Comunicata Scientiae 2(3): 135-141, 2011

Original Article

Effects of nutrient solution pH on growth parameters of alfalfa (*Medicago sativa* L.) genotypes

Maurício Marini Köpp[1*], Leônidas Paixão Passos[2], Rui da Silva Verneue[2], Francisco José da Silva Lédo[2], Jefferson Luis Meirelles Coimbra[3], Antonio Costa de Oliveira[4]

[1]Embrapa Pecuária Sul, Bagé, RS, Brasil
[2]Embrapa Gado de Leite, Juiz de Fora, MG, Brasil
[3]Centro Agroveterinário, Universidade do Estado de Santa Catarina, Lages, SC, Brasil
[4]Universidade Federal de Pelotas, Pelotas, RS, Brasil
[*]Autor Correspondente, e-mail: mauricio.kopp@cppsul.embrapa.br

Abstract

The effects of nutrient solution pH (4.0, 5.0, 6.0 and 7.0) were examined on growth features of 12 alfalfa genotypes in relation to characters: leaf blade, epicotyl, hypocotyl, first leaf petiole, trifoliate leaf petiole and root fresh weight and epicotyl, hypocotyl, first leaf petiole, trifoliate leaf petiole and root length. Significant quadratic effects of pH solution were detected for all studied parameters. The pH value which resulted in maximum growth varied, according to the studied parameter, between 5.0 and 6.0. The genotypes Victoria, Esmeralda, Crioula and F-708 exhibited superior performance when data were pooled for all studied pH values. The results indicate that the initial growth is affected by pH variation in the nutrient solution, and that contrasting genotypes tend to perform alike.

Key words: *Medicago sativa* L., genetic resources, abiotic stress, phytotoxicity.

Efeitos do pH da solução nutritiva sobre o crescimento de genótipos de alfafa (*Medicago sativa* L.)

Resumo

Foram estudados os efeitos do pH (4.0, 5.0, 6.0 e 7.0) da solução nutritiva sobre o crescimento de 12 genótipos de alfafa com relação às seguintes características: peso fresco da lâmina foliar e comprimento do epicótilo, hipocótilo, pecíolo da primeira folha, pecíolo do trifólio e raiz. Foram encontradas respostas quadráticas significativas devido a variação do pH da solução nutritiva para todas as características avaliadas. O valor de pH que resultou em crescimento máximo variou entre 5.0 e 6.0 conforme a característica estudada. Os genótipos Victoria, Esmeralda, Crioula e F-708 apresentaram desempenho superior, quando os dados foram agrupados para todos os valores de pH estudados. Os resultados indicam que o crescimento inicial é afetado pela variação de pH da solução nutritiva e que genótipos contrastantes de alfafa tendem a mostrar comportamento similar.

Palavras chave: *Medicago sativa* L., estresse abiótico, recursos genéticos, fitotoxidez.

Received: 15 October 2010 **Accepted:** 29 March 2011

Introduction

Alfalfa (*Medicago sativa* L.) is considered the most important forage legume in the world with nearly 32 million hectares cultivated chiefly in temperate regions (Mizukami et al., 2006; Du et al., 2009). In Brazil, the crop is being increasingly utilized in animal production systems, due to its high quality forage and satisfactory yield (Ferreira et al., 2008). However, expansion of alfalfa growing in tropical areas is hampered by several constraints, mostly associated with climatic and soil parameters, and soil acidity is reported as one of the leading problems (Newman et al., 2007), with special reference to causing failure in the establishment of an effective symbiosis with indigenous and inoculated rhizobia (Mahler, 1983; Papa et al., 1999).

In fact, acid soils represent a major barrier to agricultural production due to the direct effects that pH has on the root environment and plant growth (Fageria, 2001; Kochian et al., 2004; Fageria & Baligar, 2008; Chen et al., 2009). With low pH, root growth is reduced or halted, plasma membrane permeability is increased, and forthright toxicity occurs as H^+ ion concentration builds up (Waisel et al., 2002; Vitorello et al., 2005; Chen et al., 2009). Besides, toxic levels of aluminum (Al) and manganese (Mn), and deficient levels of calcium (Ca), magnesium (Mg) and phosphorus (P) are frequent under those conditions (Kochian et al., 2004; Le et al., 2008). Optimal pH values are predominantly reported between 5.5 and 6.5 and below or above this range root damage tends to occur, causing overall growth inhibition (Passos et al., 1999; Fageria, 2001; Kochian et al., 2004; Chen et al., 2009; Fageria et al., 2009; Moreira & Fageria, 2010). In the particular case of alfalfa, liming has been recommended whenever applicable so as to raise soil pH to around 6.0 (Gomes et al., 2002; Díaz & Gambaudo, 2007; Fageria et al., 2009).

