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PROMOTION OF LATERAL ROOT GROWTH AND LEAF QUALITY OF FLUE-CURED TOBACCO BY THE COMBINED APPLICATION OF HUMIC ACIDS AND NPK CHEMICAL FERTILIZERS

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SUMMARY

In this study, the effects of HA combined with NPK fertilizer (HANPK) on root growth and leaf quality of tobacco plants were investigated in tobacco fields. Results indicated that the application of HA alone did not enhance the growth of tobacco obviously, while HANPK increased tobacco biomass by 36.9% and stimulated the growth of lateral roots significantly. The number of the second-order lateral roots was increased by 89.3% compared with the control. Furthermore, HANPK raised the ratio of root biomass in 0–20 cm soil layer over the whole soil layer and increased the proportion of fine roots over the total roots. Tobacco leaf yield, output value, and benefit of HANPK were 12.2%, 29.4% and 35.5% higher than those of the control, respectively. The above results suggest that the combined application of HA and NPK chemical fertilizer is an economical pattern for improving tobacco growth.

INTRODUCTION

HA are organic C-rich materials, which comprise the major fractions of humic substances (Canellas *et al.*, 2002). They exhibit beneficial effects on reducing soil compaction and increasing nutrient availability (Nardi *et al.*, 2002). Many reports showed that HA enhanced seed germination and root, shoot and leaf growth in different plant species, such as cucumber, maize, pelargonium and wheat (Chen *et al.*, 2004; Mora *et al.*, 2010; Philippe *et al.* 2010). Treated plants showed a faster development and reached reproductive stage 3 to 5 days earlier than the control. However, the mechanism responsible for the function of HA is not yet clear. Some authors propose that humic substances promote plant growth by affecting hormone metabolism. HA had auxin activity on lateral root proliferation (Canellas *et al.*, 2002; Zandonadi *et al.*, 2007). Humic substances and its derivatives activated the auxin synthetic reporter (Dobbss *et al.*, 2010). The further research found that the beneficial effects of humic substances on shoot development could be directly associated with several active cytokinins and polyamines (Mora *et al.*, 2012). On the other hand,

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Bidegain *et al.* (2000) found that humic substances from the chemically decomposed sawdust could increase total P uptake and yield in ryegrass by enhancing root development. Humic substances also could activate the root plasma membrane H^+-ATPase activity and increase nitrate uptake rates in roots (Nardi *et al.*, 2002; Quaggiotti *et al.*, 2004). In a low organic matter soil, there was a synergistic effect of HA and fertilizer on plant growth (Hartz and Bottoms, 2010). The above results suggested that humic substances can increase root uptake of mineral nutrients. It is possible that the combined application of HA and chemical fertilizer can accelerate the growth of plants. To investigate this hypothesis, the effects of HANPK chemical fertilizer were examined in field tobacco plants.

MATERIALS AND METHODS

Material incubation and experimental design

The experiments were performed in paddy soils of Binhe village, Guandu small town, Liuyang County, Hunan Province. The basic properties of paddy soil were 12.3 g kg^{-1} of organic matter, 130.5 mg kg^{-1} of alkali-hydrolyzable N, 33.6 mg kg^{-1} of readily available P, 105.2 mg kg^{-1} of readily available K, and pH 5.8. Four treatments were set as follows: the control; NPK; HA and HANPK. NPK indicated an application of 225 kg ha^{-1} of NPK (30-10-20) chemical fertilizer. HA indicated an application of 150 kg ha^{-1} of HA (Water solubility: 1000g kg^{-1}, available N: 43.2 mg kg^{-1}, available P_2O_5: 26.1 mg kg^{-1}, available K_2O: 87 mg kg^{-1}, Produced by Shandong Hangao Biological Engineering Limited Company). HANPK indicated the mixture of 150 kg ha^{-1} of HA and 225 kg ha^{-1} of NPK. The control indicated no HA or NPK fertilizer application. 2000 kg ha^{-1} of organic manure was applied in each treatment as basal fertilizers. At the transplanting stage, 80% of HA, NPK or HANPK fertilizer was applied and 20% of HA, NPK or HANPK was applied at seedling stage (20 days after transplanting). Each treatment had four replicates. The particular application pattern was as follows: During transplanting, HA, NPK or HANPK was applied to a cultivation hole, and then a tobacco seedling was transplanted in the cultivation hole. After that, the seedling roots were covered with soil. There were 16,500 tobacco plants ha^{-1}. The tobacco variety was K326, provided by China Tobacco Hunan Industrial Co. LTD, Changsha, China.

