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pH, Nitrogen and Calcium Concentration Affect Germination and Seedling Growth In Pepper (*Capsicum annuum* L.)

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ABSTRACT

The study reports the effects of solution pH and external calcium (Ca) and nitrogen (N) applications on seed germination, and early growth of pepper cv. Demre 8 seedlings in perlite culture. The percentage of pepper seeds that germinated was significantly affected by solution pH, and ranged from 55% at pH 3.0 to 90% at pH 6.5. Seedling growth was more sensitive to pH as was evident with significant declines in root and shoot dry mass, root and shoot length, number of leaves per plant, lateral root formation and root surface area. Increasing solution Ca concentration diminished the adverse effects of low pH on germination and seedling growth. Nitrogen was also effective in enhancing the germination and seedling growth. Lateral root formation, shoot and root dry mass increased with increasing N and Ca concentrations. It is suggested that germination and early growth of pepper can be significantly improved in strongly acid soils if adequate Ca and N is made available to the germinating seed.

pH, Azot ve Kalsiyum Konsantrasyonları Biberde (*Capsicum annuum* L.) Çimlenme ve Fide Büyümesini Etkiler

Anahtar Kelimeler
Büyüme
Çıkış
Fide beslenmesi
Kuru ağırlık
Tohum
Toprak pH'sı

ÖZET

Çalışmada, perlit kültüründe yetiştirilen Demre 8 biber çeşidinde solusyon pH'sı ve dışsal kalsiyum (Ca) ve azot (N) uygulamalarının tohum çimlenmesi ve fide büyümesi üzerindeki etkileri araştırılmıştır. Çimlenen biber tohum yüzdesi solusyon pH'sından önemli derecede etkilenmiş ve çimlenme % 55 (pH 3.0) ile % 90 (pH 6.5) arasında değişmiştir. Fide büyümesinin pH'ye karşı oldukça duyarlı olduğu kök ve sürgün kuru ağırlığı, kök ve sürgün uzunluğu, bitki başına yaprak sayısı, lateral kök oluşumu ve kök yüzey alanında görülen önemli azalmalarla kanıtlanmıştır. Solusyon Ca miktarının arttırılması pH'nın çimlenme ve fide büyümesi üzerindeki olumsuz etkilerini ortadan kaldırmıştır. Azot uygulaması da çimlenme ve fide büyümesini arttırmada etkili olmuştur. Lateral kök oluşumu, ve sürgün ve kök kuru ağırlığı yükselen N ve Ca konsantrasyonları ile artış göstermiştir. Çalışma sonucunda, çimlenen biber tohumlarına yeterli Ca ve N sağlandığı takdirde, kuvvetli asidik topraklarda ve büyüme ortamlarında çimlenme ve fide büyümesinin önemli derecede iyileştirilebileceği ileri sürülmüştür.

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Erciyes Üniversitesi Fen Bilimleri Enstitüsü Dergisi 26 (3): 270-282 (2010)

INTRODUCTION

Plant growth on acidic soils is rather slow due to the availability of toxic concentrations of hydrogen (H+), aluminum (Al) or manganese (Mn), and the deficiencies of minerals including calcium (Ca), magnesium (Mg), nitrogen (N), phosphorus (P), potassium (K), molybdenum (Mo), and zinc (Zn) [1, 2, 3, 4, 5, 6]. The specific causes of weak plant growth on acidic soils vary with soil pH, clay mineral type and clay content, kind and content of organic matter, levels of salts, and with plant species and genotype [1]. Low soil pH stress and Ca deficiency restrict growth and development of many vegetable species including pepper (*Capsicum annuum L.*) in acid soils [4, 7, 8, 9, 10]. Excess H^+ ions can increase plant requirements for Ca and other nutrients in the growth medium due to the effects on nutrient uptake and retention by plant roots [4]. Calcium deficiency leads to low yield, quality, seed germination and disease resistance [11]. The direct effects of H+ toxicity or Ca deficiency on plant growth in acid soils are difficult to determine, because at soil pH<4.0, Al, Mn, and other mineral elements may be present in toxic concentrations, and also, the availability of other elements essential for plant growth may be suboptimal [4]. Since the effects of the H^+ concentration and the effects of Ca are confounded by other factors in acid soils [2], researchers use nutrient solutions or sand and perlite cultures to study the effects of low pH or Ca.

Similarly, nitrates stimulate germination of many dormant seeds [12]. One cause for this effect on germination is reported to be that nitrate acts as an oxidizing substrate in a metabolic regulatory process involving NADPH - $NADP^+$ in the pentose pathway of glucose metabolism [13]. On the other hand, both promotive and inhibitive actions of ammonium salts have been reported. For instance, ammonium salts repressed germination of Timothy grass (*Phleum pratense*) seeds, possibly due to the known actions of ammonium salts as inhibitors of L-amino acid oxidase and as uncouplers of oxidative phosphorylation [14]. However, ammonium salts showed marked promotive activity for scurvy grass (*Barbarea verna*) and yellow rocket (*Barbarea vulgaris*) seeds, for which they served as metabolic substrates [15]. The promotion of germination is thought to depend on coupling of peroxidase action to NADPH oxidation, which can regulate the pentose pathway of D-glucose 6-phosphate use [15].

