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Full Length Article

Strong Acidic Conditions Impaired Photosynthesis, Root System and Seedling Growth of Flue-Cured Tobacco

Jin Chen[1,2†], Hanhui Deng[1†], Yuanhuan Li[1], Lili Yang[1], Yongsheng Deng[1], Xiaohua Deng[1,3*], Zhicheng Zhou[3], Shuguang Peng[3], Yongjun Liu[3], Minghui Tian[4] and Zhifeng Chen[5]

[1‡]*College of Agronomy, Hunan Agricultural University, Changsha 410128, P.R. China*
[2‡]*Ningxaing Branch, Changsha Tobacco Company, Hunan, Ningxiang 410600, P.R. China*
[3]*Agricultural Laboratory Institute in Central South of China Tobacco, Changsha 410128, P.R. China*
[4]*Xiang Xi Autonomous Prefecture Branch of Hunan Tobacco Industrial Co., Ltd., Jishou 416000, P.R. China*
[5]*Changsha Tobacco Company of Hunan,Changsha 410128, P.R. China*
[*]For Correspondence: yzdxh@163.com
[†]Contributed equally to this work and are co-first authors
[‡]Jointly contributed to this paper
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Abstract

Soil pH is closely related to soil microbial activity, conversion and release of crop nutrient elements, and crop growth. In this study, tobacco was grown in solution culture at varying solution pH levels (solution pHs 4, 5, 6, 7 and 8). Data regarding tobacco growth, dry matter accumulation, activities of membrane-lipid peroxidation and protective enzymes, chlorophyll contents and photosynthetic rate were recorded at 10, 17, 24, 31, 38 and 45 days after transplanting tobacco seedlings. Results showed that growth and physicochemical characteristics of tobacco seedlings were different under varying solutions pH. With increasing solution pH, tobacco root length, volume, surface area, tip number and vitality, the ratio of root dry weight, and malondialdehyde contents increased, whereas the ratio of stem dry weight and superoxide dismutase activity was decreased. Tobacco seedling growth was strongly impaired at pH 4 while solution pH 6–7 seemed superior with respect to tobacco growth. At pH 6, the chlorophyll and photosynthesis rate of tobacco leaves reached at the highest values while pH 8 was beneficial for roots growth. In crux, tobacco growth was inhibited under strong acid and alkaline conditions due to over generation of reactive oxygen species, decreased activity of protective enzymes along with cell membrane damage leading to substantial decline in photosynthesis. Therefore, soils having pH in the range of 6–7 seemed more productive for flue-cured tobacco growth.

Keywords: Tobacco; Solution pH; Root growth; Physiological characteristics; Biochemical characteristics

Introduction

The soil pH relates closely to microorganism activity (Song *et al.* 2019), conversion and release of soil nutrient elements (Schwamberger and Sims 1991), and effective micro-element in the soil (Deng *et al.* 2017). Tobacco (*Nicotiana tabacum* L.) could normally grow in the soil of pH 4.5~8.5 (Cao 1991); yet different pH is bound to affect its growth. The volume, dry weight and vitality of tobacco root increased with ascending pH in hydroponic culture in pH 4.5~8.5; nevertheless, declining to the lowest at pH 8.5 (Xu *et al.* 2004). In another study, tobacco growth varied as the reversed parabolic curve, and dry matter accumulation of plant reached to the top at pH 6.5 (Zhu *et al.* 2012). The soil pH was adjusted from 5.0 to pH 5.5~6.6 by putting calcium oxide on soil, which greatly facilitated the root growth of tobacco (Tang and Xiong 2002). In root extension period, tobacco grows more prosperously under weak acid conditions comparing with medium and weak alkaline ones, which was unveiled by adjusting to pH environment using sand cultivation (Ren *et al.* 1995). Different tobacco varieties have different demands of pH for optimum root growth (Zhou *et al.* 2000). Slightly acid condition (pH 5.5~6.5) was beneficial to leaf dry matter accumulation in the stage of the root extension period, and comparatively higher pH (7.0~8.0) was more beneficial to leaf dry matter accumulation in the middle and late stage of flue-cured tobacco (Han *et al.* 1992). Another research demonstrated that soil pH affects photosynthetic features of tobacco leaves and inner activity of the protective enzymes as well (Wang *et al.* 2005).