Measurement of root number, root length and root thickness

In tobacco fields, tobacco seedlings were grown for 45 days, and then harvested for root parameter measurements as shown in Tables 1 to 4. Tobacco roots were dug out, soaked in tap water and rinsed thoroughly with deionized water. Then, the numbers of the first- and second-order lateral roots were counted in water respectively. The length of tap root or lateral roots was measured with a ruler. When root length is less than 5 mm, the length or the number is not included in the calculation. Root diameter was measured at the middle point of root length manually and the data were classified into $<$2mm, 2–5mm and $>$5mm. After classification, root biomass was measured. In a hydroponic experiment, tobacco seedlings were grown for 16 days after germination.

Table 1. Effect of different treatments on the growth of tobacco seedlings.

Treatment	Shoot biomass dry weight (g plant^{-1})	Root biomass dry weight (g plant^{-1})	Ratio of root to shoot
Control	46.3 ± 6.54* b	6.44 ± 0.78 b	0.139 a
NPK	56.2 ± 5.60 ab	7.59 ± 0.92 ab	0.135 a
HA	50.4 ± 3.96 b	6.84 ± 0.65 b	0.136 a
HANPK	63.8 ± 6.62 a	8.35 ± 0.86 a	0.131 a

Note: *Data indicated mean ± SE ($n = 4$). The data with different letters in the same column indicated significant differences at $p \leq 0.05$ (Duncan's test).

Roots with less than 5 mm-length or 0.5 mm-diameter are not included and counted. Average root length indicated the ratio of the total root length over the total root numbers. The data were shown in Table 6. Mature tobacco leaves were collected and used for analysis of leaf quality indices and leaf yield, please see the data in Tables 7 to 8.

Measurement of root activity

Root activity of tobacco seedlings were measured by the method of α-naphthylamine oxidation. After 15-, 30-, 45- or 60-days transplanting, tobacco roots were dug out and washed in tap water and 55 mg L^{-1} of $CaCl_2$ solution, and then submitted to a solution containing 50 mg L^{-1} of α-naphthylamine and pH 6.0 phosphate buffer for 10-min shaking slightly. After absorption, 2 mL of the solution was diluted with 10 mL of deionized water. Then, the diluted solution was reacted with 1 mL of sulfamic acid and 1 mL of sodium nitrite. After 25-min incubation, the solution was measured at 510 nm with a 754 spectrophotometer. Root activity can be calculated according to the oxidation of α-naphthylamine. For the details, please see Zhou (1995).

Effect of TIBA on the growth of lateral roots

After germination, tobacco seedlings were grown in a nutrient solution (consisting of 240 mg L^{-1} $CO(NH_2)_2$, 505 mg L^{-1} KNO_3, 118 mg L^{-1} $Ca(NO_3)_2 \cdot 4H_2O$, 136 mg L^{-1} KH_2PO_4, 246 mg L^{-1} $MgSO_4 \cdot 7H_2O$, 22.5 mg L^{-1} EDTA-Fe, 0.125 mg L^{-1} $CuSO_4 \cdot 5H_2O$, 2.8 mg L^{-1} H_3BO_3, 2.4 mg L^{-1} $ZnSO_4 \cdot 7H_2O$, 2 mg L^{-1} $MnSO_4 \cdot H_2O$, 0.098 mg L^{-1} $(NH_4)_2Mo_7O_4 \cdot 4H_2O$) for 12 days, then submitted to nutrient solution containing 10 μmol L^{-1} 2,3,5-triiodobenzoic acid (TIBA), a chemical inhibitor of polar auxin transport (Lomax *et al.*, 1995; Zhou *et al.*, 2003) for 6 days. After treatment, the number and length of tobacco roots were counted using a ruler. The treatment solution was changed each week. TIBA was firstly dissolved in dimethyl sulfoxide (DMSO) as a stocking solution. When treatment was performed, TIBA was applied to the nutrient solution and its concentration was 10 μmol L^{-1}. DMSO in the solution was less than 1 μmol L^{-1}. The effect of DMSO on root growth was negligible in the experiment.

