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EFFECT OF GROWING MEDIUM pH ON GERMINATION AND INITIAL DEVELOPMENT OF SOME GRASSLAND PLANTS

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Summary. The effect of pH of the growing medium on germination and initial development phases of seedlings of four plant species: *Festuca pratensis* Huds. and *Dactylis glomerata* L., *Trifolium repens* L. and *Medicago sativa* ssp. *falcata* × ssp. *sativa* L. in hydroponic cultures was investigated. Hoagland growing media with pH: 6.5; 6.0; 5.5; 5.0; 4.5; 4.0 were used. In this study we determined the germination energy and capacity of the seeds, the length of green part and fresh and dry matter of seedlings after one month of growth. The obtained results were analysed statistically, which involved making the one-direction analysis of variance and building regression equations. A negative effect of the growing medium acidity on studied features of seedlings was observed, whereas in the case of germination it referred mainly to pH = 4.0. Reduction in pH had the greatest effect on seedling dry matter, particularly in the case of *Trifolium repens* and *Medicago sativa*.

Keywords: alfalfa, cocksfood, growing medium pH, hydroponic culture, meadow fescue, white clover

INTRODUCTION

Many species of plants have strictly specified physiological requirements concerning the soil pH. They show particularly clear in agricultural crops. There are many plant species utilized by man, where acid pH is necessary to grow, buy there are also species intolerant of soil acidity. In some cases this results from toxic properties of aluminium ions, responsible for soil hydrolytic acidity [Lima de and Copeland 1990, Samuels *et al.* 1997, Deska and Jankowski 2001, Alamgir and Sufia 2009]. However, sometimes the presence of H^+ ions only has a negative effect on plant development [Slootmaker 1974, Budagovskaya 1995, Chohura *et al.* 2004]. This refers mainly to early phases of plant development, i.e. seed germination and development of seedlings

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[Mayer and Poljakoff-Mayber 1989, Carver and Ownby 1995, Jankowski *et al.* 2000]. The study by Marschner [1991] proves that in the case of some plants their growth in acid soil is possible, but seed germination must take place in less acidified environment. This phenomenon is caused by the need for maintaining the appropriate pH of the soil solution for amylolityc enzymes initiating germination [Lee 1998]. Acid soil pH also determines the availability of macro- and microelements necessary for plant development [Kabata-Pendias and Pendias 1999].

Acid soil pH stimulates initial development phases of some species. These include plants with thick seed coats [Turner *et al.* 1988, Vleeshouwers *et al.* 1995, Yost 2000]. The effect of acid pH may be direct, manifesting itself in dissolving of the seed coat or indirect, which involves stimulating conditions for development of some species of fungi whose action causes perforation of the seed coat [Vleeshouwers *et al.* 1995].

The knowledge of the optimal pH for plant seed germination is important in the case of species sown in mixtures and particularly on grasslands. Therefore, the aim of this study was to estimate the effect of pH of the growing medium on the germination and initial development stage of some species of monocotyledonous and dicotyledonous plants, commonly used in mixtures for green forage production.

MATERIAL AND METHODS

A laboratory experiment carried out in hydroponic cultures was established in 2008 as one-factorial in the complete randomized design with 5 replications. The experiment involved testing 4 plant species: 2 – from monocotyledons: meadow fescue (*Festuca pratensis* Huds.) of the cultivar Skra and cocksfoot (*Dactylis glomerata* L.) of the cultivar Tukan and 2 species of dicotyledonous plants: white clover (*Trifolium repens* L.) of the cultivar Podkowa and alfalfa (*Medicago sativa* ssp. *falcata* × ssp. *sativa* L.) of the cultivar Kometa. Seeds of those plant species are often sown in mixtures on permanent grasslands. The studied factor was the pH value of the growing medium. In this experiment, 6 levels of pH value were applied: 6.5; 6.0; 5.5; 5.0; 4.5; 4.0. The pH of growing media was regulated using appropriate amounts of 1.0 and 0.1M H_2SO_4 to prepare it. The pH value in the course of growth was controlled using a pH-meter and where necessary, the solutions were acidified with appropriate amounts of 0.01M H_2SO_4. Before sowing, plant seeds were subjected to disinfection for 24 hours by bath in C_2H_5OH and 8 hours in 3% solution of $HClO_4$ [Dąbrowska *et al.* 2000].