Although the effects of growth regulators, cultivar, and environmental conditions on germination and early growth of seedlings of pepper have been studied [8, 9, 16, 17], very little attention has been given to the effects of pH, N and Ca deficiency *per se* on germination and seedling growth. This study examines the effect of pH and external Ca and N concentrations on germination and early growth of pepper in perlite culture.

1. MATERIALS AND METHODS

Experiments were conducted at the experimental laboratory of Suleyman Demirel University, Isparta, Turkey. Germination and early growth of the pepper cultivar 'Demre 8' were tested for response to solution pH that was varied

independently or in combination with solution Ca and N concentrations.

Effect of pH, N and Ca on germination

The effects of eight pH values (3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0 and 6.5) and the control (distilled water) on germination of pepper cv. Demre 8 were investigated. Healthy pepper seeds obtained from Genagri Inc. Antalya, Turkey, were germinated in 9 cm Petri dishes containing two sheets of moist filter paper (Filtrak GmBH, Germany) to which 4 mL of distilled water (control) and distilled water at different pH values were added. The pH of the water was adjusted to the desired levels by adding an appropriate amount of 0.1 M HCl. There were eight replications for each treatment combination with each replication consisting of a single Petri dish of 50 seeds. Petri dishes were incubated in the dark in a temperature-controlled growth chamber (NUVE EN 500, Gaziantep, Turkey) at 25ºC for 14 days [18] and then germination was assessed. The treatments were arranged in a split plot experimental design. Seeds showing radicle emergence (5 mm) were recorded as germinated [19] and the germinated seeds were counted every other day during the experimental period. The experiment was repeated four times.

To determine the effects of solution pH levels (3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, and 6.5), N (0, 2, 4 and 6 mM) and Ca (0, 1, 1.5, and 2 mM) concentrations in combination on germination, the solution described above with the desired Ca or N concentration was used. The desired Ca and N concentrations were obtained by adding the appropriate amounts of calcium acetate and ammonium nitrate, both from Sigma (USA), to the solution respectively. There were eight replications consisting of 50 seeds per treatment combination, resulting in a total of 400 seeds per treatment. The germination conditions were similar to those described above, and the experiment was repeated four times.

Influence of pH, N and Ca on seedling growth

Seedling growth experiments were carried out in growth chambers. To determine the effects of pH, N and Ca concentrations on early seedling growth, the basal nutrient solution described below, but without Ca and N was utilized. The desired Ca and N concentrations were obtained as described above. For each treatment combination, 25 pre-germinated seeds were planted 2.5 cm deep in perlite culture in 35x30x15 cm deep vials and kept at 25ºC and 95% relative humidity under a 16-hr photoperiod with a light intensity of 72 w/m^2 at the plant tops provided by a combination of fluorescent and incandescent lamps. Moreover, the same amount of pre-germinated seeds were planted in 1:1 (v/v) perlite and peat moss (pH: 5.5; EC: 250 mmhos/cm; N: 300 mg L^{-1}; P_2O_5: 300 mg L^{-1}; K_2O: 400 mg L^{-1}; organic matter: 2%) and used as the control [18]. The sterilized media was moistened with a dilute nutrient solution which comprised (µM): 250 K, 250 N, 300 Ca, 400 S, 100 Mg, 10 Fe (as EDTA), 10 Cl, 3 B, 0.25 Zn, 0.10 Mn, 0.07 Cu, and 0.02 Mo. This solution had a pH of 6.5, and was titrated with either 0.1 M NaOH or 0.1 M HCl to obtain the target treatment pH values. The pH treatments were similar to those in the previous experiment, and the sterilized media was kept moist by periodic irrigation with the

same nutrient solution. The experiment was conducted with four replications. After emergence, the seedlings were allowed to grow for 28 days during which seedling mortality was assessed at seven-day intervals. On day 28 of emergence, the surviving healthy plants were harvested, and roots were separated from their tops. To determine treatment effects on seedling growth, root length and dry mass, shoot length and dry mass, lateral root formation, root surface area, number of leaves per plant and leaf total chlorophyll content were evaluated. Root length and root surface area were estimated using the GLS root scanner (HP Scanjet 3C, USA). Total root length was obtained by summing the lengths of all roots of a seedling. The root surface area was measured in mm^2. The plant shoots and roots were oven-dried at 80ºC for 48 h to determine the dry mass. Shoot length was measured with a ruler. Leaf total chlorophyll content was determined using the procedure of Knudson et al. [20]. The experiment was repeated four times.

Data analysis

Germination and seedling survival percentage data were arcsine transformed before statistical analysis to ensure homogeneity of variance [21]. Analysis of variance (ANOVA) was performed using the General Linear Model procedure provided by the Statistical Analysis System, USA [22]. The treatment means were separated by the Student–Newman-Keuls test.

