA)-like structural fragments in the HA, coinciding with the mode of action of this hormone or its biosynthetic precursors. Another general way to observe these effects could be explained by a possible hormonal imbalance favorable in both organs. The HA may be able to create a physiological plant response to water stress condition, regulating the content of ROS through the action of POX. Studies have also shown that the application of HA extracts and biosolids to wild plants under water-deficit stress exerts a protective effect due to high production of cytokinins and other hormones. These substances maintain a proper balance in the leaves, promoting growth and increases in

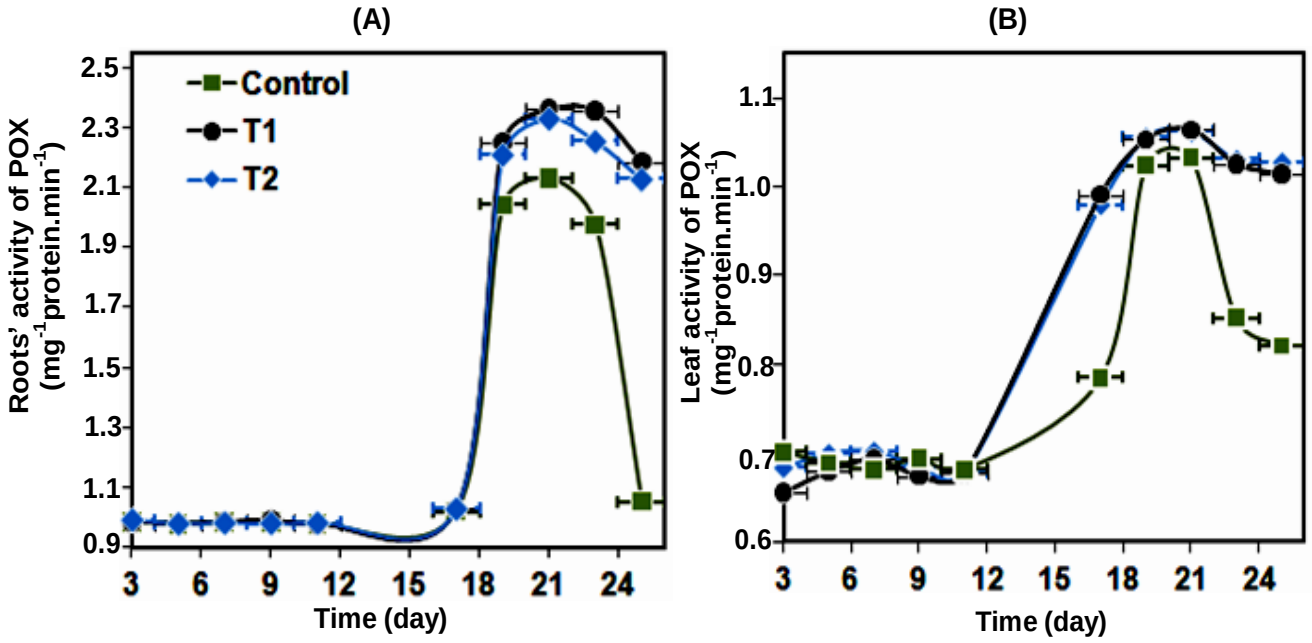

Figure 3. POX activity over time in (a) roots and (b) leaves of rice plants. Bars represent averages ± SE (standard error) from three replicate experiments.

metabolites such as proteins and amino acids and thus improving the physiological condition of the plants (Zhang and Ervin, 2004). Other authors observed that applications of bio-stimulant with different concentrations of HA, stimulated the enzyme activities of POX and APOX in plants under water stress conditions. In addition, stimulation of the enzyme CAT and generation of ERO in maize plant has been observed by applying different concentrations of HA (Cordeiro et al., 2011; Vasconcelos et al., 2009).

Hydrogen peroxide (H_2O_2) is one of the reactive oxygen species produced in cells during oxidative stress. This species, like other ROS, provokes lipid-membrane peroxidation and denaturation of proteins and nucleic acids. POX transforms these species into chemical species that are harmless to plants (Schulze et al., 2005; Verma and Dubey, 2003). The H_2O_2 content of plant leaves at 21 DAG, when POX was at their highest level is shown in Figure 4. Plants treated with HA exhibited a decrease in H_2O_2 content. Treatment T1 produced the largest reduction in H_2O_2 and was statistically different from the control. This pattern coincided with that observed for POX activity. These results confirm the effectiveness of HA in stimulating antioxidant mechanisms in plants, thus, it is reasonable to recognise the positive effect of HA under conditions of water-deficit stress, exerting a protective antioxidant effect.