Disinfected seeds were placed in an amount of 50 pieces per 1 crystallizer on a cotton mesh at 2.5 cm intervals. Testing the energy and capacity of seedlings was carried out in accordance with the guidelines comprised in ISTA [2003], using for seed moistening water solutions of H_2SO_4 with concentrations corresponding to pH: 6.0; 5.5; 5.0; 4.5; 4.0, as well as distilled water with pH = 6,5. Seed germination and seedling growth were carried out at 20^{o}C. After the second reading crystallizers were filled up with the Hoagland medium. Seedlings were kept up to 1 month from the plant emergences. Then they were removed from the supporting gauze and a measurement of the green part length and the fresh matter of whole seedlings was made, and after drying for 24 h at 105^{o}C measurements of their dry matter were carried out (Fig. 1).

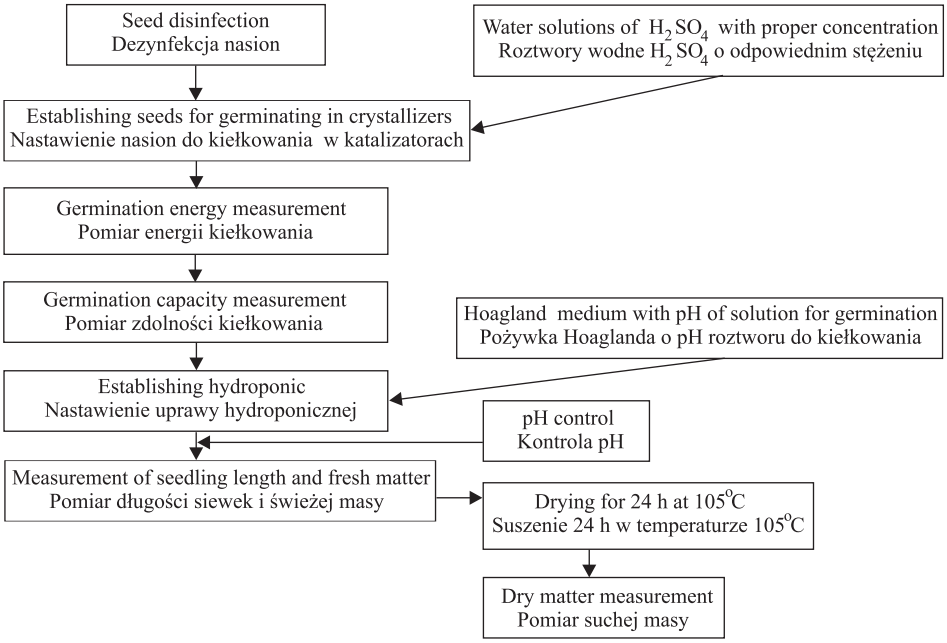

Fig. 1. Scheme of the conducted experiment
Rys. 1. Schemat przeprowadzonego doświadczenia

The complete results of the measurements were listed, comparing the average values and standard deviation for species, using the program Statistica 7 [Stanisz 2000]. For individual features of all the studied plant species also regression equations for successive levels of pH were calculated, according to the method adopted by Krzysztofiak and Urbanek [1979]. Regression equations of the lowest degree, which described the occurred relationships well enough, were used for the description. The program Microsoft Office Excel 2003 was used to build the equation.

RESULTS AND DISCUSSION

The germination energy of the studied seeds showed significant interspecific differences (Table 1). The highest value (83.9%), significantly different from others, was showed by seeds of *Festuca pratensis*, and the lowest (56.0%), significantly different from the other seeds, the seeds of *Dactylis glomerata*. Seed germination energy of both species of Fabaceae did not differ significantly from the others and was: 70.9% for *Medicago sativa* and 75.5% for *Trifolium repens*. In the course of final counting, the distribution of germination capacity was sightly different. The lowest value of this feature (70.9%), significantly different from seeds of other species, was observed in *Medicago sativa*, and the next one, significantly different (77.8%) – in *Dactylis glomerata*. For the other species values of this feature amounted to about 88% and did not differ significantly.