2. RESULTS AND DISCUSSION

Effect of pH on seed germination

Pepper seed germination was significantly affected by solution pH (Table 1). The proportion of germinated seeds significantly increased with the increase in solution pH, and the maximum number of germinated seeds was obtained from pH 6.5 treatment during the experimental period. The number of germinated seeds was similar at pH 3.5, and 4.0, but significantly lower at pH 3.0. The final germination count on day 14 ranged from 55% at pH 3.0 to 87% at pH 6.0. Germination was rather slow at pH 3.0 as compared to that at above pH 5 (not shown). From an agronomic point of view, the faster the seedling emerges the greater the likelihood of escaping pre-emergence diseases, and the less damage will be exacted by seed and seedling pathogens such as *Aspergillus flavus*, *Aspergillus niger*, *Pythium* spp, *Rhizopus* spp, *Penicillium* spp, *Fusarium* spp, *Rhizoctonia solani*, etc. [23]. Thus, the slower germination observed at pH 3.0 would make the seeds in the soil more vulnerable to soil fungal and bacterial pathogens, leading to reduced seedling emergence.

Effect of pH on seedling growth

Seedling growth was significantly affected by the growth medium pH (Tables 1 and 2).

All pre-germinated seeds emerged from the growth medium, but the number of surviving seedlings at all pH levels gradually declined starting

Erciyes Üniversitesi Fen Bilimleri Enstitüsü Dergisi 26 (3): 270-282 (2010)

Table 1. Effects of pH and various concentrations of calcium and nitrogen on pepper seed germination and seedling growth.

Treatment	Germination (%)	Root Length (m)	Lateral Root Formation	Root Surface Area (cm^2)	Total Chlorophyll (µg/mg)
pH 3.0	55 fe	0.42 ef	0 g	0.25 h	8.00 g
Ca (1.0 mM)	74 cb	0.61 def	0.33 g	0.36 h	8.26 g
Ca (1.5 mM)	75 cb	0.62 def	0.33 g	0.39 h	12.54 f
Ca (2.0 mM)	73 cb	0.64 def	0.67 fg	0.43 gh	13.12 ef
N (2 mM)	87 ab	1.24 de	3.33 cd	0.32 h	12.51 f
N (4 mM)	87 ab	0.82 edf	1.33 ef	0.36 h	18.84 de
N (6 mM)	80 ab	0.51 ef	1 f	0.34 h	15.13 ef
pH 3.5	60 e	0.39 ef	1.33 ef	0.27 h	9.15 fg
Ca (1.0 mM)	73 cb	0.74 def	1.67 ef	0.44 gh	16.26 ef
Ca (1.5 mM)	67 dc	0.59 def	1.82 ef	0.38 h	18.15 de
Ca (2.0 mM)	67 dc	0.53 ef	1.13 f	0.35 h	16.70 e
N (2 mM)	80 ab	0.62 def	2.67 d	0.40 h	13.22 ef
N (4 mM)	87 ab	0.65 def	1.33 ef	0.41 h	14.76 ef
N (6 mM)	87 ab	0.72 def	1.33 ef	0.44 gh	18.05 de
pH 4.0	60 e	0.49 ef	1.67 ef	0.30 h	10.24 fg
Ca (1.0 mM)	60 e	0.86 def	1.82 ef	0.55 gh	16.13 ef
Ca (1.5 mM)	73 cb	0.77 def	1.66 ef	0.51 gh	17.49 de
Ca (2.0 mM)	67 dc	0.70 def	1.66 ef	0.48 gh	17.52 de
N (2 mM)	90 a	0.94 de	2.67 d	0.60 fg	16.20 ef
N (4 mM)	87 ab	0.90 def	2.33 de	0.58 fg	14.17 ef
N (6 mM)	73 cb	0.69 def	2.33 de	0.48 gh	15.95 ef
pH 4.5	70 c	0.48 ef	2.67 d	0.30 h	13.65 ef
Ca (1.0 mM)	67 dc	1.16 de	2.33 de	0.82 ef	17.91 de
Ca (1.5 mM)	70 c	1.11 de	2.45 de	0.79 ef	21.06 d
Ca (2.0 mM)	70 c	0.77 def	1.67 ef	0.51 gh	22.36cd
N (2 mM)	80 ab	1.12 de	2.67 d	0.79 ef	22.53 cd
N (4 mM)	87 ab	1.10 de	3.33 cd	0.77 ef	20.76 de
N (6 mM)	73 cb	1.01 de	3.35 bc	0.74 f	18.98 de
pH 5.0	80 ab	1.01 de	3.67 bc	0.64 fg	17.80 de
Ca (1.0 mM)	78 ab	1.15 de	3.67 bc	0.80 ef	22.06 cd
Ca (1.5 mM)	78 ab	1.24 de	3.67 bc	0.88 ef	24.76 c
Ca (2.0 mM)	81 ab	1.24 de	2.33 de	0.88 ef	25.97 bc
N (2 mM)	82 ab	1.15 de	3.75 bc	0.80 ef	23.95 c
N (4 mM)	83 ab	1.26 de	3.57 bc	0.89 ef	25.64 c
N (6 mM)	80 ab	1.21 de	2.15 de	0.85 ef	23.06 cd
pH 5.5	86 ab	2.17 abc	4.33 ab	1.19 cd	18.64 de
Ca (1.0 mM)	84 ab	2.16 abc	4.33 ab	1.18 cd	21.65 cd
Ca (1.5 mM)	87 ab	1.95 bc	3.67 bc	1.06 de	20.31 de