In recent years, research institutions and agricultural

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experts have paid attention to the issues relating tobacco growing in strong acidic and alkaline conditions due to soil acidification and alkalization phenomenon in some tobacco-growing area. However, the focus of available research was to see the effects of soil pH on tobacco growth and no inclusive studies were done to unveil the mechanisms behind this growth inhibition. Therefore, this study was designed to evaluate the effects of solution pHs on root morphology, above-ground growth, dry matter accumulation and allocation, photosynthesis and anti-oxidants metabolism. The findings of this study will help to explore the mechanisms of tobacco growth reduction at strong acidic and alkaline conditions, and optimization of soil pH conducive for high-quality tobacco production.

Materials and Methods

Experimental material

The tobacco variety (*Nicotiana tabacum* L.) used in the experiment was K326, which was first planted on the holed foam board with nutrient solution under it. The nutrient solution adopted improved Hoagland formula: calcium nitrate tetrahydrate 945 mg/L, potassium nitrate 506 mg/L, ammonium nitrate 80 mg/L, potassium phosphate monobasic 136 mg/L, magnesium sulfate 493 mg/L, iron salt solution 2.5 mL, trace element solution 5 mL. Tobacco seedlings at four leaves stage were transplanted to another bigger foam board.

Experimental treatments

Tobacco was grown in hydroponic solution culture at varying solution pH levels *i.e.*, solution pHs 4, 5, 6, 7 and 8. There are 30 plants used for each treatment. Experiment was laid out following completely randomized design (CRD) with three replications. The tobacco seedlings after one-week pre-nurture, almost in the same shape, were transplanted in rectangle opaque plastic box fixed by the orderly holed foam board, whose roots were put in the nutrient solution and supplied with oxygen by a pump every day. Nutrient solution was renewed every week according to the set pH and measured by IQ 150 pH Meter produced by Spectrum Corp and adjusted to the targeted value by utilizing diluent HCl or NaOH solution.

Testing program and methods

Three samples of tobacco seedlings were taken in each treatment, first after 10 days, and then every 7 days at 17, 24, 31, 38 and 45 days after transplanting. Root indexes including length, volume, diameter, surface area, and tip number of roots were measured by the root-analyzing system of LA-2400 multi-parameter. The root vitality was measured by TTC (2, 3, 5-triphenyte-trazoliumchloride) (Li 2000). The indexes of above-ground growth and dry matter including plant height, stem thickness, leaf number, maximum leaf length and width (maximum leaf area = length × width × 0.6345) were measured according to Investigation and Measurement Method of Tobacco Agronomic Characteristics (YC/T14-2010). The physiological and biochemical indexes and photosynthetic indexes in leaves were taken from fully expanded sec-sequenced leaf. To be specific, MDA (malondialdehyde) content (Cakmak and Marschner 1992), indicated as μmol/g, FW (fresh weight), was measured by Thiobarbituric acid method and SOD (superoxide dismutase) activity was measured by Pyrogallol auto-oxidation method (Wei 2000). The SPAD-502plus portable chlorophyll tester (Japan Konica Minolta Co., Ltd.) was used to note chlorophyll SPAD value. The net photosynthetic rate of tobacco leaves was recorded by LI-6400XT Portable Photosynthesis Meter at 9:00~11:00 a.m. on sunny days (Wei 2000).

Statistical analysis

Collected data were analyzed by Microsoft Excel 2003 and SPSS 17.0 following one-way ANOVA for each sampling date to check overall significance of data. Moreover, treatments means were separated using Duncan's Multiple Range Test at 5% probability level (Steel *et al.* 1997).

