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Acid Soil Is Associated with Reduced Yield, Root Growth and Nutrient Uptake in Black Pepper (*Piper nigrum* L.)

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Abstract

Low pH is a major limiting factor for the production of black pepper (*Piper nigrum* L.) in Hainan province. Black pepper gardens often exhibit a decrease in soil pH (to 5.5 - 5.0) on orchards with a multi-year production history. An exploratory hydroponic experiment was conducted to examine the effects of increasingly acid nutrient solution pH (7.0, 5.5, 4.0, and 3.5) on seedling growth, tissue nutrient concentrations and root morphological traits. The results indicated that low pH may directly inhibit root development and function, limit K, Ca and Mg absorption and reduce seedling growth. At pH 5.5, black pepper attained maximum growth, while the minimum growth occurred at pH 3.5. It can be concluded that low pH reduces plant growth and is associated with low root nutrient concentrations of Ca and Mg, which may explain the decline of the yield in the seven pepper gardens of the Institute.

Keywords

Nutrient Concentration, Pepper Seedling Growth, pH, Root Morphology, Soil Acidification

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1. Introduction

Acid soils occupy approximately 30% of the world's land area and restrain global agricultural production [1]. Soil acidification is a growing problem in soils of Chinese agricultural systems in the tropical region [2]. Numerous factors can result in soil acidification, such as large inputs of inorganic fertilizers, high rainfall, acid deposition and greenhouse gas. As the concentration of H^+ in the soil increases, it can inhibit root growth [3], disrupt the functions of the plasma membrane [4], cell wall [5], or by increasing Al^{3+} toxic levels [6]. Deficient levels of calcium (Ca), magnesium (Mg) and phosphorus (P) are also frequent under low pH conditions.

Black pepper (*Piper nigrum* L.) is one of the important cash crops in the tropical agricultural regions of China. Hainan province, a major producer and exporter of black pepper in China, has 22,000 ha in cultivation and produces 36,000 Mg of pepper berry annually, comprising 90% of the pepper production in China. Production of pepper provides income to approximately 1 million rural growers and has become an important tropical crop industry with output value of more than 1 billion Chinese yuan.

The suitable soil pH for pepper growth is 5.5 - 7.0 [7]. However, soil pH below 5.5 occupies approximately 50% of the typical pepper gardens in Hainan province [7]. Soil pH values less than 5.5 suggest the possible presence ot toxic levels of soil aluminum. Long term continuous planting of pepper may result in significant soil acidification [8]. Many pepper gardens with low soil pH have produced pepper for more than 30 years [7] [9]. In these gardens, pepper often showed poor growth, serious plant diseases and insect pests, nutrient deficiency, low yield and poor quality [8]. These problems become detrimental for the pepper industry. Therefore, it is urgent to investigate the effects of pH on pepper growth and nutrient absorption.

The objective of this study was to determine whether the decrease in pepper productivity of aging pepper gardens was associated with the decreased soil pH and how soil acidification affected pepper growth and nutrient absorption.

2. Materials and Methods

2.1. Experiment 1: Survey of Institute Gardens with Pepper

The study was conducted in Spice and Beverage Research Institute (SBRI) at the southeast of Hainan Province of China (18˚72'N - 18˚76'N, 110˚19'E - 110˚22'E). The area is characterized by a tropical monsoon climate with an average temperature of 24.6˚C, while the absolute maximum may reach 38.7˚C and the minimum may be 11.6˚C. Average annual precipitation of the area is 2150 mm, and the annual mean relative humidity is 85%.

The study area was comprised of a selection of seven gardens, which were divided into five age groups (10, 15, 20, 25 and 30 years of production) of black pepper (*Piper nigrum* L. cv. Reyin No.1) (**Table 1**). Row spacing was 2.5 m, while spacing in the row was 2 m. Within each garden five sampling locations were selected on an S-shape sampling configuration. A series of measurements were taken at each sampling location such as soil pH and pepper yield per plant. Four soil cores at each of the five sampling locations were composited for the soil sample to be analyzed. The soils were sampled at the 0 - 20 cm soil depth. Soil pH was measured using a ratio of 10 g soil to 25 ml of water and stirred for 30 minutes.

Table 1. Survey of pepper gardens at the spice and beverage institute, Hainan Island, China.