The examination of plant growth alterations in response to pH variation is important in terms of tagging a removable source of restrained crop production and prospectively identifying genetic sources of tolerance to acid soils condition (Waisel et al., 2002; Fageria et al., 2005).

Evaluation techniques based on growing plants in nutrient solution under controlled conditions have been widely employed for characterizing the responses of contrasting genotypes to different kinds of stress (Köpp et al., 2006; 2007a). Although such evaluations do not reproduce the actual environmental pressure that exists under field plot research (Sreenivasulu et al., 2007), the latter, in turn, generates a considerable amount of experimental error, because of its greater number of uncontrolled variables (Waisel et al., 2002). Despite such dissimilarity, significant correlations between parameters obtained in field tests and those from artificial setups are reported for several plant species exposed to various types of abiotic stress (Sreenivasulu et al., 2007). Therefore, the investigation of the effects of medium pH on plant growth is expected to be feasible through the utilization of nutrient solution and controlled environment. In addition to that, due to the more precise establishment of pH and other variables, nutrient solution formulations are essential for further evaluations of toxicity in the rhizosphere, such as those produced by aluminum (Cheng et al., 2004; Langer et al., 2009).

The objective of this work was to verify the effects of nutrient solution pH variation on growth parameters of young seedlings of alfalfa. Twelve cultivars were chosen for the evaluations, based on cultivar recommendations by Oliveira & Oliveira (1999) for milk production systems.

Material and Methods

Seeds of alfalfa cvs. Alfa 200, Alto, Araucana, Costera, Crioula, Esmeralda, Falcon, F 708, Rio, Romagnola, Valley Plus and Victoria were placed in vermiculite (22 °C, darkness) and allowed to germinate. Two days after germination, six uniform seedlings of each cultivar were selected, placed in supporting lids, and immediately transferred to 2 L plastic containers with aerated nutrient solution. After 24 h, each 12-cultivar seedling group was exposed to fresh aerated nutrient solution, with pH altered to 4.0, 5.0, 6.0 and 7.0. Subsequently, pH values were checked twice a day and adjusted with HCl 1 mol L^{-1} or NaOH 1 mol L^{-1}, whenever needed. Solutions were replaced every other day and regularly brought to volume with distilled water.

Nutrient solution composition consisted of 1.5 mmol L^{-1} $Ca(NO_3)_2$, 1 mmol L^{-1} K_2HPO_4, 1 mmol L^{-1} KH_2PO_4, 1 mmol L^{-1} $MgSO_4$, 0.5 mmol L^{-1} NH_4NO_3, and micronutrients (0.32 µmol L^{-1} $CuSO_4$, 60.65 µmol L^{-1} H_3BO_3, 0.52 µmol L^{-1} MoO_3, 11.37 µmol L^{-1} $MnCl_2$, and 1.15 µmol L^{-1} $ZnSO_4.7H_2O$). In addition to that, Fe-EDTA was added to provide 89.5 mmol L^{-1} Fe (Passos, 1996).

Plants were grown for 12 days and subsequently harvested for the evaluations. Manipulations were carried out under controlled conditions (Biotronette Mark III environmental chamber, LAB-LINE Instruments), set at 26°C, 60% RH, 16 h photoperiod and 200 mol s^{-1} m^2 photosynthetically active radiation (PAR, measured with LI-190SA quantum sensor and LI-189 quantum meter, LI-COR). The experiments were carried out as a randomized block design, considering a 12 (cultivars) x 4 (pH values) factorial, with 6 replications.

Data were collected on fresh weight (FW, g) of leaf blade (LFW), and on FW and length (L, cm) of the epicotyl (EL and EFW), hypocotyl (HL and HFW), first leaf petiole (FL and FFW), trifoliate leaf petiole (TL and TFW) and root (RL and RFW). Length evaluations were carried out with a precision ruler and weighing measurements were performed in a digital analytical scale. Experimental data were statistically analyzed through ANOVA, regression establishment and Scott-Knott grouping test with

SAS software (Statistical Analysis System, 2002).