Ca (2.0 mM)	88 ab	1.96 bc	3.33 cd	1.02 de	20.75 de
N (2 mM)	84 ab	2.33 abc	4.53 a	1.25 c	26.43 bc
N (4 mM)	85 ab	2.54 ab	4.68 a	1.36 bc	25.23 c
N (6 mM)	88 ab	2.42 abc	3.11 cd	1.20 cd	23.02 cd
pH 6.0	87 ab	2.56 ab	3.11 cd	1.26 c	20.17 de
Ca (1.0 mM)	85 ab	2.62 ab	3.10 cd	1.29 c	26.02 bc
Ca (1.5 mM)	86 ab	2.53 ab	2.95 cd	1.24 c	22.91 cd
Ca (2.0 mM)	85 ab	2.53 ab	2.95 cd	1.24 c	22.27 cd
N (2 mM)	80 ab	2.74 ab	3.33 cd	1.47 bc	29.06 bc
N (4 mM)	80 ab	3.12 a	3.67 bc	1.66 a	33.39 ab
N (6 mM)	80 ab	3.05 ab	3.11 cd	1.62 ab	33.70 a
pH 6.5	87 ab	2.45 abc	2.53 d	1.23 cd	20.00 de
Ca (1.0 mM)	85 ab	2.51 ab	2.51 d	1.25 c	23.36 cd
Ca (1.5 mM)	90 a	2.48 ab	2.52 d	1.24 c	25.14 c
Ca (2.0 mM)	87 ab	2.50 ab	2.52 d	1.24 c	23.99 c
N (2 mM)	80 ab	2.66 ab	2.63 d	1.29 c	26.59 bc
N (4 mM)	87 ab	2.75 ab	2.65 d	1.32 c	27.00 bc
N (6 mM)	85 ab	2.78 ab	2.33 de	1.33 c	25.38 c
Control	83 ab	2.44 abc	1.12 f	1.25 c	20.41 de
Ca (1.0 mM)	80 ab	2.46 abc	1.21 f	1.25 c	22.92 cd
Ca (1.5 mM)	85 ab	2.48 ab	1.05 f	1.24 c	23.72 cd
Ca (2.0 mM)	83 ab	2.46 abc	1.14 f	1.24 c	23.07 cd
N (2 mM)	80 ab	2.53 ab	1.12 f	1.26 c	23.44 cd
N (4 mM)	75 cb	2.51 ab	1.24 f	1.25 c	23.92 c
N (6 mM)	73 cb	2.56 ab	1.13 f	1.27 c	23.32 cd

*Means within each column followed by the same letter are not significantly different at the 5% level of significance.

seven days after emergence (data not shown). Only 48% of the seedlings survived on day 28 of emergence at the pH 3.0 treatment, compared to 98% and 97% at pH 6.5 and control treatments, respectively (Table 2). The total root length on day 28 of emergence was 0.42 m per plant for plants grown at pH 3.0, and increased over 6 fold to 3.12 m for plants grown at pH 6.0 (Table 1). Roots thicker than 2 mm in diameter in the pH 3.0, pH 4.0 and pH 4.5 treatments exhibited visual symptoms similar to those reported [24] for H^+ injury on plant roots. These symptoms included stunted root growth, brownish colour, and little lateral root development. Some of the roots were decayed. There was no lateral root formation at pH 3.0 (Table 1). However, pepper plants started to form lateral roots as the pH increased. Even a 0.5 unit rise in pH of the medium caused significant lateral root formation. Maximum lateral root formation was obtained at pH 5.5. Hydrogen-induced root injury as observed at pH below 4.5 in our study may change root membrane permeability (membranes become leaky), interferes in absorption and transport of nutrients, increases loss of

276

Erciyes Üniversitesi Fen Bilimleri Enstitüsü Dergisi 26 (3): 270-282 (2010)

organic substrates (such as sugars and amino acids) and adsorbed cations, and reduces capacities for absorption of nutrients [4]. Thus, plants grown in acid soils are bound to be restricted from utilizing available water and nutrients when root proliferation and root function is limited by low pH [25].

Root surface area followed a similar response trend as root length (Table 1). The root surface area increased by more than 400% for plants growing at pH 6.5 compared to those growing at pH 3.0. The detrimental effects of a solution pH of 3.0 were evident within 12 days of plant growth when the shoot growth was visibly impaired, and the plant shoots and hypocotyls had a grayish-green color. Plants grown at low pH levels demonstrated slower growth than plants grown at higher pH levels (Table 1). As the pH increased shoot length increased significantly and reached 7.9 cm at pH 6.5. A similar trend was also observed for the total number of leaves per plant (Table 2). Leaf total chlorophyll content remained relatively constant up to pH 4.5 and then increased significantly with rises in pH (Table 1). The significantly higher levels of total chlorophyll content in seedlings grown at higher pH levels possibly contribute to more assimilate production and thus leading to more growth of these seedlings.