Table 1. Germination characteristic of seeds of studied plant species at pH = 6.5
Tabela 1. Charakterystyka kiełkowania nasion badanych gatunków roślin przy pH = 6,5

Plant species Gatunek rośliny	Mean value Wartość średnia $\bar{x}$	Standard deviation Odchylenie standardowe s	x_{min}	x_{max}
Germination energy – Energia kiełkowania, %				
Festuca pratensis	83.9 c	7.42	73	84
Dactylis glomerata	56.0 a	4.65	47	60
Medicago sativa	70.9 b	3.36	66	75
Trifolium repens	75.5 b	1.48	74	78
Germination capacity – Zdolność kiełkowania, %				
Festuca pratensis	87.2 c	7.71	76	97
Dactylis glomerata	77.8 b	5.19	70	84
Medicago sativa	70.9 a	3.30	66	76
Trifolium repens	88.3 c	5.62	82	96

n = 200
a, b, c – mean values marked with the same letters do not differ significantly at $P \leq 0.05$ – wartości średnie oznaczone tymi samymi literami nie różnią się istotnie przy $P \leq 0,05$

Germination capacity is the most important feature of seeds, determining seed material quality [Dąbrowska *et al.* 2000]. It is defined as the percentage share of seeds which are able to form normal seedlings. This is usually a species feature, but it also depends on cultivation factors and the seed age [Mayer and Poljakoff-Mayber 1989]. The appropriate standards [ISTA 2003] show the necessity of counting germinating seeds twice. The first counting, called initial counting or germination energy, is conducted after the minimal time needed for the seeds of the given species to germinate. The second counting, called germination capacity, is carried out after the time when theoretically all the seeds should germinate [Dąbrowska *et al.* 2000].

The obtained values, particularly for *Medicago sativa* and *Dactylis glomerata,* were lower than 90%, which is regarded as the critical value for seed germination [ISTA 2003]. Seeds of *Medicago sativa* were characterized by a particularly low level of germination, which in the case of this species, owing to its hard seeds, is a feature reported also by other authors [Yokota and Ojima 1995].

Young seedling development was highly varied, according to the biology of germination characteristic of the studied species (Table 2). The seedlings of *Festuca pratensis* were characterized by the largest length of the green part (12,54 cm), the seedlings of *Dactylis glomerata* were shorter, also significantly different from the others (9.63 cm), whereas seedlings of the genus *Faba* had the smallest length, not differing statistically (on average for *Trifolium repens* 3.43 cm and *Medicago sativa* 3.82 cm).

Fresh matter of seedlings for treatments with pH = 6.5, where germination took place in distilled water, did not differ significantly (Table 2). Only seedlings of *Dactylis glomerata* were characterized by a significantly lower fresh matter (13.57 mg) than the seedlings of the other plants. Similar proportions were found for seedling dry matter. Seedlings of *Dactylis glomerata* had the smallest dry matter content, which was significantly different from the others (1.41 mg). The seedlings of *Medicago sativa* were characterized by the next significantly different value as compared to the seedlings of the other species (2.61 mg). The highest values were found for *Festuca pratensis* and *Trifolium repens*.

Table 2. Statistical characteristic of studied features of seedlings at pH = 6.5
Tabela 2. Charakterystyka statystyczna badanych cech siewek przy pH = 6,5