Results and Discussion

The analysis of variance revealed no significant effect of the 'cultivar X pH' interaction, demonstrating a similar behavior of all studied genotypes to the varying pH in the growing medium. The result suggests that there is no genetic variability among the studied alfalfa cultivars relative to pH variation in the nutrient solution. On the other hand, there were significant effects of nutrient solution pH on growth of alfalfa seedlings. Length (Figure 1) and fresh weight (Figure 2) data pooled for all the studied cultivars revealed quadratic responses in all evaluated plant organs (R^2 ranging from 0.7127 to 0.9995). The magnitude of such effects and also the most suitable pH for growth varied among organs, but, in each one of the latter, trends for L and FW were similar, excepting epicotyls and leaf blades (in which length was not measured). In fact, RL and RFW exhibited maximal values at pH 6.0; HL, HFW, FL and FFW at pH near 5.5; and TL and TFW at pH slightly above 5.0. In contrast, while EL yielded superior results for a pH value slightly below 6.0, EFW was maximal at a pH near 5.0. Finally, LFW showed the best performance in pH variation between 5.5 and 6.0. In all cases, these growth-related values were severely depressed as pH variation was altered towards the extremes of the range.

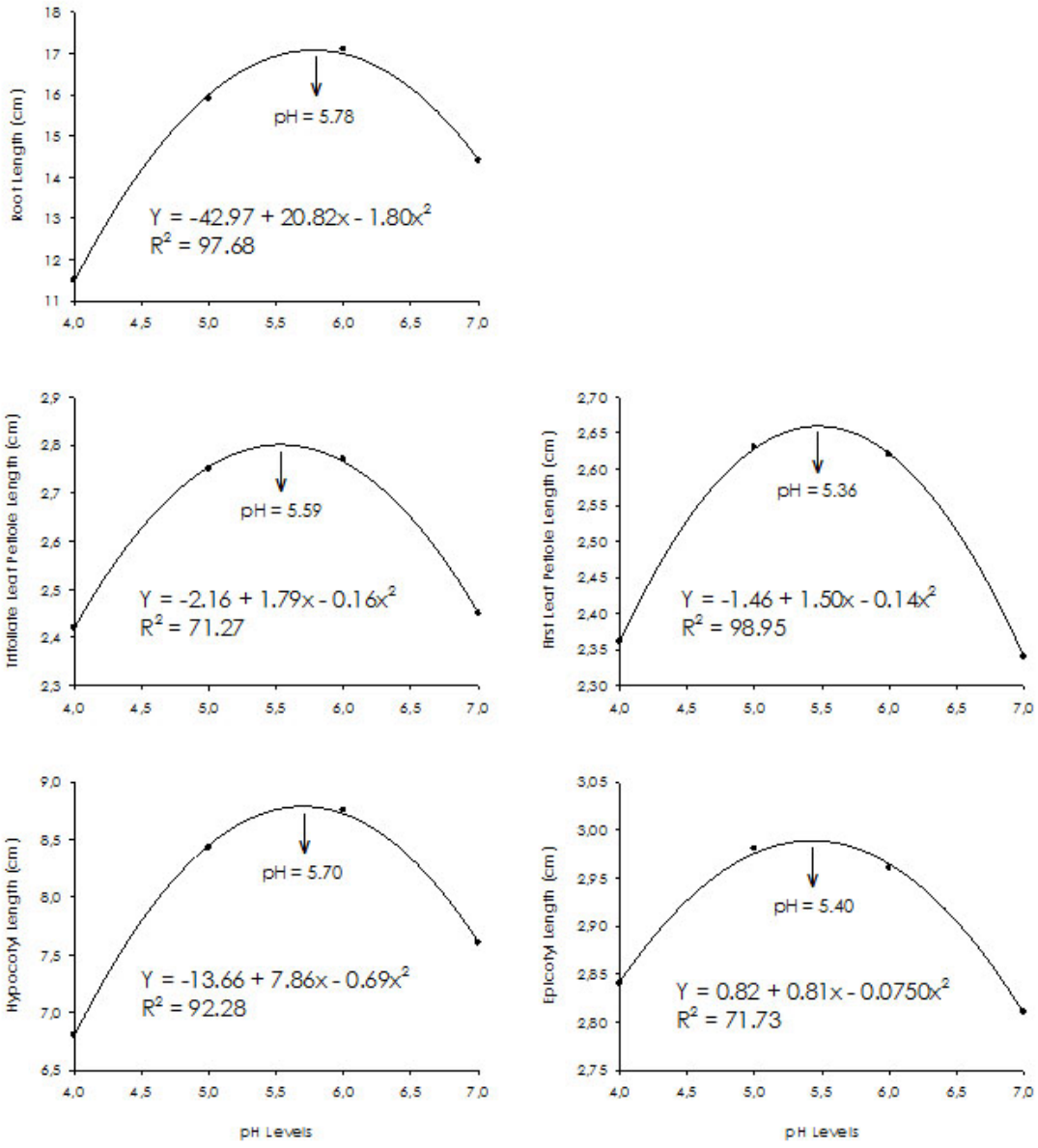

Figure 1. Length responses of alfalfa root, trifoliate leaf petiole, first leaf petiole, hypocotyl and epicotyl to varying nutrient solution pH variation.

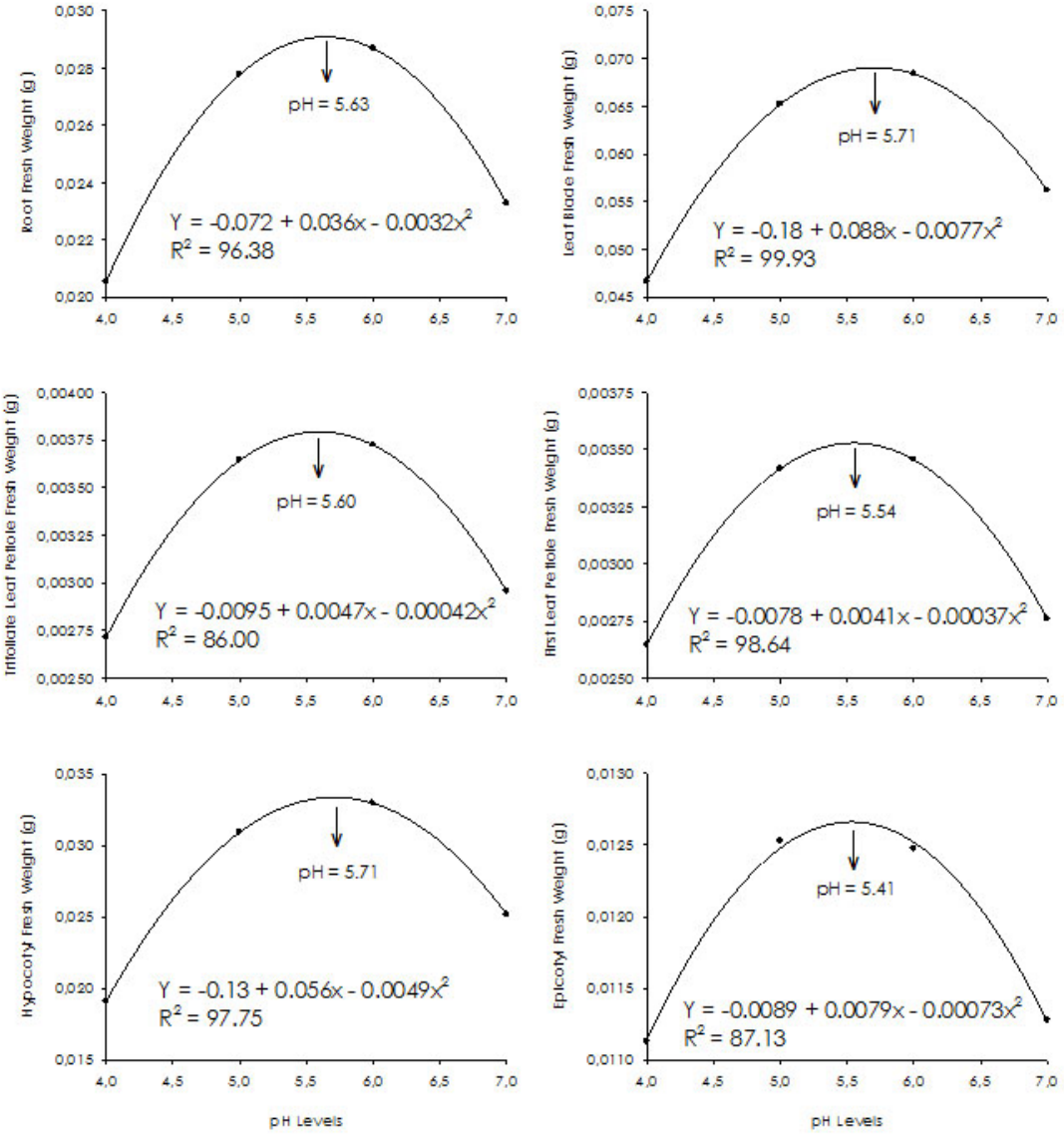

Figure 2. Fresh weight responses of alfalfa root, leaf blade, trifoliate leaf petiole, first leaf petiole, hypocotyl and epicotyl to varying nutrient solution pH variation.