Increases in pH resulted in a significant rise in shoot and root dry mass (Table 2). The shoot dry mass was not significantly different at pH 3.0, 3.5, 4.0, 4.5 and 5.0, although plants in the pH 3.0, 3.5 and 4.0 treatments displayed some symptoms of H^+ injury. Similarly, solution pH had no significant effect on root dry mass at low pH levels (pH 3.0-5.5). Low pH appears to redirect more assimilates to

the roots than to the shoot system in order to offset the adverse effects of unfavourable pH on root growth. Tang and Thomson [26] also noted that root dry mass of a number of grain legume species responded to solution pH in a similar manner to shoot dry mass, but the effect of low pH on decreasing root mass was less than on shoot mass. The adverse effects of low solution pH were greater on root surface area compared to root dry mass. We observed a considerable level of fine roots at high pH levels compared to the low pH levels where short and stubby roots were prominent. This phenomenon can be attributed to inadequate Ca uptake, which negatively affect on cell division and elongation, resulting in a shorter and denser root system [2, 7]. Furthermore, at low pH levels assimilates were accumulating in the roots, resulting in short and stubby roots, thus no differences in dry mass of the root systems were observed at different pH levels. Therefore, while root proliferation can be severely affected by inadequate Ca, the root mass may not be affected to the same extent.

Effect of pH, nitrogen and calcium on seed germination

At pH 3.0, only 55% of the seeds germinated when no Ca was applied (Table 1). As the Ca concentration increased, the negative effects of pH diminished and germination improved.

Erciyes Üniversitesi Fen Bilimleri Enstitüsü Dergisi 26 (3): 270-282 (2010)

Table 2. Effects of pH and various concentrations of calcium and nitrogen on pepper seedling growth on day 28 of emergence.

Treatment	No. of Leaves	Shoot Length (cm)	Shoot Dry Mass (gr)	Root Dry Mass (gr)	Seedling Surv. (%)
pH 3.0	4.8 fg	5.5 e	0.031 h	0.022 d	48 l
Ca (1.0 mM)	5.5 f	6.5 de	0.043 fg	0.034 cd	74 efg
Ca (1.5 mM)	6 de	6.6 de	0.046 f	0.035 cd	86 bcd
Ca (2.0 mM)	6.7 cd	6.9 cd	0.051 ef	0.038 bc	98 ab
N (2 mM)	5 fg	5.8 e	0.037 gh	0.024 d	54 jkl
N (4 mM)	5 fg	5.7 e	0.033 h	0.022 d	53 kl
N (6 mM)	5 fg	5.4 e	0.032 h	0.022 d	55 jkl
pH 3.5	5 fg	5.5 e	0.032 h	0.022 d	53 kl
Ca (1.0 mM)	5.3 f	6.4 de	0.043 fg	0.034 cd	75 efg
Ca (1.5 mM)	5.5 f	6.8 de	0.044 fg	0.036 c	87 bcd
Ca (2.0 mM)	6 de	7.1 cd	0.053 ef	0.039 bc	99 a
N (2 mM)	4.9 fg	5.4 e	0.030 h	0.021 d	56 jkl
N (4 mM)	4.9 fg	5.0 e	0.028 h	0.019 d	58 ijk
N (6 mM)	5.1 fg	5.1 e	0.029 h	0.020 d	58 ijk
pH 4.0	4.9 fg	5.1 e	0.033 h	0.022 d	72 fgh
Ca (1.0 mM)	5.5 f	6.3 de	0.039 g	0.025 d	89 bcd
Ca (1.5 mM)	6.3 d	7.8 cd	0.047 f	0.032 cd	97 ab
Ca (2.0 mM)	7.4 b	8.2 bc	0.062 cd	0.036 c	99 a
N (2 mM)	6 de	5.2 e	0.035 gh	0.022 d	78 def
N (4 mM)	7.1 bc	6.0 de	0.042 fg	0.026 d	84 cde
N (6 mM)	7.2 bc	6.3 de	0.048 ef	0.029 cd	86 bcd
pH 4.5	4.8 fg	5.7 e	0.034 gh	0.023 d	83 cde
Ca (1.0 mM)	6.2 de	6.7 de	0.037 gh	0.030 cd	95 ab
Ca (1.5 mM)	6.6 cd	7.0 cd	0.045 f	0.033 cd	97 ab
Ca (2.0 mM)	6 de	7.9 c	0.056 de	0.037 c	99 a
N (2 mM)	6 de	5.4 e	0.032 h	0.022 d	84 cde
N (4 mM)	6.5 cd	5.8 e	0.039 g	0.023 d	88 bcd
N (6 mM)	6.9 bc	5.9 de	0.040 fg	0.024 d	85 cde
pH 5.0	5.7 f	5.8 e	0.036 gh	0.023 d	88 bcd
Ca (1.0 mM)	6.3 d	6.8 de	0.046 f	0.031 cd	98 ab
Ca (1.5 mM)	6.7 cd	7.5 cd	0.053 ef	0.035 cd	100 a
Ca (2.0 mM)	6.7 cd	8.2 bc	0.064 cd	0.041 bc	100 a
N (2 mM)	6.3 d	6.0 de	0.037 gh	0.026 d	98 ab
N (4 mM)	7.7 b	7.0 cd	0.049 ef	0.033 cd	98 ab
N (6 mM)	7.5 b	7.3 cd	0.053 ef	0.035 cd	97 ab
pH 5.5	6.5 cd	6.8 de	0.046 f	0.031 cd	95 ab
Ca (1.0 mM)	6.7 cd	7.8 cd	0.059 de	0.039 bc	100 a
Ca (1.5 mM)	6 de	8.2 bc	0.062 cd	0.044 bc	100 a
Ca (2.0 mM)	6 de	8.5 bc	0.066 cd	0.046 bc	100 a