Gatunek rośliny Plant species	Mean value Wartość średnia $\bar{x}$	Standard deviation Odchylenie standardowe s	x_{min}	x_{max}
Length of seedling green part – Długość zielonej części siewek, cm				
Festuca pratensis	12.54 c	0.95	11.0	15.5
Dactylis glomerata	9.63 b	1.52	6.5	12.7
Medicago sativa	3.43 a	0.36	2.7	4.2
Trifolium repens	3.82 a	0.68	2.3	5.4
Seedling fresh matter – Świeża masa siewki, mg				
Festuca pratensis	26.01 b	2.37	22.58	29.75
Dactylis glomerata	13.57 a	1.51	11.60	15.75
Medicago sativa	21.98 b	2.00	19.90	25.12
Trifolium repens	25.87 b	2.46	22.58	29.75
Seedling dry matter – Sucha masa siewki, mg				
Festuca pratensis	3.02 c	0.33	2.62	3.56
Dactylis glomerata	1.41 a	0.13	1.25	1.63
Medicago sativa	2.61 b	0.32	2.10	2.99
Trifolium repens	3.00 c	0.39	2.50	3.56

n = 200
a, b, c – mean values marked with the same letters do not differ significantly at $P \leq 0.05$ – wartości średnie oznaczone tymi samymi literami nie różnią się istotnie przy $P \leq 0,05$

Introducing H^+ ions to the solution where seeds germinated and developed resulted in a diversified species response to the applied changes in pH.

Environment acidity up to pH = 5.5 increased the number of germinating seeds of *Dactylis glomerata* (Fig. 2). Only further lowering of the pH value had a negative effect on germination energy, decreasing its value by 20%. Similar effects for this species at a similar pH range of the solution were obtained by Pérez-Fernández *et al.* [2006], although the maximal seed germination was not higher than 43%.

Seeds of *Festuca pratensis* showed different response in comparison with the above mentioned species. Each successive level of the solution acidity had a negative effect on the proportion of germinating seeds of this species, which resulted in a decrease in the number of germinating seeds by 33% in relation to the effects for the optimal pH. The response of *Trifolium repens* seeds to acidifying was more dynamic. A difference between germination at the optimal pH (6.0) and pH = 4.0 was as much as 41%. In the seeds of *Medicago sativa* n turn, the best effects of germination were observed at pH = 6.5. Successive, lower pH values resulted in a decrease in seed germination energy of this species, yet considerably smaller than in the case of *Trifolium repens.*

Counting germinating seeds at the second date allows the assessment of germination also of seeds which are weaker or react stronger to the environmental conditions [ISTA 2003]. In the case of the studied plant species, prolonging the time of germination improved seeds germination mainly at pH = 6.5 and 6.0 (Fig. 3). This did not apply to seeds of *Medicago sativa*, for which at pH lower than 6.0 an increase in germination was small or was not observed. This is described by regression equations of seed germination capacity of those species. This may indicate that seeds with a lower germination potential do not germinate in the acid environment. Similar effects were

observed in rye by Slootmaker [1974]. They were explained by considerable distortions in the action of enzymes initiating processes of cell division at the early stages of sprout formation.