Measurement of chemical component of tobacco leaves

When tobacco leaves were mature, the 13th and 14th leaves from the top were harvested, baked and used for analysis of chemical components, including total sugar,

Table 2. Effect of different treatments on lateral roots of tobacco seedlings.

Treatment	Length of tap roots (cm)	Number of the first branch lateral roots (n plant^{-1})	Average length of the first branch lateral roots(cm)	Number of the second branch lateral roots (n plant^{-1})	Average length of the second branch roots (cm)
Control	47.6 ± 8.5* a	91 ± 11 a	35.1 ± 2.7 a	121 ± 23 c	8.6 ± 1.4 a
NPK	51.6 ± 4.9 a	98 ± 12 a	37.5 ± 3.8 a	152 ± 21 bc	8.4 ± 2.1 a
HA	55.7 ± 7.2 a	106 ± 11 a	32.2 ± 4.2 a	196 ± 25 ab	7.1 ± 1.2 a
HANPK	59.5 ± 8.3 a	105 ± 16 a	33.5 ± 5.1 a	229 ± 38 a	7.3 ± 0.9 a

Note: *Data indicated mean ± SE ($n = 4$). The data with different letters in the same column indicated significant differences at $p \leq 0.05$ (Duncan's test).

reducing sugar, nicotine, total N (nitrogen) and K (potassium). After digestion with H_2SO_4-H_2O_2, N was measured by the Kjeldahl nitrogen determination method, K was determined by a flame spectrophotometry method. Total sugar, reducing sugar and water soluble sugar were measured by a copper reduction–titration method. Nicotine was determined by a Uv-vis spectrophotometry method. Shmuck value means the ratio of water soluble sugar and total protein. For more details, please see Bao (2000) and Wang (2003).

Statistical analysis

The experiments were performed in a completely randomized design and each treatment had four replicates. The experiment was replicated twice independently. The data were analysed statistically by Microsoft Office Excel 2000 and by Duncan's New Multiple range test, $p < 0.05$. Significant difference was analysed by using SAS software (SAS 8.1).

RESULTS

Effect of HANPK on the growth of tobacco roots

Many previous reports indicated that HA from various materials and compost could promote plant growth (Adani *et al.*, 1998; Bidegain *et al.*, 2000; Daur and Bakhashwain, 2013; Mora *et al.*, 2010; Mora *et al.*, 2012). In this study, we examined the effect of HANPK on the growth of flue-cured tobacco roots in fields (Table 1). Results indicated that HANPK increased both root and shoot biomass of tobacco seedlings significantly. At 45 days after transplanting, shoot biomass with NPK, HA and HANPK treatment were 121.3%, 109% and 137.9% of that of the control. The corresponding values for root biomass were 117.9%, 106.3% and 129.7% respectively. However, HA, NPK or HANPK did not influence the ratio of root to shoot obviously.

To explore the mechanism of HANPK in enhancing tobacco growth, the number and average length of lateral roots were measured under different treatments. Results from Table 2 indicated that NPK, HA or HANPK did not influence the length of tap roots, and the number of the first-order lateral roots and their lengths significantly. Interestingly, HA or HANPK seemed to stimulate the growth of the second-order

Table 3. Effect of different treatments on root biomass under different soil layers.

Treatment	Root biomass in different soil layer (g plant^{-1})			
	0–20 cm	20–40 cm	40–60 cm	0–60 cm
Control	4.6 ± 0.6* b	1.2 ± 0.1 a	0.6 ± 0.1 a	6.4 ± 0.5 b
NPK	5.8 ± 0.7 b	1.1 ± 0.2 a	0.4 ± 0.1 a	7.3 ± 0.5 ab
HA	5.7 ± 0.6 b	0.9 ± 0.1 a	0.4 ± 0.1 a	7.1 ± 0.5 b
HANPK	6.9 ± 0.6 a	1.1 ± 0.3 a	0.5 ± 0.1 a	8.6 ± 0.7 a

Note: *Data indicated mean ± SE ($n = 4$). The data with different letters in the same column indicated significant differences at $p \leq 0.05$ (Duncan's test).

lateral roots. Compared with the control, the numbers of the second-order lateral roots under HA and HANPK were increased by 61.9% and 89.3% respectively. However, the average length of the second-order lateral roots did not vary greatly under different treatments (Table 2). Canellas *et al.* (2002) also reported that treatment with HA could produce a hyper-induction of maize lateral roots. The proliferation of lateral roots induced an increase in both total radicular length and root surface area, which would benefit the uptake of water and nutrients.