Pepper garden identification	Pepper garden age, yr	Number of plants reaching fruiting age
No. 13	26	424
No. 14	28	415
No. 15	28	382
No. 19	21	85
No. 21	15	312
No. 22	15	310
No. 24	10	377

2.2. Experiment 2: Solution Culture Experiment

A solution culture experiment of black pepper (*Piper nigrum* L. cv. Reyin No. 1) seedlings was carried out to quantify the response of black pepper seedlings to increasing acidity. Seedlings were cultured in sand until the extensive development of roots. Seedlings were weighed and transferred to 15 L plastic pots with nutrient solution consisting of K_2SO_4 (750 μM), $MgSO_4$ (650 μM), KCl (100 μM), KH_2PO_4 (250 μM), $Ca(NO_3)_2$ (2000 μM), Fe-EDTA (100 μM), H_3BO_3 (100 μM), $MnSO_4$ (1 μM), $ZnSO_4$ (1 μM), $CuSO_4$ (0.1 μM), $(NH_4)_6Mo_7O_{24}$ (0.005 μM) [10]. The experimental design structure was a completely randomized combination of four solution pH treatments (3.5, 4.0, 5.5, and 7.0) with 8 replications. The pH of the nutrient solution was adjusted once a day by HCl. Nutrient solutions were renewed every 4 days and continuously aerated with a pump. The pots were re-randomized every 20 days. Pepper seedlings were grown in a glasshouse at the SBRI with a photoperiod of 11 $h \cdot d^{-1}$, average temperature and photosynthetic photon flux density were 23˚C and 234 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at the shoot level and a relative humidity of 85%.

After 65 d of growth, seedlings were harvested and separated into leaves, vines and roots. Fresh weight was determined. Root morphological parameters, such as total root length, root surface area, number of lateral roots, average diameter and volume of roots, were determined from scanned root samples with an Epson V700 scanner and the software WinRHIZO according to the method described by Liu *et al*. [10]. Dry weight of the root (WR) and shoot (WS) were measured after drying in an oven at 70˚C.

2.3. Statistics

Data were analyzed using regression and ANOVA using SAS (version 8.02) statistical software. Lines of best fit for the curves and error bars for the bar chart were calculated using GraphPad Prism (Version 5, GraphPad Software) [11].

3. Results

3.1. Variation in Soil pH with Pepper Garden Age

Across the seven gardens, soil pH significantly declined with increasing garden age ($P = 0.018$) (**Figure 1**). According to the regression equation initial pH was approximately 5.84, while soil in 10-year-old orchards averaged 5.64, and 15-year-old gardens were characterized by a pH of 5.54. The 28-year-old garden averaged pH 5.28.

3.2. Pepper Seedling Response to Reduced pH (Solution Culture Experiment)

3.2.1. Fresh Weight Growth

Fresh weight (shoot plus root) growth decreased significantly with decreasing solution pH. Pepper accumulated the maximum fresh weight of both shoots and roots (average 90.1 g) at pH 5.5 (**Figure 2**), and a minimum fresh

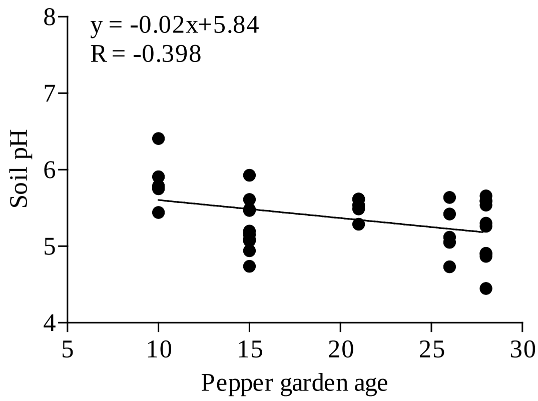

Figure 1. Variation in soil pH with planting year across seven pepper gardens at the SBRI, Hainan Island, China.

weight (average 56.5 g) at pH 3.5. There was no significant difference in fresh weight between pH 7 and pH 5.5, both of which were much higher than plant fresh weight at pH 3.5. At pH 4.0, the fresh weight was much lower (68.3 g) compared with the weight in solutions of pH 5.0 (90.1 g). There was no significant difference of fresh weight between pH 4.0 and pH 3.5 (**Figure 2**).