Results

Root traits

Different solution pHs had significant effect on roots traits (root length, root volume, root surface area, root diameter, root tip number and root vitality) of tobacco grown in hydroponic conditions at all sampling dates *i.e.*, 10, 17, 24, 31, 38 and 45 days after transplanting (DAT) (Table 1; Fig. 1). Strong acidic conditions (solution pH 4) resulted in substantial reduction in entire roots traits (root length, root volume, root surface area, root diameter, root tip number and root vitality) at all sampling dates *i.e.*, 10, 17, 24, 31, 38 and 45 DAT with the only exception of non-significant effect of solution pHs on root vitality at 10 DAT (Table 1; Fig. 1). Nonetheless, neutral to slightly alkaline conditions, solution pH 8 in particular, improved tobacco root length, root volume, root surface area, root diameter, root tip number and root vitality recorded at 10, 17, 24, 31, 38 and 45 DAT (Table 1; Fig. 1).

Above-ground growth of tobacco seedlings

Different solution pHs had significant effect on plant height, stem thickness and maximum leaf area of tobacco grown in hydroponic conditions at all sampling dates *i.e.*, 10, 17, 24, 31, 38 and 45 DAT with the exception of non-significant effect on plant height at 10 DAT and on stem thickness at 10 and 17 DAT. However, solution pH had non-significant effect on tobacco leaf number at all sampling dates (Table 2).

Table 1: Comparison of tobacco root traits under different solution pH

Days	Medium	Root length (cm)	Root volume (cm^3)	Root surface area (cm^2)	Root diameter (mm)	Roots tip number
10	pH 4	170 ± 36.9b	1.27 ± 0.34b	65.9 ± 14.2a	0.81 ± 0.14a	636 ± 85.3b
	pH 5	195 ± 34.0a	1.40 ± 0.13a	63.2 ± 6.74a	0.93 ± 0.11a	826 ± 15.0a
	pH 6	203 ± 29.1a	1.54 ± 0.44a	60.7 ± 24.9a	0.85 ± 0.22a	898 ± 64.5a
	pH 7	199 ± 21.1a	1.56 ± 0.89a	75.9 ± 21.8a	0.84 ± 0.13a	889 ± 78.8a
	pH 8	188 ± 30.0 a	1.53 ± 0.66a	62.0 ± 16.4a	0.96 ± 0.11a	923 ± 67.9a
17	pH 4	206 ± 50.9c	2.29 ± 0.61c	75.2 ± 19.5c	1.02 ± 0.05b	686 ± 84.0c
	pH 5	243 ± 50.5b	2.32 ± 0.73c	87.8 ± 27.8b	1.16 ± 0.16a	879 ± 26.6b
	pH 6	256 ± 27.2 b	2.53 ± 0.47b	93.9 ± 20.8a	1.22 ± 0.12a	961 ± 79.3a
	pH 7	309 ± 70.0 a	2.78 ± 0.99ab	98.8 ± 13.8a	1.24 ± 0.10a	969 ± 87.6a
	pH 8	297 ± 64.4 a	3.36 ± 0.65a	101 ± 11.7a	1.23 ± 0.29a	1035 ± 91.5a
24	pH 4	226 ± 65.6d	2.36 ± 0.85d	85.8 ± 23.0c	1.05 ± 0.06c	746 ± 79.3c
	pH 5	297 ± 44.4c	2.85 ± 0.24cd	98.6 ± 19.5c	1.26 ± 0.19b	936 ± 31.8b
	pH 6	301 ± 15.9b	3.06 ± 0.12c	103 ± 16.0b	1.48 ± 0.33a	1014 ± 75.6b
	pH 7	412 ± 87.1a	3.77 ± 0.29b	107 ± 17.7b	1.41 ± 0.17a	1115 ± 99.7b
	pH 8	421 ± 78.1a	4.17 ± 0.66a	114 ± 27.1a	1.49 ± 0.40a	1319 ± 94.4a
31	pH 4	322 ± 33.6d	2.66 ± 0.46c	92.7 ± 17.9c	1.12 ± 0.12c	784 ± 81.4c
	pH 5	362 ± 69.9c	3.06 ± 0.36b	109 ± 7.55c	1.49 ± 0.24b	970 ± 86.3b
	pH 6	416 ± 39.7b	3.16 ± 0.61b	114 ± 6.46b	1.75 ± 0.05a	1056 ± 42.6b
	pH 7	457 ± 24.5ab	3.13 ± 0.03b	116 ± 21.8b	1.60 ± 0.48a	1162 ± 56.8b
	pH 8	499 ± 62.1a	4.70 ± 0.94a	121 ± 15.3a	1.70 ± 0.75a	1443 ± 75.3a
38	pH 4	365 ± 97.5c	2.95 ± 0.22c	103 ± 18.8c	1.21 ± 0.07c	900 ± 106c
	pH 5	460 ± 11.8b	3.36 ± 0.60b	123 ± 6.89b	1.69 ± 0.28b	1081 ± 89.7b
	pH 6	484 ± 43.6b	3.38 ± 0.87b	137 ± 18.0b	1.57 ± 0.14b	1459 ± 107a
	pH 7	559 ± 24.0ab	3.67 ± 0.92b	138 ± 23.1b	1.72 ± 0.00ab	153 ± 46.1a
	pH 8	592 ± 55.8a	5.02 ± 0.78a	158 ± 29.1a	1.85 ± 0.10a	1602 ± 73.1a
45	pH 4	385 ± 59.0c	3.17 ± 0.87d	111 ± 13.7c	1.39 ± 0.13c	931 ± 73.5c
	pH 5	520 ± 39.0 b	3.94 ± 0.23c	152 ± 11.1b	1.44 ± 0.18c	1174 ± 42.1b
	pH 6	582 ± 83.9ab	4.60 ± 0.94b	157 ± 21.5b	1.68 ± 0.08b	1540 ± 44.7a
	pH 7	607 ± 82.5a	5.36 ± 0.58a	161 ± 14.4b	1.65 ± 0.21b	1611 ± 93.6a
	pH 8	616 ± 33.2a	5.34 ± 0.53a	182 ± 28.9a	2.00 ± 0.36a	1644 ± 91.7a