In tobacco fields, the distribution of root biomass was also investigated in different soil layers using a root-digging method. The total soil profile was divided into three layers, i.e. 0–20 cm, 20–40 cm and 40–60 cm. Results indicated that most of root biomass was localized in 0–20 cm soil layer (Table 3). Root biomass in 0–20 cm soil layer was 72.9% to 81.6% of that in the total soil profile among different treatments. Compared with the control, HANPK increased root biomass by 51.1% in 0–20 cm soil layer and 34.9% in 0–60 cm soil layer respectively. A single application of NPK or HA influenced the distribution of root biomass in total soil layer slightly.

In addition, we measured the effect of HANPK on root thickness of tobacco seedlings (Table 4). Results indicated that root biomass with the diameter less than 2 mm under HA and HANPK was 43.7% and 64.8% higher than that of the control. NPK treatment did not enhance root biomass with less than 2 mm diameter in comparison to the control. The root biomass with 2–5 mm root diameter showed a similar varying pattern to the root biomass less than 2 mm root thickness among different treatments. No significant differences of root biomass with the diameter bigger than 5 mm were observed among different treatments. The results suggested that treatment with HA or HANPK contributed to the growth of more fine roots in comparison to the control or NPK treatment.

Effect of different treatments on the activity of tobacco roots

Except root biomass, we also measured the activity of tobacco roots at different transplanting days. Results from Table 5 indicated that HANPK stimulated the activity of tobacco roots obviously at early growth stages. Root activity with HANPK treatment was 128.4% and 143.2% of the control at 15 and 30 days after transplanting respectively. While treatment with NPK or HA influenced the activity of tobacco roots slightly in comparison to the control. At later growth stages (45 days or 60 days after

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Table 4. Effect of different treatments on root thickness of tobacco seedlings.

Treatment	Root biomass with different root diameters (g plant^{-1})		
	<2 mm	2–5 mm	>5 mm
Control	1.42 ± 0.16* b	1.87 ± 0.52 b	3.05 ± 0.76 a
NPK	1.54 ± 0.38 b	1.94 ± 0.51 b	3.74 ± 0.41 a
HA	2.04 ± 0.42 a	2.35 ± 0.66 a	2.75 ± 0.38 a
HANPK	2.34 ± 0.35 a	2.84 ± 0.53 a	3.31 ± 0.43 a

Note: *Data indicated mean ± SE ($n = 4$). The data with different letters in the same column indicated significant differences at $p \leq 0.05$ (Duncan's test).

Table 5. Effect of different treatments on root activity of tobacco seedlings.

Treatment	Activity of tobacco roots (μg g^{-1} FW·h)			
	15 days	30 days	45 days	60 days
Control	7.4 ± 0.4* b	12.5 ± 1.1 b	13.6 ± 1.4 b	14.2 ± 1.6 a
NPK	8.4 ± 0.5 ab	14.1 ± 1.3 b	15.3 ± 1.1 ab	15.4 ± 1.3 a
HA	8.9 ± 0.6 a	15.7 ± 1.4 a	16.2 ± 2.1 a	16.5 ± 1.5 a
HANPK	9.5 ± 0.8 a	17.9 ± 1.3 a	17.6 ± 1.5 a	16.8 ± 1.7 a

Note: *Data indicated mean ± SE ($n = 4$). The data with different letters in the same column indicated significant differences at $p \leq 0.05$ (Duncan's test).
FW = fresh weight.

transplanting), no significant differences of root activity were observed among different treatments (Table 5).

Inhibitory effect of TIBA on lateral root growth

HANPK stimulated the growth of lateral roots of flue-cured tobacco seedlings significantly (Table 2). To explore whether auxin was involved in or not, the effect of TIBA, an inhibitor of polar auxin transport on lateral root growth was investigated hydroponically as shown in Table 6. Results showed that the length of the first-order lateral roots and the number of the second-order lateral roots with HANPK treatment was increased by 32.3% and 58.6% in comparison to the control. But the addition of 10 μmol L^{-1} of TIBA to HANPK solution inhibited HANPK stimulating effect obviously (Table 6). Average length of the first- and the second-order lateral roots and number of the second-order lateral roots were reduced by 32.5%, 33% and 48.2%, respectively. Interestingly, TIBA did not influence the length of tap roots. Since the function of TIBA mainly blocked polar auxin transport, it was suggested that the stimulating effect of HANPK on lateral roots might be associated with indole-3-acetic acid (IAA) synthesis or transport (Table 6).