Furthermore, the measurement of electrical conductivity over time is an effective and practical method to evaluate membrane permeability. Control plants showed a high level of membrane damage caused by oxidative stress generated by the water deficit (Figure 6). Ion efflux across the membranes was favoured, promoting increased conductivity in the medium (Li-Ping et al., 2006). According to the results, it is appreciated that although the HA treated plants suffered some damage to the permeability of the membrane (visibly displayed on the increased value of conductivity during the first 27 min of measurement), the damage in the leaves of plants treated with HA was always less to the damage to the control plants (also visibly displayed within 30 min of evaluation). At the end of the experiment, the conductivity values of plants treated with T1 and T2 were approximately 60% lower than the values of the control plants.

Proline content is an important also indicator of abiotic stresses in plants, even when the total amino acid content decreases due to the stimulation of protein biosynthesis. Among other roles, proline acts as an osmolyte under conditions of water deficit and salinity induced stress. Under water deficit conditions, free proline content increased in both HA treatments, especially treatments T2 and T1 did not seem to affect the synthesis of proline or other osmolytes (the plants in this treatment exhibited decreased levels of carbohydrates, which also have an osmoregulatory function). However, treatment T2 appeared to affect the synthesis of metabolites with osmotic regulatory functions. Increase in proline content has been linked to an increase in plant tolerance to different stresses, especially drought stress. Proline is recognized as an osmo-protectant compounds, stabilizing proteins, inhibiting lipid peroxidation and scavenging of ROS (Ashraf and Foolad, 2007; Trovato et al., 2008). Inducement in the proline synthesis under drought stress may mean a decrease of the cytosolic

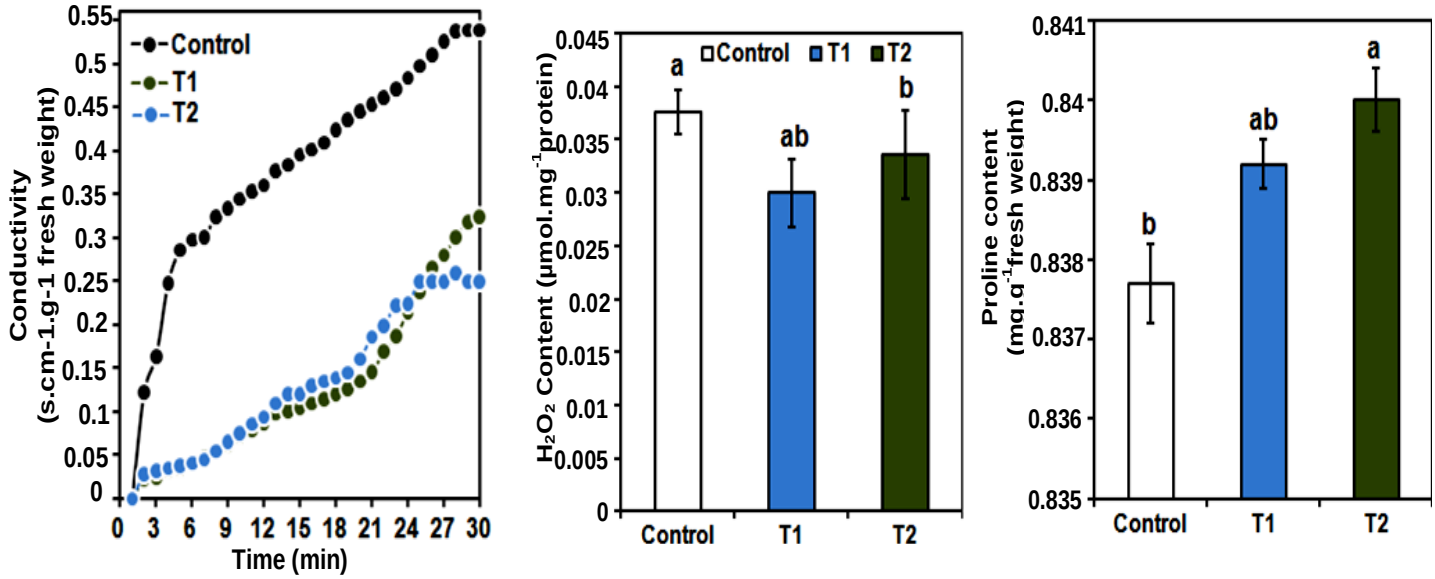

Figure 4. Leaf content of H_2O_2, electrical conductivity of the solutions in contact with the leaves and free proline content in the leaves of plants at 21 DAG. Different letters indicate significant differences according to Tukey's test , $p \leq 0.05$)