Means ± standard deviation with different letters, in the same group, differ significantly from each other at $P \leq 0.5$ according to Duncan's Multiple Range Test

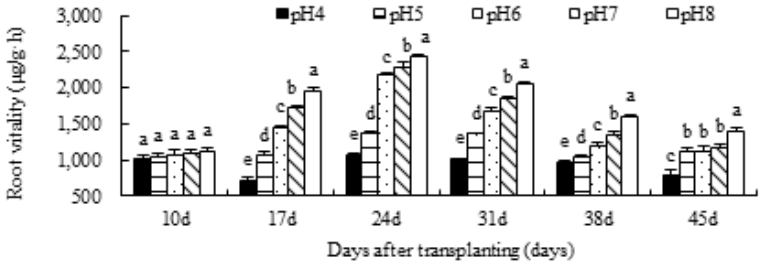

Fig. 1: Effect of solution pH on tobacco root vitality
Bar charts represent means ± standard deviation and means with different letters differ significantly from each other at 5% probability level

Tobacco grown in strong acidic conditions (solution pH 4) and alkaline conditions (pH 8) observed significant decrease in plant height, stem thickness and leaf area; however, tobacco grown under slight acidic to neutral conditions (pH 6~7) recorded more plant height, stem thickness and leaf area at all sampling dates (Table 2).

Belowground and aboveground dry weight of tobacco seedlings

Different solution pHs had significant effect on belowground root dry weight and its ratio, and dry weight of above-ground stem and leaf and its ratio in hydroponic conditions at all sampling dates *i.e.*, 10, 17, 24, 31, 38 and 45 DATS. Slight acidic to neutral conditions (pH 6~7) observed the greatest belowground root dry weight and dry weight of above-ground stem and leaf, which dropped to the least grown in strong acidic conditions (pH 4) at all sampling dates (Fig. 2–3). There was an obvious increase in the ratio of root to whole plant, and a substantial reduction in the ratio of stem and leaf to whole plant with increasing solution pH at all sampling dates (Fig. 2–3).