Effect of different treatments on tobacco leaf chemical components

Leaf chemical components are the major indices of tobacco leaf quality, which include total sugar, reducing sugar, nicotine, total nitrogen, potassium, etc. When these components were coordinated with each other, the quality of flue-cured tobacco

Table 6. Effect of TIBA and HANPK on lateral root growth of tobacco seedlings.

Treatment	Length of tap roots (cm)	Number of the first branch of lateral roots (n plant^{-1})	Average length of the first branch of lateral roots (cm)	Number of the second branch of lateral roots (n plant^{-1})	Average length of the second branch of lateral roots (cm)
Control	5.5 ± 0.6* a	6.5 ± 0.7 a	3.5 ± 0.6 b	18.6 ± 1.5 b	0.5 ± 0.3 a
HANPK	6.6 ± 0.8 a	7.7 ± 0.9 a	4.7 ± 0.7 a	29.5 ± 1.8 a	0.6 ± 0.2 a
HANPK + TIBA	6.3 ± 0.9 a	5.9 ± 0.8 a	3.2 ± 0.5 b	15.1 ± 2.1 b	0.4 ± 0.2 b

Note: *Data indicated mean ± SE ($n = 4$). The data with different letters in the same column indicated significant differences at $p \leq 0.05$ (Duncan's test).

Table 7. Effect of different treatments on the content of chemical components of flue-cured tobacco leaves.

Treatment	Total sugar (%)	Reducing sugar (%)	Nicotine (%)	Nitrogen (%)	Potassium (%)
Control	19.91 ± 1.56* a	15.22 ± 1.48 b	2.17 ± 0.16 b	1.55 ± 0.08 b	2.05 ± 0.08 b
NPK	21.98 ± 1.25 a	17.56 ± 1.81 a	2.59 ± 0.38 a	1.79 ± 0.07 a	2.28 ± 0.11 ab
HA	21.56 ± 2.87 a	16.62 ± 1.37 a	2.35 ± 0.34 ab	1.62 ± 0.08 ab	2.12 ± 0.09 ab
HANPK	23.15 ± 2.52 a	19.51 ± 3.18 a	2.45 ± 0.25 ab	1.88 ± 0.11 ab	2.45 ± 0.23 a

Note: *Data indicated mean ± SE ($n = 4$). The data with different letters in the same column indicated significant differences at $p \leq 0.05$ (Duncan's test).

leaves tended to be harmonious (Wang, 2003; Xu *et al.*, 2008). Results from Table 7 indicated that different chemical components showed various varying patterns greatly. Total sugar and reducing sugar did not change greatly among different treatments although HANPK tended to increase their contents. HANPK treatment increased K uptake significantly. In comparison to the control, leaf K content with NPK, HA and HANPK treatment was elevated by 11.2%, 3.4% and 19.5%, respectively. NPK treatment increased nicotine content by 19.4%, while no differences of nicotine content were observed among HANPK, HA and the control. The results suggested that HA combined NPK compound fertilizer contributed to the elevation of the quality of tobacco leaves. Wang (2003) indicated that the quality of tobacco leaves increased with increasing sugar content. Low N content of tobacco leaves was better than that of high N content. The content of nicotine ranging from 1.5 to 3.5 was suitable for tobacco leaf quality. The data of Table 6 were within the scope of the above values.

Effect of different treatments on the main quality index ratio of flue-cured tobacco leaves

We also examined the main quality indices including shmuck value (water soluble sugar/total protein), nitrogen/nicotine, reducing sugar/nicotine and reducing sugar/total nitrogen as shown in Table 8. Shmuck value was not different among different treatments. Many reports indicated that it is suitable for highly qualified tobacco leaves when shmuck value approaches 3, and the ratio of reducing sugar to nicotine and the ratio of reducing sugar to nitrogen range from 6 to 10 (Wang, 2003; Xu *et al.*, 2008). In this study, the above two indices ranged from 6.8 to 10.4, which were within the above scope. Noteworthy, HANPK increased the ratio of reducing

Table 8. Effect of different treatments on main quality indices of flue-cured tobacco leaves.