Pooling data for all studied pH values revealed significant differences among genotypes. Regarding length of organs (Table 1), greater contrasts were found in roots, with cvs. Victoria, Araucana, Falcon, F-708, Crioula and Valley Plus showing superior results than Rio, Alto, Alfa 200, Esmeralda, Costera and Romagnola. A superior cluster (cvs. Victoria, Araucana, Falcon, F-708, Crioula, Rio, Alto, Alfa 200, and Esmeralda) was also observed with hypocotyls. No significant differences among genotypes were found in length of epicotyls, first leaf petioles or trifoliate leaf petioles.

Relative to FW, differences among cultivars were detected in all studied organs (Table 2). Performance of cv. Victoria was the most remarkable, since it ranked first cluster in all cases. Also, cvs. Crioula, Esmeralda, and F-708 exhibited outstanding behavior, because they ranked first cluster in four of the evaluated organs,

Table 1. Length (cm) of seedling organs of alfalfa genotypes exposed to different nutrient solution pH variation (4.0, 5.0, 6.0 and 7.0)[1].

Cultivar	Root	Epicotyl	Hypocotyl	FL[2] Petiole	TF[3] Petiole
Victoria	16.65 A	3.04 A	8.87 A	2.83 A	3.20 A
Araucana	16.42 A	2.95 A	7.63 A	2.68 A	2.65 A
Falcon	16.25 A	2.99 A	7.85 A	2.24 A	2.40 A
F-708	16.00 A	3.08 A	7.90 A	2.56 A	2.80 A
Crioula	15.00 A	3.24 A	9.48 A	2.46 A	2.46 A
Valley Plus	15.23 A	2.89 A	6.85 B	2.42 A	2.44 A
Rio	14.17 B	2.92 A	7.53 A	2.40 A	2.43 A
Alto	13.74 B	2.71 A	7.93 A	2.41 A	2.54 A
Alfa 200	13.62 B	2.73 A	8.71 A	2.37 A	2.58 A
Esmeralda	13.09 B	2.83 A	8.74 A	2.72 A	2.68 A
Costera	12.59 B	2.67 A	6.03 B	2.36 A	2.60 A
Romagnola	12.14 B	2.81 A	6.50 B	2.50 A	2.37 A

[1] For each column, means followed by the same letter are not significantly different by the Scott-Knott grouping test; [2] First leaf; [3] Trifoliate leaf.

with special reference to the roots. In turn, the poorest behavior was observed with cvs. Costera, Rio, Romagnola and Valley Plus, which have ranked second cluster in all cases. The cv. Valley showed a distinct behavior, yielding a superior-class root length, but a contrastingly low root FW. The similar pattern observed in organ responses clearly indicates that optimal pH for growth of alfalfa seedlings ranges from 5.0 to 6.0. Besides, extreme pH values markedly inhibit growth, or as a reduction of pH below 4.0 or increase it to 7.0, within the zone of optimal pH, caused sharp reductions in length and FW of all organs. For evaluations of initial sensitivity of alfalfa genotypes to pH levels, it is reasonable to consider root length as the most feasible parameter to be taken into account, because of the rapidity of its measurement and lesser possibility of tissue damage. For field evaluations in which root systems are not to be disturbed, leaf blade FW appears to be the most adequate parameter to be examined.

Table 2. Fresh weight (g) of seedling organs of alfalfa genotypes exposed to varying nutrient solution pH variation (4.0, 5.0, 6.0 and 7.0)[1].