Membrane-lipid peroxidation and protective enzyme activities of tobacco leaves

Different solution pHs had significant effect on MDA and SOD of tobacco seedlings grown in hydroponic conditions at all sampling dates *i.e.*, 10, 17, 24, 31, 38 and 45 days after transplanting (DAT). Acidic to alkaline (pH 5~8) conditions

Table 2: Comparison of above ground parts tobacco growth under different solution pH

Days	Medium	Plant height (cm)	Stem thickness (cm)	Leaf number	Maximum leaf area (cm^2)
10	pH 4	13.0 ± 1.42	2.00 ± 0.08	4.50 ± 0.58	125 ± 13.7b
	pH 5	11.7 ± 1.24	2.13 ± 0.19	5.00 ± 0.00	123 ± 8.60b
	pH 6	12.4 ± 1.64	1.93 ± 0.17	4.25 ± 0.50	150 ± 28.1a
	pH 7	13.0 ± 4.72	2.18 ± 0.36	4.25 ± 0.96	146 ± 30.7a
	pH 8	11.8 ± 1.67	1.83 ± 0.21	4.25 ± 0.50	122 ± 15.6b
17	pH 4	13.4 ± 2.17b	2.15b ± 0.21	5.75 ± 0.50	164 ± 24.7c
	pH 5	17.3 ± 2.71a	2.55a ± 0.30	6.50 ± 0.58	179 ± 35.7b
	pH 6	17.3 ± 1.55a	2.60a ± 0.29	7.00 ± 0.00	181 ± 39.4b
	pH 7	17.0 ± 2.92a	2.68a ± 0.22	7.00 ± 0.00	207 ± 22.8a
	pH 8	13.5 ± 1.59b	2.53a ± 0.21	6.75 ± 1.50	157 ± 36.7c
24	pH 4	14.8 ± 0.61c	2.23 ± 0.13c	7.50 ± 0.58	178 ± 20.9c
	pH 5	17.6 ± 1.33b	2.80 ± 0.24b	7.50 ± 0.58	193 ± 14.0b
	pH 6	18.6 ± 1.22b	2.95 ± 0.17ab	7.75 ± 0.50	224 ± 35.9a
	pH 7	21.9 ± 1.88a	3.03 ± 0.17a	7.25 ± 0.50	208 ± 34.4ab
	pH 8	18.1 ± 1.44b	2.85 ± 0.06b	7.00 ± 0.00	178 ± 40.8c
31	pH 4	15.6 ± 1.03c	2.83 ± 0.17b	8.75 ± 0.96	165 ± 20.9b
	pH 5	18.6 ± 2.58b	2.83 ± 0.17b	9.75 ± 0.50	208 ± 17.1a
	pH 6	20.8 ± 0.89b	2.98 ± 0.17ab	9.75 ± 0.50	232 ± 27.4a
	pH 7	24.1 ± 1.19a	3.15 ± 0.13a	10.0 ± 0.00	223 ± 39.7a
	pH 8	21.4 ± 2.89b	2.85 ± 0.30b	9.25 ± 0.50	210 ± 12.7a
38	pH 4	15.8 ± 3.51c	2.90 ± 0.18b	9.25 ± 1.50	185 ± 42.6d
	pH 5	20.3 ± 1.45b	2.95 ± 0.24b	10.8 ± 0.50	210 ± 47.3c
	pH 6	23.0 ± 2.09b	3.03 ± 0.15b	10.8 ± 0.96	254 ± 8.16a
	pH 7	25.6 ± 3.78a	3.25 ± 0.13a	10.0 ± 0.82	226 ± 15.0b
	pH 8	21.7 ± 3.07b	2.95 ± 0.26b	10.3 ± 0.50	224 ± 28.4b
45	pH 4	21.2 ± 5.10c	2.90 ± 0.26b	10.0 ± 0.82	195 ± 21.6d
	pH 5	25.0 ± 3.21b	3.00 ± 0.33b	11.0 ± 0.00	221 ± 28.2c
	pH 6	27.7 ± 1.68ab	3.20 ± 0.08a	11.3 ± 0.50	270 ± 32.8a
	pH 7	29.9 ± 2.58a	3.33 ± 0.35a	10.5 ± 0.58	248 ± 20.7b
	pH 8	22.0 ± 1.54c	2.98 ± 0.10b	10.5 ± 1.00	233 ± 46.6c