Table 3. Average content of photosynthetic pigments in the plants at 30 DAG.

Treatment	mg.g^{-1} (dry mass)			
	Chl a	Chl b	Car	(Chl a + Chl b)/Car
Control	0.810 ± 0.051^{b}	0.690 ± 0.056^{b}	0.257 ± 0.016^{b}	5.844 ± 0.125^{a}
T1	0.995 ± 0.048^{a}	0.919 ± 0.055^{ab}	0.302 ± 0.014^{ab}	6.327 ± 0.131^{ab}
T2	1.007 ± 0.040^{a}	0.939 ± 0.048^{a}	0.344 ± 0.016^{a}	5.650 ± 0.132^{b}

Different letters indicate significant differences according to Tukey's test, $p \leq 0.05$.

acidity and maintenances on the values $NADP^+$: NADPH, compatible with good metabolic performances. Another important advantage is the ability of proline to enhance the pentose-phosphate pathway, which is very important in antioxidative defence mechanism (Hare and Cress, 1997) even in rice (Demiral and Türkan, 2005).

Photosynthetic pigments, reducing carbohydrates, proteins and amino acids

Table 3 shows the average content of photosynthetic pigments in the plants at 25 DAG. Plants exposed to HA contain more photosynthetic pigments, indicating that pigment biosynthesis continued even under conditions of great stress. The total content of chlorophyll a and b and carotenoids was 15% greater in treatment T1 than in the control treatment. These results indicate that plants in contact with HA maintained a more favourable physiological status, ensuring the continuation of photosynthesis for energy production even under stressful conditions. The photosynthetic-pigment measurements are particularly interesting because reports in the literature have indicated the possibility that humic substances affect chlorophyllase activity and thus promote the degradation of pigments (Yang et al., 2004), a hypothesis our results do not however support.

The average content of carbohydrates, proteins and amino acids in the seedlings is shown in Figure 5. Protein biosynthesis was stimulated in plants of both HA treatments, as reflected by the protein content in both organs of these plants. The highest level of stimulation was observed in the roots, especially in treatment T1, with a rate more than 20% greater than that observed in the control treatment. A similar difference in stimulation was observed in the foliar organs. These results for protein content support the dry-mass data in indicating increased biomass content in plants exposed to HA. Proteins that may be involved in this process include POX enzymes, aquaporin proteins and regulators of osmolyte action, water flow and osmotic pressure (Souza-Filho et al., 2003; Zhao et al., 2008). On the other hand, amino-acid content in the leaves of plants exposed to HA did not differ significantly from that of control plants. The average amino-acid concentrations in the roots of T1 and T2 plants were even lower than those of