N (2 mM)	7.5 b	7.5 cd	0.052 ef	0.037 c	95 ab
N (4 mM)	7.7 b	8.5 bc	0.064 cd	0.046 bc	98 ab
N (6 mM)	8.3 a	8.2 bc	0.063 cd	0.044 bc	98 ab
pH 6.0	6.5 cd	7.0 cd	0.051 ef	0.033 cd	95 ab
Ca (1.0 mM)	6.4 cd	7.4 cd	0.053 ef	0.035 cd	99 a
Ca (1.5 mM)	6.6 cd	7.6 cd	0.056 de	0.037 c	100 a
Ca (2.0 mM)	6.5 cd	8.0 bc	0.064 cd	0.042 bc	100 a
N (2 mM)	7 bc	9.2 ab	0.076 b	0.049 ab	98 ab
N (4 mM)	7.4 b	9.8 a	0.083 ab	0.054 ab	100 a
N (6 mM)	7.6 b	9.8 a	0.083 ab	0.054 ab	100 a
pH 6.5	6.0 de	7.9 c	0.062 cd	0.040 bc	98 ab
Ca (1.0 mM)	6.1 de	9.0 ab	0.074 b	0.047 b	99 a
Ca (1.5 mM)	6.1de	9.0 ab	0.075 b	0.047 b	98 ab
Ca (2.0 mM)	6.0 de	9.2 ab	0.077 b	0.049 ab	100 a
N (2 mM)	7.0 bc	10.0 a	0.085 a	0.057 a	98 ab
N (4 mM)	7.4 b	9.4 ab	0.080 ab	0.051 ab	98 ab
N (6 mM)	7.5 b	9.0 ab	0.074 b	0.047 b	100 a
Control	6.0 de	6.6 de	0.043 fg	0.029 cd	96 ab
Ca (1.0 mM)	6.2 de	9.6 ab	0.076 b	0.053 ab	97 ab
Ca (1.5 mM)	6.4 cd	8.7 bc	0.065 cd	0.048 ab	96 ab
Ca (2.0 mM)	6.3 d	7.4 cd	0.055 de	0.037 c	97 ab
N (2 mM)	7.2 bc	9.4 ab	0.081 ab	0.051 ab	99 a
N (4 mM)	7.5 b	8.0 bc	0.060 d	0.042 bc	98 ab
N (6 mM)	7.7 b	7.5 cd	0.055 de	0.036 c	98 ab

*Means within each column followed by the same letter are not significantly different at the 5% level of significance.

Germination percentages of up to 90% were obtained with Ca concentrations of 1.0 mM or higher, irrespective of the pH (Table 1). However, there were no significant differences among the three Ca concentrations evaluated in our study in any of the pH treatments. The effects of Ca on seed germination were more apparent at lower pH values and as the pH increased its effects started to diminish. At pH 5.0 or higher treatments, there was no significant difference between control (no Ca) and Ca treatments. Thus Ca acts to alleviate the negative effects of low pH on seed germination. In one contrasting report, Pierce et al.[27] did not find any effects of Ca on the germination of large crabgrass when soil was amended with $CaCO_3$. This could be due to differences between plant species [1].

N diminished the negative effects of low pH, increasing seed germination significantly at all low pH levels (Table 1). However, the greatest effect was observed at pH 3.0. While control seeds showed 55% germination at pH 3.0, the germination increased to 87% in the 2 mM N treatment. As in the case for Ca, the effect of N on pepper seed germination was not significant at higher

Erciyes Üniversitesi Fen Bilimleri Enstitüsü Dergisi 26 (3): 270-282 (2010)

pH levels. Stimulation of germination by nitrate and ammonium has been documented for several species [10, 18, 28, 29]. It was suggested that N oxides induce the germination of these species by oxidizing the sub-dermal cuticle and thus increasing its permeability [30]. The emergence and stand establishment of pepper seeds are often slow and extremely erratic, particularly under stress conditions. Pepper seeds have a well defined non-starchy endosperm. It has become clear that the endospermic tissue enclosing the radicle tip of the embryo offers a mechanical barrier to the growing embryo, thus affecting germination [17, 31, 32].