Means ± standard deviation with different letters, in the same group, differ significantly from each other at $P \leq 0.5$ according to Duncan's Multiple Range Test

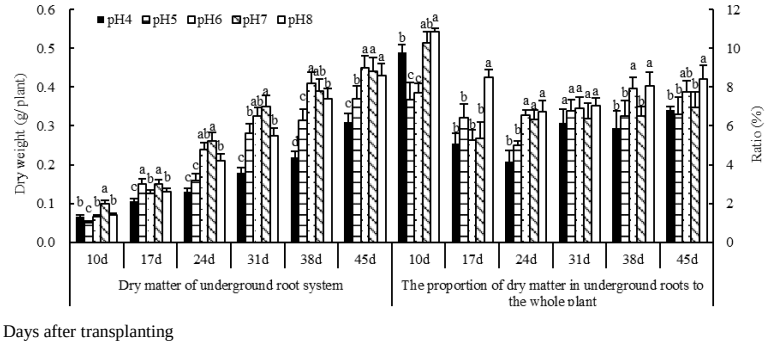

Fig. 2: Effect of solution pH on root dry weight and its ratio of tobacco
Bar charts represent means ± standard deviation and means with different letters differ significantly from each other at 5% probability level

observed an increase in MDA of tobacco seedlings, and a decrease in SOD at all sampling dates with rising pH (Fig. 4). Moreover, solution pH 4 and pH 8 had more significant effects on MDA and SOD contents than other solutions (Fig. 4). It was shown that a strong acid condition did more harm to cell membrane than the alkaline in the preliminary stage of tobacco transplant, and vice versa in the late stage.

Chlorophyll content and photosynthetic rate of tobacco leaves

Different solution pHs had significant effects on SPAD values (proportional to chlorophyll content) and photosynthetic rate of tobacco leaves in hydroponic conditions at all sampling dates *i.e.*, 10, 17, 24, 31, 38 and 45 DATS. SPAD values and photosynthetic rate of tobacco leaves were maximum at pH 6 compared with strong acidic (pH 4) and alkaline conditions (pH 8) at all sampling dates (Fig. 5). SPAD values and photosynthetic rate were lowest at pH 8 recorded at 10, 17 and 24 DAT and at pH 4 recorded at 31, 38, and 45 DAT (Fig. 5).

Discussion

Results of this hydroponic study unveiled that slightly acidic to neutral conditions (pH 6~7) improved root growth and germination, dry weight accumulation, and root activity of

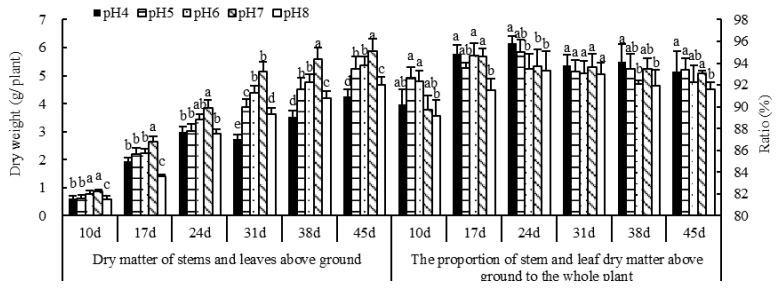

Fig. 3: Effect of solution pH on stem dry weight and stem and leaf dry weight ratio of tobacco
Bar charts represent means ± standard deviation and means with different letters differ significantly from each other at 5% probability level