3.2.2. Black Pepper Shoot and Root Growth and Nutrient Concentrations in Response to Solution pH

The effects of solution pH on root and shoot fresh weights are also shown in **Table 2**. Low pH decreased root

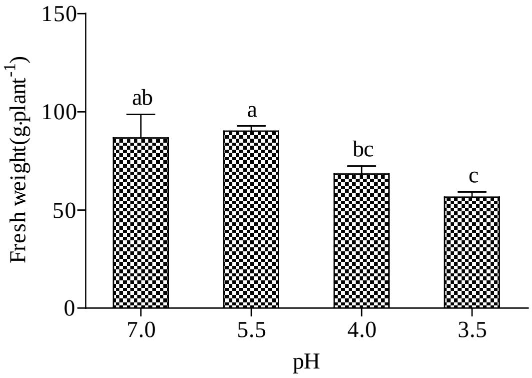

Figure 2. Growth yield of *Piper nigrum* L. cultured with four solution pHs (3.5, 4.0, 5.5, 7.0). Error bars indicate the standard error of the mean (n = 3); columns not sharing the same letter indicate significant differences according to the ANOVA in SAS ($P = 0.05$).

Table 2. Pepper (*Piper nigrum*, L.) seedling fresh weight and nutrient concentrations in the root and shoot of black pepper at 65 days after transplanting as affected by solution pH.

pH	Fresh weight (g·plant^{-1})	Nutrient concentrations				
		N (mg·g^{-1})	P (mg·g^{-1})	K (mg·g^{-1})	Ca (mg·g^{-1})	Mg (mg·g^{-1})
			Root			
7.00	8.17 ± 0.73	33.77 ± 1.54	2.41 ± 0.10	10.24 ± 0.87	12.63 ± 0.29	3.69 ± 0.13
5.50	7.77 ± 0.20	42.70 ± 1.81	3.05 ± 0.09	9.68 ± 0.67	8.90 ± 0.27	2.83 ± 0.12
4.00	6.70 ± 0.40	43.89 ± 1.06	4.45 ± 0.38	3.80 ± 1.20	8.00 ± 0.48	1.70 ± 0.32
3.50	5.77 ± 0.62	53.00 ± 2.06	3.71 ± 0.37	3.63 ± 1.33	5.54 ± 0.33	1.09 ± 0.06
$LSD_{0.05}$	1.73	5.41	0.89	3.42	1.15	0.61
			Coefficients of regression equation[b]			
y_0	3.86	66.33	5.85	−3.87	−0.17	−1.31
a	0.65**	−4.60***	−6.49**	2.14***	1.79***	0.73***
R^2	0.55	0.76	0.62	0.72	0.89	0.92
			Shoot			
7.00	78.50 ± 11.29	27.70 ± 0.99	1.30 ± 0.09	21.78 ± 0.27	10.68 ± 0.45	4.09 ± 0.57
5.50	82.33 ± 0.83	34.89 ± 2.42	1.66 ± 0.27	20.86 ± 0.89	10.51 ± 0.40	4.52 ± 0.30
4.00	61.57 ± 3.80	37.58 ± 2.83	1.37 ± 0.12	25.61 ± 1.07	11.09 ± 0.85	3.86 ± 0.07
3.50	50.73 ± 3.35	35.67 ± 0.71	1.62 ± 0.19	21.98 ± 2.16	8.22 ± 0.23	3.80 ± 0.65
$LSD_{0.05}$	20.70	6.38	ns[a]	ns	1.74	ns
			Coefficients of regression equation			
y_0	27.95	46.12	1.73	25.61	8.00	3.47
a	8.07*	−2.43**	−0.05 ns	−0.61 ns	0.43 ns	0.12 ns
R^2	0.50	0.51	0.05	0.11	0.19	0.05

Each value represents the mean of three replicates ± s.e.m. [a]ns, no significance; ** and ***, significance at $P < 0.01$ and $P < 0.001$ level, respectively. [b]The general regression equation is $y = y_0 + ax$, where y is the molar ratio of nutrient and x is level of nutrient solution pH.

and shoot fresh weights of pepper seedlings ($P < 0.01$).The extremely low pH caused a greater decrease in shoot than in root weights. The smallest root and shoot fresh weights were observed at pH 3.5 with decreases by 29% and 35% when compared with pH 7.0. However, little or no injurious effect was observed on root and shoot fresh weights at pH 5.5.