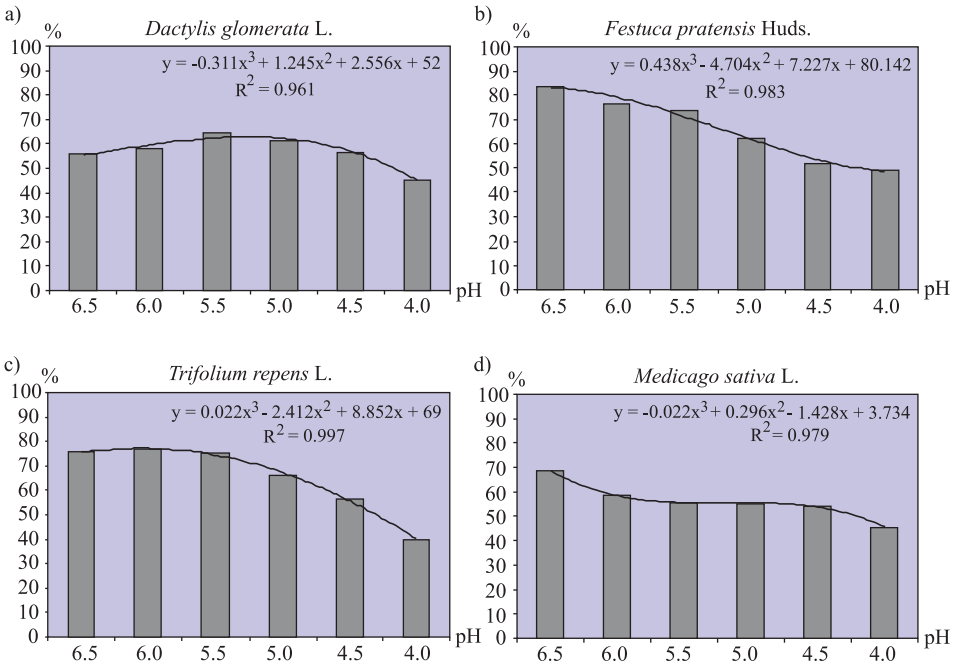

Fig. 2. Relationship between pH of growing medium and germination energy
Rys. 2. Zależność energii kiełkowania od pH pożywki

Unfavourable changes occurring at early stages of germination contribute to a weaker development of plant seedlings [Mayer and Poljakoff-Mayber 1989]. Acid soil pH has also an effect on taking up nutrients by roots during the process of seedling growth, although some cases of the proper growth of plants negatively responding to low pH during germination were observed [Zeigler *et al.* 1995]. In the present study lowering pH from 6.5 to 5.5 resulted in a considerable decrease in the length of seedling green parts in *Dactylis glomerata* and *Medicago sativa* (Fig. 4). Successive lowering pH values did not cause significant changes in seedling length. In the case of *Trifolium repens* a decrease in the length of this part of seedlings was observed at the smallest three values of pH of the growing medium. The highest length of leaves of *Festuca pratensis* seedlings was measured at pH = 5.5, which indicates resistance of this plant to soil acid pH, which was also reported by other authors [Stańko 1961, Kozłowska 2005].

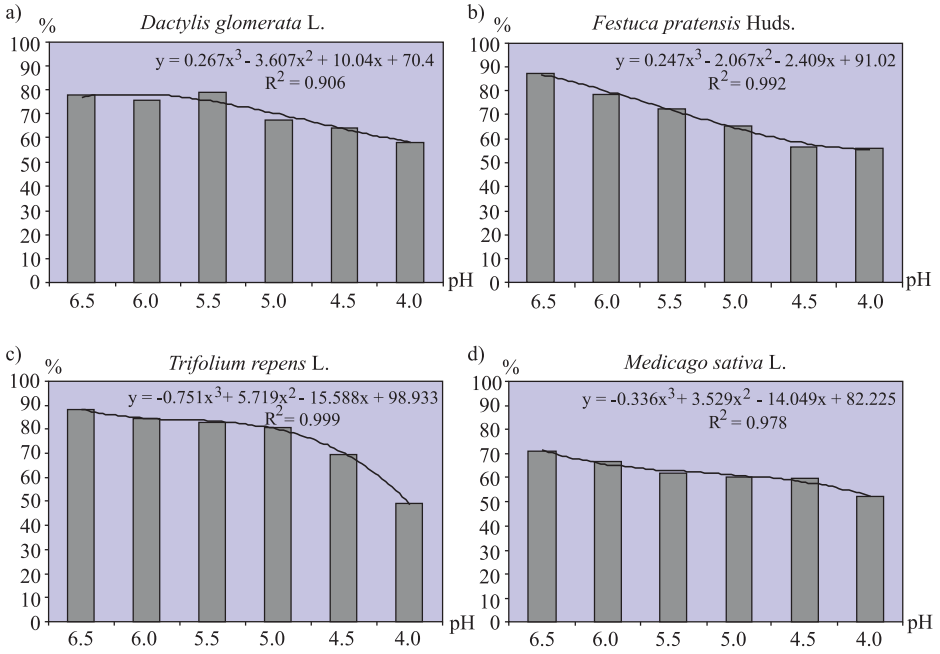

Fig. 3. Relationship between pH of growing medium and germination capacity
Rys. 3. Zależność zdolności kiełkowania od pH pożywki