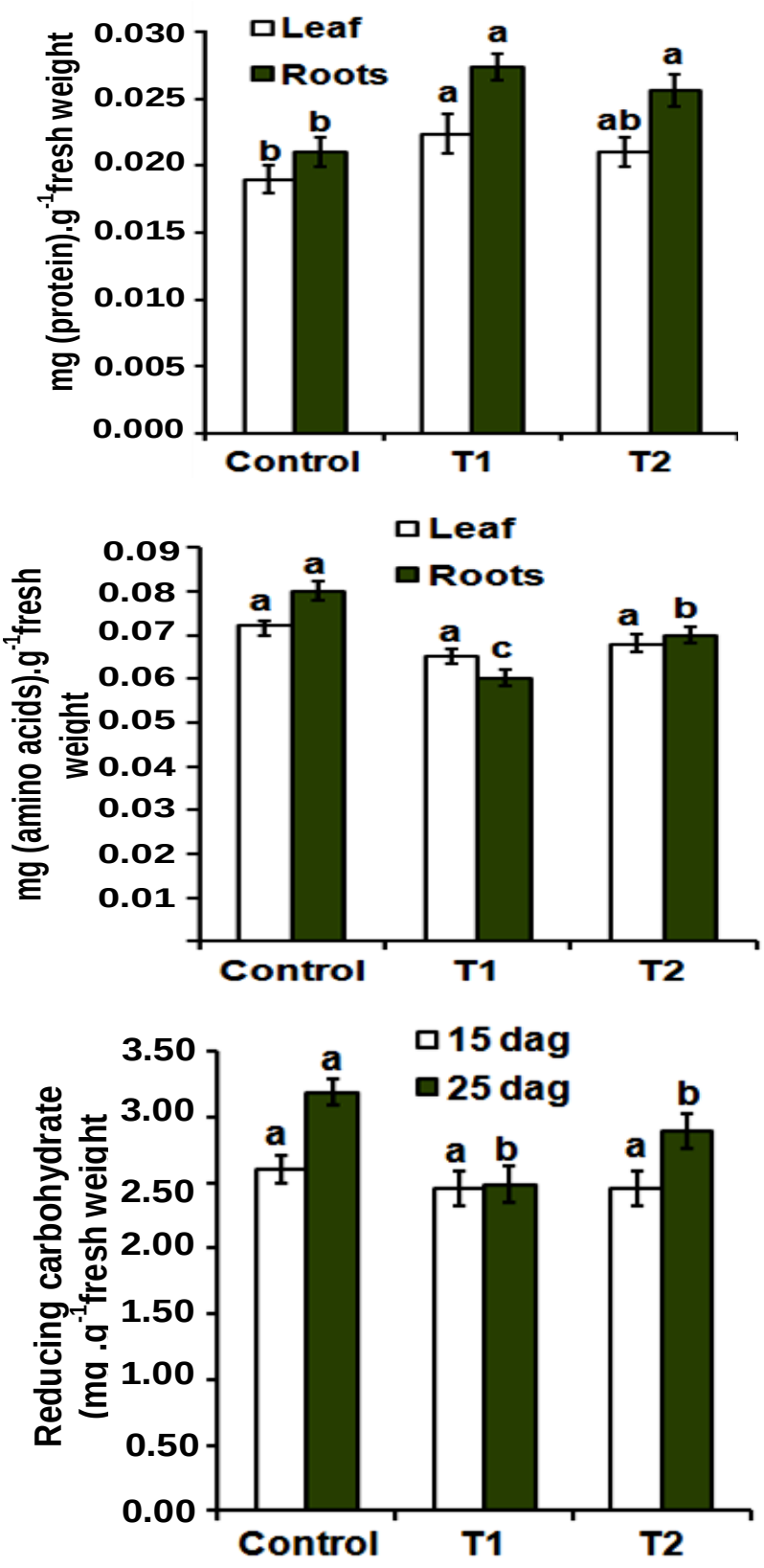

Figure 5. Protein, carbohydrate and total amino acid contents in roots and leaves in the plants. Different letters indicate significant differences according to Tukey's test, $p \leq 0.05$). Bars represent averages ± SE (standard error) from three replicate experiments.

control plants. These results suggest that when plants treated with HA are subjected to water-deficit stress, protein biosynthesis is stimulated to high levels, resulting in rapid consumption of amino acids. Although, no significant differences in carbohydrate content were observed at 15 DAG, a slight trend toward lower average values in HA-treated plants was observed even when environmental conditions were appropriate for plant development at the time of the evaluation. At 25 DAG, however, HA-treated plants showed a significant decrease in reducing-carbohydrate content. A previous study on the use of HA in maize plants has demonstrated that treated plants utilise carbohydrates more rapidly and efficiently (Nardi et al., 2007).

Conclusion

The results of this study showed that rice plants subjected to water-deficit conditions received increased protection against oxidative stress from the application of HA. Plants treated displayed higher growth rates and water content than untreated plants. POX enzyme activity was stimulated in the leaves and roots by HA in plant-treated and its stimulation of POX caused a reduction in H_2O_2 content. In addition, membrane permeability was less affected by stress. This study therefore provides new evidence of the role of HA in plant defensive mechanisms and suggests new directions in the utilisation of these remarkably versatile humic substances as effective and ecological pathway to maintain the yield agriculture under drought stress conditions.

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