Cultivar	Leaf	Root	Epicotyl	Hypocotyl	FL[2] Petiole	TF[3] Petiole
Victoria	0.7194 A	0.3140 A	0.1490 A	0.33587 A	0.3861 A	0.4474 A
Araucana	0.5540 B	0.2478 B	0.1163 B	0.25325 B	0.3396 A	0.3846 A
Falcon	0.6102 A	0.2437 B	0.1176 B	0.2893 A	0.2712 B	0.2917 B
F-708	0.6345 A	0.2869 A	0.1219 B	0.2827 A	0.3087 B	0.3846 A
Crioula	0.6387 A	0.2950 A	0.1440 A	0.3272 A	0.3083 B	0.3013 B
Valley Plus	0.5439 B	0.2443 B	0.1212 B	0.2273 B	0.2927 B	0.2886 B
Rio	0.5652 B	0.2275 B	0.1165 B	0.2339 B	0.2704 B	0.2704 B
Alto	0.5859 A	0.2402 B	0.1137 B	0.2585 B	0.2735 B	0.3274 B
Alfa 200	0.5994 A	0.2430 B	0.1114 B	0.3014 A	0.2929 B	0.3224 B
Esmeralda	0.6494 A	0.2874 A	0.1192 B	0.3361 A	0.3661 A	0.3422 B
Costera	0.4384 B	0.1612 B	0.1023 B	0.1739 B	0.2509 B	0.3109 B
Romagnola	0.5192 B	0.1866 B	0.0855 B	0.1889 B	0.2704 B	0.2704 B

[1] For each column, means followed by the same letter are not significantly different by the Scott-Knott grouping test; [2] First leaf, [3] Trifoliate leaf.

The detected optimal pH range approaches or matches results reported previously for several species (Passos et al., 1999; Islam et al., 1980; Kochian et al., 2004; Epstein & Bloom, 2005; Fageria et al., 2005). The suggested specific pH confirms observations with alfalfa exposed to inorganic nitrogen or inoculated with nodule bacteria (Correa et al., 2001). However, it differs from the optimal pH (5.0) observed to have the maximum effect on growth peaks in potato (Cao & Tibbitts, 1994) and elephantgrass (Passos et al., 1999) under controlled environments. This difference is likely to be caused by inherent morphological and physiological profiles of the considered species.

The occurrence of growth inhibition below pH 5.0 confirms results verified with other species (Passos et al., 1999; Waisel et al., 2002; Köpp et al., 2007; Fageria & Baligar, 2008; Fageria et al., 2009). The detrimental effects found in pH level above 6.0 corroborate reports regarding depressed root expansion and root and shoot FW (Tang et al., 1993; Tang & Robson, 1993; Fageria et al., 2005; Epstein & Bloom, 2005). However, this contrasts with previous field recommendations for maintaining pH of alfalfa rhizosphere around 6.0 (Gomes et al., 2002; Diáz & Gambaudo, 2007; Fageria et al., 2009). In addition to that, a recent work has shown that maximal alfalfa production under tropical conditions is obtained with soil pH at 5.4 (Moreira & Fageria, 2010). Notwithstanding, it should be pointed out that the optimum pH in field conditions can vary with soil texture, organic matter and other chemical properties of soils, and that intensive liming may cause yield depressions, depending on application rates and relative composition of some essential elements (Fageria, 2001; Epstein & Bloom, 2005; Godsey et al., 2007; Chen et al., 2009). Finally, the reduced availability of metal minor nutrients that tend to betide in growing media showing higher pH may cause diverse responses among plant species or ecotypes within the same species (Kochian et al., 2004; Epstein & Bloom, 2005; Fageria et al., 2005; Fageria & Baligar, 2008).

Distinct responses of genotypes to pH variation in root medium have been verified in some plant species (Passos et al., 1999; Köpp et al., 2007). In alfalfa, Buss et al. (1975) noticed differences among three clones in the nutritional status of seven essential elements, as soil pH was lowered. In our study, such interactions were not detected, probably because modern alfalfa cultivars, albeit being synthetic and broad-based with more than 100 parents each, lack the introduction of more complex traits, so that continued alfalfa breeding remains recommended for gains in adaptation into hostile environments such as saline or acidic soils or in the presence of high levels of aluminum (Waisel et al., 2002; Kochian et al., 2004; Godsey et al., 2007; Fageria et al., 2009). However, considering the extent of the known alfalfa germplasm, further investigations on ecotypes aiming at pH sensitivity should not be ruled out. For such an approach, the selection of the most suitable parameters to allow rapid and low-cost genotype selection should be emphasized.

Conclusions

Nutrient solution pH variation significantly affected growth of alfalfa seedlings with best responses occurring in the pH range from 5.0 to 6.0, according to maximal length and fresh weight of root, hypocotyl, epicotyl, first leaf petiole, trifoliate leaf petiole and leaf blade. Outside this range, growth was progressively inhibited towards extreme pH values.