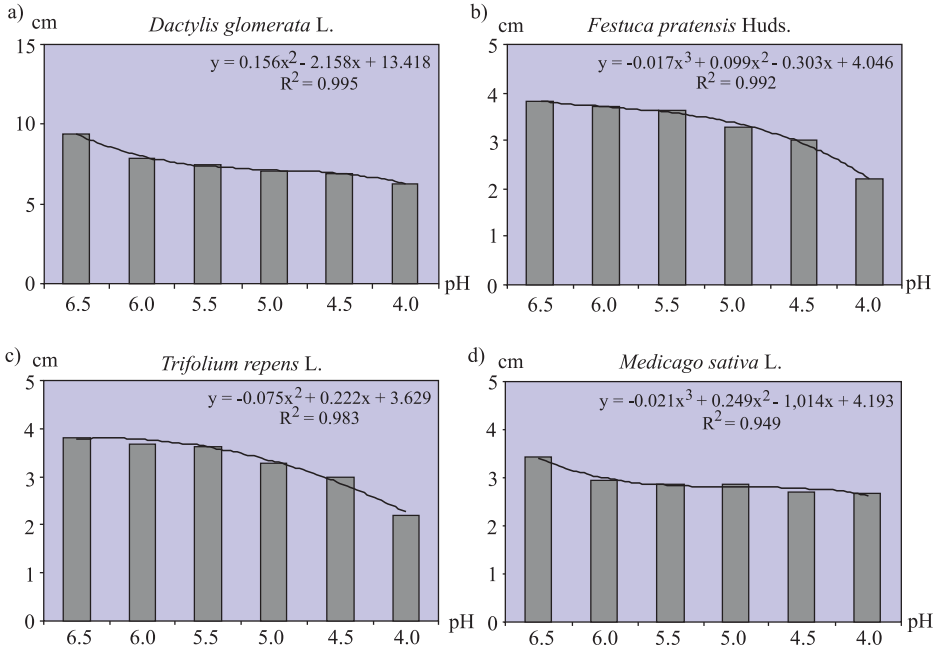

Fig. 4. Relationship between pH of growing medium and length of seedling green part
Rys. 4. Zależność długości części zielonej siewek od pH pożywki

The fresh weight of seedlings reflects both utilization of nutrients from the soil (growing medium) and water uptake and its content in plant cells, which can be also disrupted by the acid pH of the environment of plant growth [Haller 1983]. The fresh weight of seedlings of *Festuca pratensis* and *Trifolium repens* decreased directly proportionally to a decrease in the medium pH to the value = 4.5, which is shown by the mathematical form of the trend curves for those species. The average matter of *Festuca pratensis* seedlings did not differ significantly at pH of the media 4.5 and 4.0. A similar effect occurred in the seedlings of *Medicago sativa*. In the case of *Dactylis glomerata* a decrease in seedling matter was smaller and stayed in accordance with the third degree polynomial (Fig. 5).