Treatment	Shmuck value	Nitrogen/nicotine	Reducing sugar/nicotine	Reducing sugar/Nitrogen
Control	2.81 ± 0.32* a	0.71 ± 0.08 a	7.01 ± 0.56 b	9.82 ± 0.78 a
NPK	2.75 ± 0.09 a	0.69 ± 0.07 a	6.78 ± 0.64 b	9.81 ± 0.65 a
HA	2.87 ± 0.11 a	0.69 ± 0.05 a	7.07 ± 0.35 b	10.26 ± 0.56 a
HANPK	2.85 ± 0.12 a	0.77 ± 0.09 a	7.96 ± 0.57 a	10.37 ± 0.97 a

Note: *Data indicated mean ± SE ($n = 4$). The data with different letters in the same column indicated significant differences at $p \leq 0.05$ (Duncan's test).

Table 9. Effect of different treatments on main economic indices of flue-cured tobacco.

Treatment	Yield (kg ha^{-1})	Proportion of superior and medium tobacco (%)	Average price (USD kg^{-1})	Output value (USD ha^{-1})
Control	2655.8* b	85.1 b	3.36 b	8921.8 c
NPK	2812.5 ab	84.6 b	3.39 b	9536.1 b
HA	2741.2 b	89.5 ab	3.67 a	10065.3 b
HANPK	2981.6 a	93.7 a	3.88 a	11553.8 a

Note: *Data indicated mean of four results. The data with different letters in the same column indicated significant differences at $p \leq 0.05$ (Duncan's test).

Table 10. Costs, benefits and output input ratio of tobacco leaves under different treatments.

Treatment	Input (USD ha^{-1})	Output (USD ha^{-1})	Benefit (USD ha^{-1})	Output input ratio
Control	3390.6* c	8921.8 d	5531.2 d	2.63 c
NPK	3554.7 b	9536.1 c	5981.4 c	2.68 c
HA	3552.3 b	10065.3 b	6513 b	2.83 b
HANPK	3716.4 a	11553.8 a	7837.4 a	3.11 a

Note: *Data indicated mean of four results. The data with different letters in the same column indicated significant differences at $p \leq 0.05$ (Duncan's test).

sugar to nicotine by 13.6%. However, shmuck value and nitrogen/nicotine did not vary significantly (Table 8).

Effect of different treatments on the economic characteristic indices of flue-cured tobacco leaves

Tobacco yield, average price, output value and proportion of superior and medium leaves of flue-cured tobacco were the main economic characteristic indices, which reflected the quality and economic effect of tobacco leaves (Wang, 2003). Results from Table 9 showed that HANPK significantly increased tobacco yield, proportion of superior and medium tobacco leaves, and output value in comparison to the control. The corresponding values were increased by 12.2%, 10.1% and 29.4%, respectively. The results suggested that HANPK treatment enhanced leaf yield and quality of flue-cured tobacco plants (Tables 8 and 9).

Cost, benefits and output input ratio of tobacco leaves were also calculated under different treatments as shown in Table 10. Among four treatments, the input, output and benefit of HANPK were the highest, while those of the control were the lowest.

NPK and HA treatment had similar inputs, but the output, benefit and output input ratio were different. HA treatment had a higher output, benefit and output input ratio than NPK treatment. The results suggested that the treatment of HA was better than NPK treatment. Results from Table 10 suggested that HANPK application was a great benefit to the production of tobacco leaves among these four treatments (Table 10).