Determinations of root length should be the priority in evaluations of alfalfa genotypes regarding sensitivity to pH, allowing rapidity and lesser chances of tissue damage. Regarding

verifications in the aerial part, leaf blade FW appears to be the most adequate parameter to be used.

The cultivars examined in this study differed in growth, with cvs. Victoria, Esmeralda, Crioula and F-708 exhibiting superior performance, but did not show differential behavior concerning pH level alterations. However, the extent of the known alfalfa germplasm apparently justifies additional efforts, through broader ecotype screening and searching of suitable selection parameters.

Acknowledgements

The authors are grateful to Mr. Sebastião de Castro Evaristo for his assistance during the study.

References

Buss, G.R., Lutz, J.A., Hawkins, G.W. 1975. Effect of soil pH and plant genotype on element concentration and uptake by alfalfa. *Crop Science* 15: 614-617.

Cao, W., Tibbitts, T.W. 1994. Responses of potatoes to solution pH levels with different forms of nitrogen. *Journal of Plant Nutrition* 17: 109-126.

Chen, G., Li, G.D., Conyers, M.K., Cullis, B.R. 2009. Long-term liming regime increases prime lamb production on acid soils. *Experimental Agriculture* 45: 221-234.

Cheng, Y., Howieson, J.G., O'hara, G.W., Watkin, E.L.J., Souche, G., Jaillard, B., Hinsinger, P. 2004. Proton release by roots of *Medicago mure*x and *Medicago sativa* growing in acidic conditions, and implications for rhizosphere pH changes and nodulation at low pH. *Soil Biology & Biochemistry* 36: 1357-1365.

Correa, O.S., Aranda, A., Barneix, A.J. 2001. Effects of pH on growth and nodulation of two forage legumes. *Journal of Plant Nutrition* 24: 1367-1375.

Díaz, M.Z., Gambaudo, S. 2007. Fertilización y encalado em alfafa. In: Basigalup DH (Ed.) *El Cultivo de Alfafa en la Argentina*. INTA, Buenos Aires, p. 227-246.

Du, W.H., Tian, X.H., Cao, Z.Z., Humphries, A. 2009. Effects of micronutrients on seed yield and yield components of alfalfa. *Journal of Plant Nutrition* 32: 809-820.

Epstein, E., Bloom, A.J. 2005. *Mineral Nutrition of Plants: Principles and perspectives*. Sinauer Associates, Sunderland, USA, 400 p.

Fageria, N.K. 2001. Improving nutrient use efficiency of annual crops in Brazilian acid soils for sustainable crop production. *Communications in Soil Science and Plant Analysis* 32: 1303-1319.

Fageria, N.K., Baligar, V.C. 2008. Ameliorating soil acidity of tropical oxisols by liming for sustainable crop production. *Advances in Agronomy* 99: 345-399.

Fageria, N.K., Baligar, V.C., Clark, R.B. 2005. *Physiology of Crop Production*. Haworth Press, New York, 345 p.

Fageria, N.K., Baligar, V.C., Li, Y.C. 2009. Differential soil acidity tolerance of tropical legume cover crops. *Communications in Soil Science and Plant Analysis* 40: 1148-1160.

Ferreira, R.P., Rassini, J.B., Rodrigues, A.A., Freitas, A.R., Camargo, A.C., Mendonça, F.C. 2008. *Cultivo e Utilização da Alfafa nos Trópicos*. Embrapa Pecuária Sudeste, São Carlos, 469 p.

Godsey, C.B., Pierzynski, G.M., Mengel, D.B., Lamond, R.E. 2007. Changes in soil pH, organic carbon, and extractable aluminum from crop rotation and tillage. *Soil Science Society of America Journal* 71: 1038-1044.

Gomes, F.T., Borges, A.C., Neves, J.C., Fontes, P.C.R. 2002. Influência das doses de calcário com diferentes relações cálcio:magnésio na produção de matéria seca e na composição mineral de alfafa. *Pesquisa Agropecuária Brasileira* 37: 1779-1786.

Islam, A.K.M.S., Edwards, D.G., Asher, C.J. 1980. pH optima for crop growth Results of a flowing solution culture experiment with six species. *Plant and Soil* 54: 339-357.

Kochian, L.V., Hoekenga, O.A., Piñeros, M.A. 2004. How do crop plants tolerate acid soils? Mechanisms of aluminum tolerance and phosphorous efficiency. *Annual Review of Plant Biology* 55: 459-493.