Most of the variation of nutrient concentrations in both root and shoot came from different pH treatments (**Table 2**). Root nutrient concentrations differed with the nutrient. Compared to pH 7.0, N and P concentrations increased by 146% and 54% at pH 3.5, respectively. In contrast, root potassium (K), calcium (Ca) and magnesium (Mg) decreased sharply with the decreasing pH. At pH 3.5, K, Ca and Mg decreased 65%, 56% and 70%, respectively.

3.3. Prediction of *Pepper nigrum* L. Growth from Root Nutrient Concentrations

The relationship between pepper growth and nutrient concentrations in roots varied with the nutrient (**Figure 3**). All nutrients were significantly related with growth except for P. The relationship was strongest for Mg (R^2 = 0.63), followed by Ca (R^2 = 0.44) and K (R^2 = 0.44). However, growth was significantly negatively related with N concentration in root.

3.4. Effects of Solution pH on Root Morphology

Pepper root growth decreased consistently with decreased solution pH (**Figure 4**). The root morphology of pepper was sensitive to the change in solution pH, with root length, surface area and number of laterals apparently

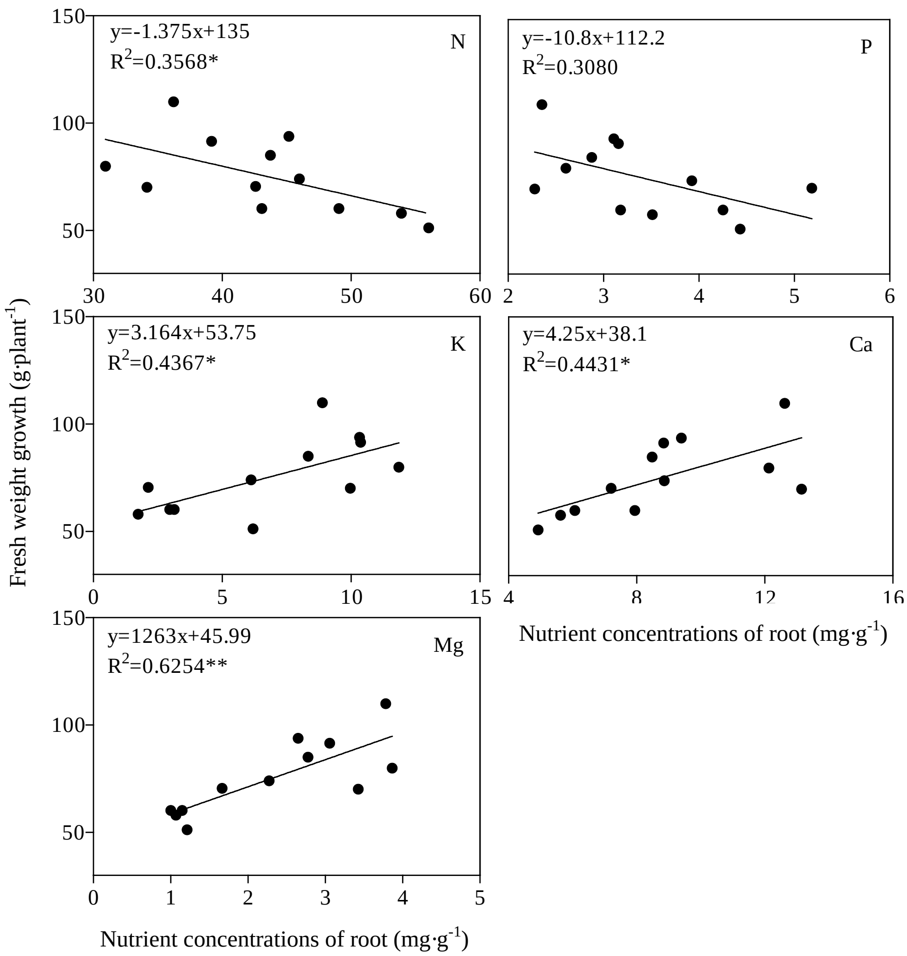

Figure 3. The prediction of *Piper nigrum* L. growth from root nutrient concentrations after 65 d growth.