Effect of pH, nitrogen and calcium on seedling growth

On day 28 of emergence, the solution pH, N and Ca concentration significantly affected seedling growth (Tables 1 and 2). At pH levels below 5.0, Ca concentrations had significantly higher effects on seedling survival and growth compared to that at pH above 5.0. As pH decreased seedling survival percentages declined (Table 2). More than 95% of the seedlings survived at pH 6.0, regardless of the Ca concentrations of the nutrient solution. Both Ca and N concentrations significantly affected root length and lateral root formation (Table 1). The greatest effect was observed at lower pH levels. For instance, at pH 3, root length increased 35% and 132% with 2 mM Ca and 2 mM N applications, respectively. Similarly, no lateral root formation was observed at pH 3.0 but seedlings produced significant amount of lateral roots in response to N treatment. At higher pH levels (pH>4.5) there were no significant effects of N and Ca on root length and lateral root formation except pH 5 and 5.5 at which 2.0 mM Ca significantly decreased lateral root formation. Root surface area followed the same response trends as root length (Table 1). Total root surface area in the control treatment did not differ significantly from that at pH 6.0, but was 404% higher than that at pH 3.0. Ca treatment significantly increased root surface area only at pH below 5.0. However, N was effective at both high and low pH levels (Table 1). N gradually increased root surface area up to pH 6.0 and then its effect declined. N also significantly increased root surface area at pH 6.0 as compared to the control. Ca significantly increased root dry mass even at pH 3.0, but N showed no significant effect on root dry mass. Better root growth was observed when solution pH was favourable (Table 1 and 2). The root dry mass was the highest at pH 6.5, and ranged from 0.022 g plant^{-1} with no Ca or N to 0.057 g plant^{-1} at 2.0 mM N. There was a greater reduction in the development of the finer roots than of the thicker roots and taproot at the lower pH levels and lower Ca concentrations in agreement with the findings of Sanzonowicz et al. [33] who reported that H^+ toxicity inhibited the length of lateral roots of soybeans more than that of taproots. In their study, a 50% reduction in lateral root length occurred at pH 5.1, whereas a similar reduction in taproot length occurred at pH 4.7.

The effects of pH, N and Ca concentrations on shoot dry mass were significant, with shoot dry mass increasing as pH, and N and Ca

concentrations increased (Table 2). The interaction effects between pH and Ca and between pH and N concentrations were also significant, showing a greater impact of pH on shoot dry mass at intermediate Ca and low N concentrations. Plants grown with solution Ca concentration of 2.0 mM produced similar dry mass up to pH 5.5. Yan et al. [34] documented similar results in their studies on maize and broad beans, which showed that higher levels of solution Ca counteracted the negative effects of low solution pH on growth of the two crops. N was not as effective at low pH as Ca was. The effect of N on shoot dry mass was not significant up to pH 5.0 but thereafter it increased shoot dry mass significantly confirming the findings of Guzman and Olave [35] who reported that shoot dry mass and shoot length of melon (*Cucumis melo*) were significantly enhanced in response to N application.

Overall, the root growth was more affected by solution pH than the shoot growth. Other researchers have also shown that the reductions in shoot growth of grain legumes at low pH are associated with more severe depressions in root growth [36]. Yan et al. [34] observed an 80% reduction in root elongation of faba bean grown at pH 4.0 compared to that grown at pH 6.5, while Beusichem [37] observed a 40% reduction in root dry mass of peas without a decrease in shoot dry mass.

Both Ca and N demonstrated significant effects on shoot length and number of leaves per plant. Ca was more effective in increasing shoot length at low pH. At pH 3.0, 3.5, 4.0 and 4.5, 25, 29, and 61% increases in shoot length were obtained with 2 mM Ca treatment. On the other hand, there was no significant increase in shoot length in response to N application up to pH 5.0, thereafter N significantly stimulated shoot length. However, there was no significant difference between N doses at all pH levels. Similar trends were also observed for the number of leaves per plant. Both N and Ca application significantly enhanced leaf formation. While Ca was effective at low pH, N effects were more prominent at higher pH levels. Total chlorophyll content was also significantly affected by both increasing Ca and N concentrations (Table 1). At pH 3, total chlorophyll content ranged from 8.00 μg mg^{-1} to 13.12 and 18.84 μg mg^{-1} in 2.0 mM Ca and 4 mM N treatments, increasing over 64 and 135%, respectively. At pH 4.5, 64 and 65% increases were evident in 2mM Ca and 2 mM N treatments, respectively. The effects of both Ca and N gradually diminished as pH increased in agreement with the findings of Pierce at al. [27] who reported that increasing soil pH reduced the growth of crabgrass and melon seedlings. This may be due to a greater number and size of the cells of secondary xylem tissues.

3. CONCLUSIONS

The results indicate that low pH has a major impact on the germination of pepper seeds, and significantly influences the seedling survival and early growth. The germination of pepper seed was not tolerant of low solution pH; given that at pH 3.0 a germination percentage of 55% was attained, and that increasing the pH in the range 3.0–6.5

enhanced germination to over 90%. The adverse effects of low pH on germination and seedling growth were more pronounced in the absence of Ca, and became progressively less as the solution Ca concentration increased. Seedling growth was more sensitive to the effects of pH than seed germination, and both parameters were improved as the Ca and N concentrations and pH values were increased. Ca was more effective at low pH levels but N effects were observable at both low pH (less than 4.5) and more so at higher pH levels (pH>5.0). Pepper seedlings performed best in the pH range from 5.0 to 6.5. Seedling growth (root and shoot growth) also enhanced in response to Ca and N treatments. The combination of low Ca and low pH severely retarded shoot and root growth. These results imply that germination and early growth of pepper can be significantly improved in strongly acid soils if adequate Ca and N are made available to the germinating seed.