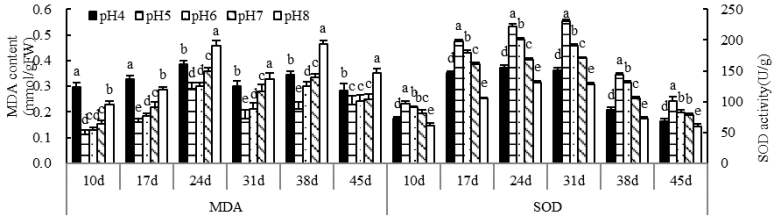

Fig. 4: Effect of solution pH on MDA content and superoxide dismutase (SOD) activity of tobacco leaf
Bar charts represent means ± standard deviation and means with different letters differ significantly from each other at 5% probability level

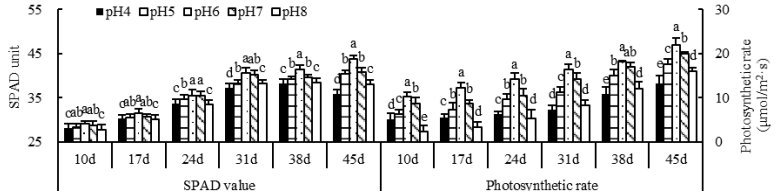

Fig. 5: Effect of solution pH on chlorophyll SPAD value and photosynthetic rate of tobacco leaf
Bar charts represent means ± standard deviation and means with different letters differ significantly from each other at 5% probability level

tobacco seedlings. However strong acidic conditions curbed root growth, and strong acid and alkaline conditions impaired aboveground growth of tobacco (Tables 1, 2). In another hydronic study as nutrient solution changed from pH 4.5 to 8.5, tobacco growth observed a reversed parabolic curve, and maximum dry matter accumulation was recorded at pH 6.5 (Zhu *et al.* 2012).

Decrease in tobacco growth under strong acidic conditions might be linked with decreased root activity and growth. This reduced root vitality and growth might be resulted in poor nutrient and water uptake leading to impaired above-ground growth and metabolism (Tong 2005). Besides, the destroyed internal mechanism of tobacco in strong acidic conditions, and the comparative higher speed of nutrient consumption than merging, gives rise to the low accumulation of above-ground dry weight (Li *et al.* 2009). The negative effects on tobacco growth and germination under alkaline conditions might be due to reduced absorption of early nitrogen and the contribution of nutrient elements for tobacco growth (He and Yuan 2014). Moreover, both pH extremes (pH 4 and 8) elevated oxidative stress (as was evident from higher values of MDA contents) and damaged cell membrane (Fig. 4). Elevated membrane damage coupled with decreased chlorophyll contents resulted in lower photosynthesis and ultimately less accumulation of biomass.

It is also evident from the research results that strong acidic (pH 4) and alkaline (pH 8) environment increased the MDA contents (oxidative damage) on one hand while also decreased the activity of protective enzymes like superoxide dismutase (SOD) on the other hand. Therefore, this two-way damage accelerated the membranes damage and ultimately reduced the carbon fixation potential of tobacco seedlings. Despite the insignificant influence of environmental pH on inside plant cell pH (Raven and Smith 1980), solution pH directly or indirectly affected the properties and state of cell membrane, intracellular matter transport, metabolism, and uptake of mineral elements by plant roots. If resistance or defense limits of plants are surpassed, the merging of membrane protective enzyme activity and protective content would be curbed. As a result, the process of membrane lipid peroxide was intensified leading to more cell membrane damaged (Wolfe 1991). Hence these differences of root vitality, membrane-lipid peroxidation, protective enzyme activity, chlorophyll content and photosynthetic rate of tobacco leaves under different pH environment were the key

reasons to influence tobacco growth, and the accumulation and allocation of dry matter.

Conclusion

Solution pH has significant impacts on growth and germination of flue-cured tobacco and physiological and biochemical characteristics of its leaf. Strong acid and alkaline conditions impaired tobacco growth due to over generation of reactive oxygen species, decreased activity of protective enzymes along with cell membrane damage leading to substantial decline in photosynthesis. Therefore, soils having pH in the range of 6–7 seemed more suitable for flue-cured tobacco growth.

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