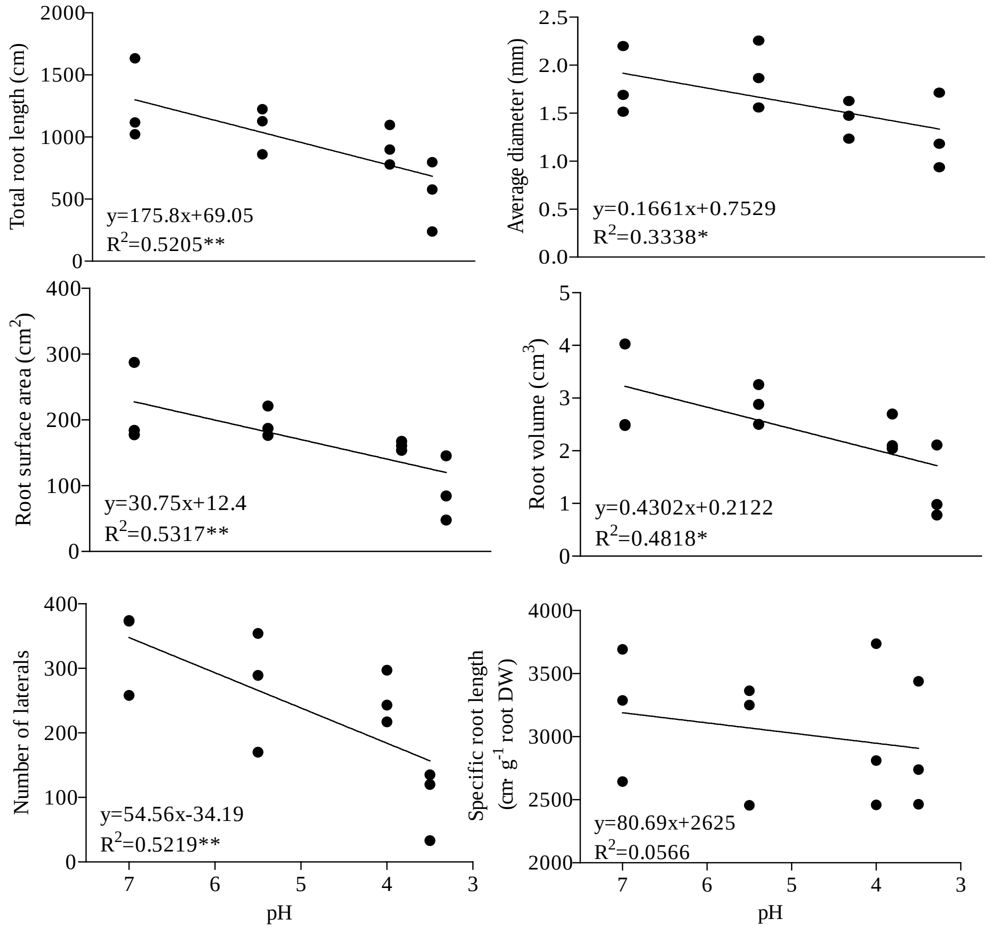

Figure 4. The relationship between solution pH with root morphological traits of *Piper nigrum* L. grown for 65 d.

more sensitive to acidity than were root diameter and volume (data not shown). No significant difference was detected in terms of specific root length.

The increasing acidity was apparently toxic, reducing total root length by 15%, 26% and 57% at pH 5.5, 4.0 and 3.5, respectively (**Figure 4**). Pepper root surface area was reduced by 10%, 26% and 57%, respectively. The surface area of root showed strongest negatively correlation with pH ($r^2 = 0.53$). The number of laterals was reduced by 19%, 25% and 71% in lower pH solutions. Low pH also appeared to be inhibitory for root diameter and volume of roots but less so than for root length, surface area and laterals. The specific root length was not inhibited greatly under low pH (**Figure 4**).

4. Discussion

In the present study, soil pH exhibited a significant decrease with increasing pepper garden age. The results from a hydroponic experiment suggested that low pH considerably inhibited seedling fresh weight and root morphological parameters. Root nutrient concentrations of potassium, calcium and magnesium decreased, while nitrogen and phosphorus increased under low solution pH. Indeed, a decrease in favorable root morphology does not mean a weak ability to acquire N and P from solution, as illustrated by the results. These differences are likely related to differences in K, Ca and Mg absorption and fresh weight growth. We further explore this in the following sections to provide insights into the variation that we have obtained.