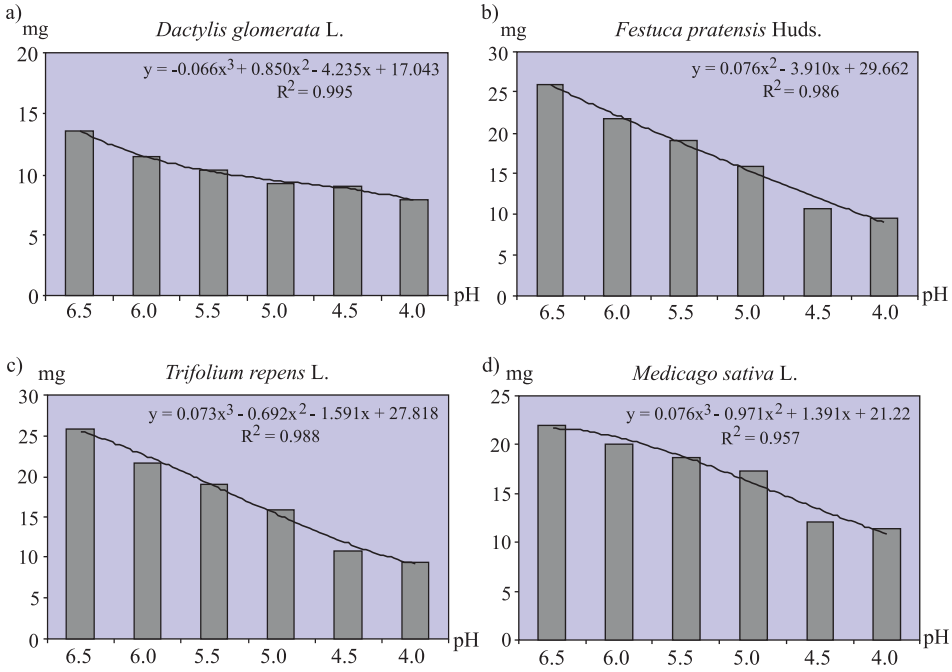

Fig. 5. Relationship between pH of growing medium and seedling fresh matter
Rys. 5. Zależność świeżej masy siewek od pH pożywki

Seedling dry matter reflecting the nutrient uptake may undergo considerable changes under the influence of the soil pH [Haller 1983]. The effect of pH on taking up fertilizer components by plants is described by many authors [Marschner 1991, Budagovskaya 1995, Kabata-Pendias and Pendias 1999], who sometimes also report a direct effect of H^+ ions on a reduction in taking up cations by root cells. This disorganizes cellular processes and consequently, reduces plant matter growth.

Lowering pH of the growing medium significantly reduced the seedling mass of all the studied plant species (Fig. 6), which is shown by regression equation indexes. In the case of every species, the largest decrease in the matter was observed at lowering pH from 6.5 to 6.0. The largest effect of acid pH of the solution on a reduction in seedling matter growth was observed in dicotyledonous plants: *Trifolium repens* (from 3.05 mg to 1.12 mg) and *Medicago sativa* (from 2.64 to 1.25 mg).

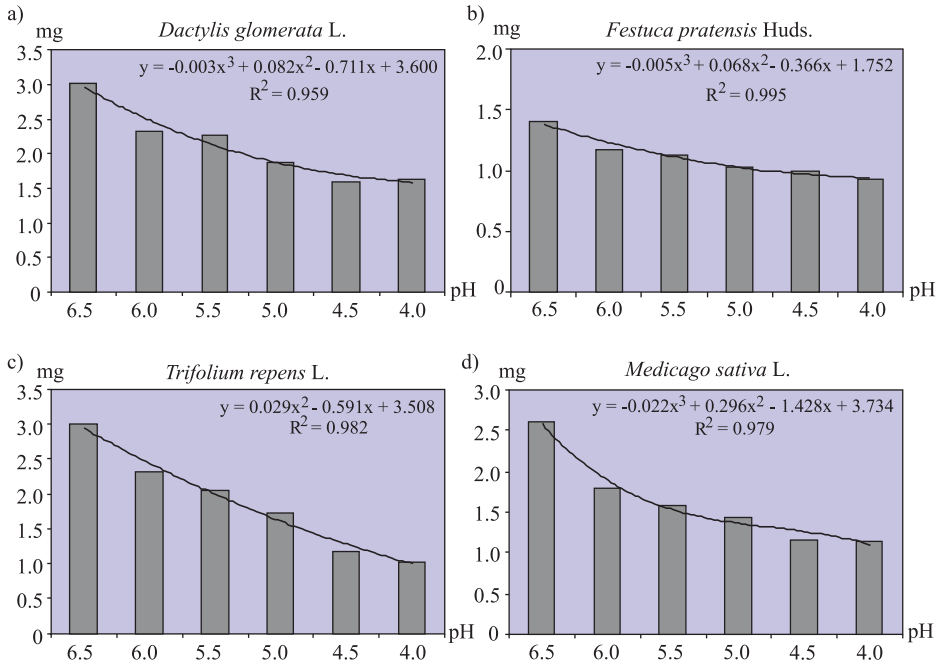

Fig. 6. Relationship of pH of growing medium and seedling dry matter
Rys. 6. Zależność suchej masy siewek od pH pożywki

The obtained results confirm the widely accepted view about a negative effect of too acid pH of the environment on the development of fodder plants. The lack of studies concerning cultivars of fodder plant species grown currently in Poland makes it impossible to more precisely evaluate the obtained results of the study.