DISCUSSION

Many studies have shown that HA could not only enhance root, leaf and shoot growth, but also stimulate the seed germination of various crop species (Adani *et al.*, 1998; Bama and Selvakumari, 2009; Bidegain *et al.*, 2000; Canellas *et al.*, 2002; Daur and Bakhashwain 2013; Mora *et al.*, 2010; Mora *et al.*, 2012; Tahir *et al.*, 2011). These positive effects are explained by the direct and indirect interaction of HA and roots at different physiological and metabolic processes. According to the previous results reported in research literatures (Fagbenro and Agboola, 1999; Jindo *et al.*, 2012), the positive effect of HA was first observed on plant growth such as the length or biomass of roots and shoots. Our results from Table 1 showed that the combined application of HANPK stimulated the growth of both roots and shoots of flue-cured tobacco plants significantly (Tables 1 and 2). Furthermore, HANPK increased K uptake by 19.5% (Table 7). The results suggested that the combined use of HA and NPK chemical fertilizer not only stimulated tobacco growth, but also enhanced the quality of flue-cured tobacco. In consistent with our results, Zhang *et al.* (2013) performed an experiment to examine the effect of combination of chemical compound fertilizer and HA on yield and quality of apple. They found that the addition of HA to chemical compound fertilizers increased apple yield by 35%. In rice, a similar result was also observed that the application of HA combined with chemical fertilizer produced pronounced effects on root volume, root CEC and root length (Bama and Selvakumari, 2009). Cimrin and Yilmaz (2005) indicated that a single application of HA to lettuce did not improve its yield, while the combined application of 120 kg ha^{-1} of phosphorus together with 300 kg ha^{-1} of HA increased the head weight of lettuce very much. The combination of HA and NPK chemical fertilizer might improve the availability of rhizosphere nutrients, and enhance the activities of microorganisms and enzymes, which thus stimulate plant growth (Du *et al.*, 2013; Nardi *et al.*, 2002).

Turgay *et al.* (2011) indicated that the application of humic-fulvic substances alone showed different inclinations on selected soil characteristics in consecutive cropping seasons. Application of HA did not show significant effects on plant growth when soil organic carbon was high enough (Bernal *et al.*, 2009). Our results from Tables 1 and 9 supported the above view that a single application of HA did not influence tobacco growth and leaf yield significantly. This phenomenon might be associated with high content of active organic carbon in paddy soils. Since tobacco seedlings were planted in paddy soils in the system of tobacco–rice rotation, due to continuous straw returning, the contents of dissolved organic carbon and active organic carbon were high, which might retard the stimulating the effect of a single application of HA.

According to Pinton *et al.* (1999) and Schmidt *et al.* (2007), the beneficial effects of HA on plant nutrition have been attributed to the promotion of root development. Our results agreed with this observation that the combination of HANPK increased both dry weight and root numbers of tobacco plants significantly (Tables 1 and 2). Furthermore, HANPK increased the emergence of the second-order lateral roots greatly. The number of the second-order lateral roots was 189.3% of that of the control (Table 2). Similar results were also observed by other authors (Canellas *et al.*, 2002; Hartwigsen and Evans, 2000). In *Arabidopsis*, HA altered developmental programmes at an early stage of root cell differentiation, which resulted in an increased root absorptive surface area (Schmidt *et al.*, 2007). Modification of root structure might underline the beneficial effects of HANPK on early stages of tobacco growth. Unlu *et al.* (2010) found that foliar and soil HA fertilization could increase the accumulation of antioxidant compounds such as carotenoids and phenolic compounds in pepper fruit. Increased root activity might be associated with an increase of antioxidant enzyme activity in tobacco roots at under HANPK treatment (Table 5).

HA molecules have been reported to produce similar effects to IAA on root growth (Canellas *et al.*, 2002; Schmidt *et al.*, 2007; Trevisan *et al.*, 2010; Zandonadi *et al.*, 2007). Interaction between plant hormones and humic substances might contribute to the effects of humic molecules on plant growth and development. Inhibition of IAA synthesis and transport would result in a negative effect on plant growth. Our hydroponic experiment supported the above hypothesis that the addition of TIBA, a polar auxin transport inhibitor, resulted in decreases of the number and average length of lateral roots (Table 6). The beneficial effects of HANPK on plant development suggest a possible involvement of auxin synthesis and transport. The phytohormone auxin was required to regulate the initiation and emergence phases of lateral root development (Casimiro *et al.*, 2001). Jindo *et al.* (2012) found that HA of different origins could increase the number of lateral roots by 22 to 111% in maize. HA from composted materials contain more carboxylic groups and more hydrophobic character, and thus produce morphological and biochemical effects on plant growth. In this study, HA from composted materials might exert a significant enhancing effect on lateral roots, and thus the number of lateral roots was increased by 58.6% in a hydroponic experiment (Table 6).

CONCLUSION

In conclusion, within the experimental conditions studied, we show that HANPK chemical fertilizer exhibits positive effects on tobacco growth and leaf quality. These results suggest that the combined use of HANPK fertilizer may generate various scientific and economic advantages.

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