REFERENCES

4. Clark, R.B., In: Adams, F., (Ed.) Soil Acidity and Liming. American Society of Agronomists, Madison, WI, (1984) 99–147.

5. Fageria, N.K., V.C. Baligar and D.G. Edwards, In: Baligar, V.C., Duncan, R.R. (Eds.), Crops as Enhancers of Nutrient Use, Academic Press Ltd. San Diego, CA, (1990) 475–507.

6. Foy, C.D., Comm. Soil Sci. Plant Anal. 19: (1998) 959–978.

7. Foy, C.D., Adv. Soil Sci. 19: (1992) 97–133.

8. Kamprath, E.J. and C.D. Foy, In: Engelstad, O.P., (Ed.), Fertilizer Technology and Use, Soil Sci. Soc. Am. Madison, WI, (1985) 91–151.

9. Sanchez, P.A. and M. Uehara, Management consideration for acid soils with high phosphorus fixation capacity. Soil Sci. Soc. America, Madison, WI, (1980) 471–514.

10. Bennett, W.F., In: Bennett, W.F. (Ed.), Nutrient Deficiencies and Toxicities in Crop Plants. APS Press. St. Paul, MN, USA, (1993) 1–7.

11. Carter, A.K. and C.S. Vavrina., HortSci. 36: (2001) 645-814.

12. Korkmaz, A., HortSci. 40: (2005) 5-271.

13. Pérez-Fernández, M.A., E. Calvo-Magro, J. Montanero-Fernández and J.A. Oyola-Velasco, J. Environ. Biol. 27(1): (2006) 13-20.

14. Gascho, G.J. and J.G. Davis, In: Smartt J. (Ed.), The Groundnut Crop: A Scientific Basis for Improvement. Chapman and Hall. London, UK, (1994) 215–227.

15. Steinbaier, G.P. and B. Grisby, Weeds 5: (1957) 175-182.

16. Roberts, E. H., In: W. Heydecker (Ed), *Seed Ecology*. Pennsylvania State Ulniversity Press, University Park (1973) 189-218.

17. 1Lehninger, A. L., The Mitochondrion. New York, (1964) 124.

18. Hendriks, S.B. and R.B. Taylorson, Plant Physiol. 54: (1974) 304-309.

19. Mbonu, O.A., O.A. Opara–Nadi and K.G. Okoroh, Int. J. Agric. Rural Dev. 7(2): (2006) 134-140.

20. Watkins, J.T., J.D. Cantlife, D.J. Huber and T.A. Nell, J. Amer. Soc. Hort. Sci. 110: (1985) 61-65.

21. ISTA, International Rules for Seed Testing. Seed Sci. Tech. 24, Supplement, Rules, (1996).
22. Mayer, A.M. and A. Poljakoff-Mayber, The Germination of Seeds. 2nd Ed. Pergamon Press. New York (1975).
23. Knudson, L.L., T. W. Tibbitts and G.E. Edwards, Dept. Hort. Univ. Wisconsin. Madison. Plant Physiol. 60: (1977) 606-608.
24. Badger, K.S., and I.A. Ungar, Can. J. Bot. 67: (1989) 1420–1425.
25. SAS, SAS User's Guide: Statistics, Version 5 Ed.; Statistical Analysis System Institute. Cary, NC (1985).
26. Melouk, H.A. and P.A. Backman, In: H.A. Melouk, & F.M. Shokes (Eds), *Peanut Health Management* APS Press. St. Paul, MN (1995) 75–82.
27. Lund, Z.F., Soil Sci. Soc. Amer. J. Proc. 34: (1970) 456–459.
28. Goldman, I.L., T.E. Carter and R.P. Patterson, Agronomy J. 81: (1989) 461–463.
29. Tang, C. and B.D. Thomson, Plant Soil 186: (1996) 321–330.
30. Pierce, G.L., S.L. Warren, R.L. Mikkelsen, H.M. Linker, (1999) Weed Technol. 13(2): 421-424.
31. Cohn, M.A., In: Taylorson, R.B.1974 , (Ed.), Recent Advances in the Development and Germination of Seeds. Plenum Press, New York , NY, (1989) 261–267.
32. Adkins, S.W., and A.L. Adkins, Weed Sci. 42: (1994) 353–357.
33. Keeley, J.E. and C.J. Fotheringham, J. Ecol. 86: (1998) 27–36.
34. Groot, S.P.C. and C.M. Karssen, Planta 171: (1987) 525-531.
35. Andreoli, C., and A.A. Khan, HortSci. 28: (1993) 708-709.
36. Sanzonowicz, C., T.J. Smith and D.W. Israel, J. Plant Nut. 21 (2): (1998) 387–403.
37. Yan, F., S. Schubert and K. Mengel, Plant Physiol. 99: (1992) 415–421.
38. Guzman, M. and J. Olave, J. Food Agric. Environ. 4(1): (2006) 163-165.
39. Jayasundara, H.P.S., B.D. Thomson and C. Tang, Adv. Agronomy 63: (1998) 77–151.
40. Beusichem, M.L.V., N. J. Agric. Sci. 30: (1982) 85–97.