Köpp, M.M., Coimbra, J.L.M., Luz, V.K., Sousa, R.O., Carvalho, F.I.F., Oliveira, A.C. 2006. Organic acid tolerance in M_3 families of oat mutants. *Crop Breeding and Applied Biotechnology* 7: 59-66.

Köpp, M.M., Coimbra, J.L.M., Luz, V,K., Farias, D.R., Carvalho, F.I.F., Oliveira, A.C. 2007a. Butyric acid tolerance in mutant M_4 rice families. *Crop Breeding and Applied Biotechnology* 7: 373-380.

Köpp, M.M., Luz, V.K., Silva, V.N., Coimbra, J.L.M., Maia, L.C., Carvalho, F.I.F., Oliveira, A.C. 2007b. Efeito do pH da solução nutritiva na fitotoxidez causada por ácidos orgânicos em arroz. *Magistra* 19: 40-46.

Langer, H., Cea, M., Curaqueo, G., Borie, F. 2009. Influence of aluminum on the growth and organic acid exudation in alfalfa cultivars grown in nutrient solution. *Journal of Plant Nutrition* 32: 618-628.

Le, T.M.H., Collins, R.N., Waite, T.D. 2008. Influence of metal ions and pH on the hydraulic properties of potential acid sulfate soils. *Journal of Hydrology*

356: 261-270.

Mahler, R.L. 1983. Influence of pH on yield and N and P nutrition of alfalfa grown on an andic mission silt loam. *Agronomy Journal* 75: 731-735.

Mizukami, Y., Kato, M., Takamizo, T., Kanbe, M., Inamil, S., Hattori, K. 2006. Interspecific hybrids between *Medicago sativa* L. and annual *Medicago* containing alfalfa weevil resistance. *Plant Cell, Tissue and Organ Culture* 84: 79-88.

Moreira, A., Fageria, N.K. 2010. Liming influence on soil chemical properties, nutritional status and yield of alfalfa grown in acid soil. *Revista Brasileira de Ciência do Solo* 34: 1231-1239.

Newman, Y.C., Sinclair, T.R., Blount, A.S., Lugoc, M.L., Valencia, E. 2007. Forage production of tropical grasses under extended day length at subtropical and tropical latitudes. *Environmental and Experimental Botany* 61: 18-24.

Oliveira, P.P.A., Oliveira, W.S. 1999. Estabelecimento da cultura. In: Peixoto, A.M., Moura, J.C., Silva, S.C., Faria, V.P. (Ed.) *Alfafa*. FEALQ, Piracicaba, p. 67-93.

Papa, M.F., Balangué, L.J., Sowinski, S.C., Wegener, C., Segundo, E. 1999. Isolation and characterization of alfalfa-nodulating rhizobia present in acidic soils of central Argentina and Uruguay. *Applied and Environmental Microbiology* 64: 1420-1427.

Passos, L.P., Vidigal, M.C., Pereira, A.V., Ferreira, R.P., Verneque, R.S. 1999. Responses of elephantgrass to nutrient solution pH levels. *Journal of Plant Nutrition* 22: 701-708.

Passos, L.P. 1996. *Métodos analíticos e laboratoriais em fisiologia vegetal*. Embrapa Gado de Leite, Coronel Pacheco, Brasil. 223 p.

Sreenivasulu, N., Sopory, S.K., Kavi-Kishor, P.B. 2007. Deciphering the regulatory mechanisms of abiotic stress tolerance in plants by genomic approaches. *Gene* 388: 1-13.

Statistical Analysis System-SAS. 2002. *Statistical Analysis System - Getting Started with the SAS Learning Edition*. SAS Institute, Cary, 86 p.

Tang, C., Kuo, J., Longnecker, N.E., Thomson, C.J., Robson, A.D. 1993. High pH causes disintegration of the root surface in *Lupinus angustifolius* L. *Annals of Botany* 71: 201-207.

Tang, C., Robson, A.D. 1993. pH above 6.0 reduces nodulation in *Lupinus* species. *Plant and Soil* 152: 269-276.

Vitorello, V.A., Capaldi, F.R., Stefanuto, V.A. 2005. Recent advances in aluminum toxicity and resistance in higher plants. *Brazilian Journal of Plant Physiology* 17: 129-143.

Waisel, Y., Eshel, A., Kafkafi, U. 2002. *Plant roots: the hidden half*. M. Dekker, New York, 1136 p.