4.1. The Soil pH Changed over Time

In Hainan province, soil pH of common pepper garden appeared to decrease after many years of production.

Large inputs of chemical fertilizers, particularly ammoniacal fertilizers, may be one of the key factors reducing pH. White pepper production and fertilizer N applications reached 1 and 0.58 kg·plant^{-1} nationally in recent years, respectively, reducing 50% and increases 154% as compared with year 1990 [12]. These applications result in large amounts of N fertiliser (1,167 kg·ha^{-1} each year). Furthermore, forms of applied N are usually urea-N, synthetic fertilizer-N (15-15-15) and manure-N, and, the percentage of inorganic-N is 77%. High rates of N fertilization can cause soil acidification both directly and indirectly [13] [14]. Consequently the decreasing soil pH may have affected pepper growth.

4.2. Fresh Weight Growth Inhibition under Low Solution pH

The solution culture showed that fresh weight growth of pepper seedlings was significantly inhibited at pH 4.0 and 3.5. Potassium, calcium and magnesium concentrations of roots were also significantly inhibited under low solution pH. A regression analysis was performed to examine the effect of root nutrient concentrations on the seedling fresh weight. The inhibition of K, Ca and Mg uptake was associated with poor fresh weight growth.

4.3. Root Growth and Nutrient Absorption Limitation in Response to Low pH Conditions

Among the various plant parts, the roots are directly or indirectly affected by the pH of the growth medium. Low pH injury or H^+ injury is one of the factors responsible for growth retardation in acid conditions [5]. In the present study, decreasing growth medium pH significantly reduced root growth and nutrient content. Root growth was obviously inhibited by low pH, especially surface area. Root surface area decreased linearly with declining solution pH ($P = 0.0071$). Number of laterals and total root length increased significantly, but less than surface area with increasing pH ($P = 0.0080$). Root diameter and volume showed significant positive correlation with pH ($P < 0.05$). Excess H^+ in the growth medium affects plant growth by two processes: a) H^+ may cause injury to the root tissue, therefore root elongation, lateral branching, surface area and volume stretching were inhibited [5], and b) Specific effects on root ion fluxes via H^+ competition with base cations for uptake and H^+ damage to the ion-selective carrier in root membranes. For example H^+ decreases the function of the plasma membrane and promotes K loss or the inhibition of K uptake, and consequently brings about poor root growth [5].

A decrease in the K, Ca and Mg concentrations in seedling roots was observed under low pH conditions. A previous study showed that an increase in the hydrogen-ion concentration of the medium generally caused a decrease in the rate of cation absorption, probably as a result of competition between the similarly charged ions for binding and carrier sites [5]. The abnormal morphology caused by acidic conditions may be another reason for the reduced absorption of cations. Root N concentration decreased with increasing pH in the range of 3.5 - 7.0, however, plant N content did not appear to decrease.

5. Conclusions

The decline in soil pH with years under pepper production may correlate with high levels of N fertilization, which usually leads to reduced soil pH. Solution culture studies of the effect of low solution pH indicated inhibition of *Piper nigrum* L. seedling growth and nutrient uptake in high H^+ conditions. Pepper seedling fresh weight was reduced 34% at pH 3.5 compared to pH 7.0. The root K, Ca and Mg absorption were restricted by the low pH. The decrease in fresh weight was clearly affected by the K, Ca and Mg decline. The root morphology was significantly modified, usually reduced at the low pH. Root elongation, lateral branching, surface area and volume stretching usually enhance the ability to take up nutrients of K, Ca and Mg from acid solution. Alternatively, excessive H^+ decreases the function of the plasma membrane and promotes K, Ca and Mg loss or the inhibition of K, Ca and Mg uptake, and consequently brings about poor root growth.

Nutrient solution pH significantly reduced growth of pepper seedlings with most reduction occurring in the pH range from 4.0 to 3.5, according to minimal fresh weight, morphology of root and root K, Ca, Mg concentrations. Outside this range, growth was progressively increased with suitable pH values. Determinations of root morphology should be the priority in evaluations of pepper regarding growth yield in response to pH.

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