In the study by Aleshire and Teutscha [2005] of grasses of the genus *Digitaria*, carried out in the pH range 4.8-6.4, no negative effect of pH on the germination or development of the studied plant seedlings was observed. In the study of germination in *Pisum sativum* carried out by Bukvic *et al.* [2007], a higher seed germination affected by lowering pH to 5.0 was observed, yet a further development of seedlings was better at a higher pH.

Occurring relationships between pH values and the studied seedling features are described most precisely by regression equations of the third degree, which take to consideration a more complex course of the relationship (Figs. 2-6). A large diversification in indexes in the derived regression equations indicates varied responses of plants to changing pH values of the growing medium. The low values of the index at components of the second and third degree and high values of indexes of the first degree, indicating almost rectilinear relationships, were found in the case of the effect of pH on seedling length (Figs. 5a-d).

Varied tolerance of agricultural crops to acidity of soils or growing media is confirmed in studies published at other research centres [Bona *et al.* 1995, Zeigler *et al.* 1995, Benedycki *et al.* 1998, Narro *et al.* 2001, Pérez-Fernández *et al.* 2006]. They indicate a diversification in plant response not only within species but also cultivars. Similar resistance of fodder plants sown in multi-species mixtures to soil acidity is of

particular importance for production, since using plants with different responses to medium acidity in mixture may cause dying out plants with low tolerance towards low pH from the mixture [Howieson 1995].

Although the present study involve 4 species of grassland plants, they show a large diversification in the response of the studied features to the level of the medium acidity. More thorough look at the studied problem will be possible after extending the experiments to other plant species sown on grasslands. The obtained results indicate that caution should be exercised in selection of species sown in mixtures on grassland, where soils are characterized by low pH values.

CONCLUSIONS

1. Studied species of plants grown on grasslands were characterized by diversification in germination capacity and energy as well as the initial development of seedlings, determined by species peculiarities.
2. Lowering pH of the solution reduced germination of seeds of all the studied plant species, assessed both in its initial and final phases. Low pH of the growing medium resulted in a particularly large reduction in germination of seeds of *Trifolium repens*.
3. Low pH of the medium had a negative effect on green part length of the seedlings, particularly in *Trifolium repens*.
4. Largest changes in seedling dry matter were caused by decreasing pH of the growing medium in *Trifolium repens* and *Medicago sativa*.
5. Lowering pH already from 6.5 to 6.0 resulted in a decrease in germination capacity and energy and seedling dry matter in *Festuca pratensis* and *Medicago sativa*.

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WPŁYW PH POŻYWKI NA KIEŁKOWANIE I POCZĄTKOWY ROZWÓJ WYBRANYCH ROŚLIN UŻYTKÓW ZIELONYCH

Streszczenie. W kulturach hydroponicznych badano wpływ odczynu pożywki na kiełkowanie i początkowe fazy rozwoju siewek czterech gatunków roślin: *Festuca pratensis* Huds. i *Dactylis glomerata* L., *Trifolium repens* L. i *Medicago sativa* ssp. *falcata* × ssp. *sativa* L.). Zastosowano pożywki Hoaglanda o pH: 6,5; 6,0; 5,5; 5,0; 4,5; 4,0. W badaniach określono energię i zdolność kiełkowania nasion, długość części zielonej oraz świeżą i suchą masę siewek po miesiącu wegetacji. Uzyskane wyniki poddano analizie statystycznej, wykonując jednokierunkową analizę wariancji oraz budując równania regresji. Stwierdzono negatywny wpływ zakwaszenia pożywki na badane cechy siewek, przy czym w przypadku kiełkowalności dotyczyło to głównie pH = 4,0. Obniżanie pH najsilniej wpływało na suchą masę siewek, szczególnie w przypadku *Trifolium repens* i *Medicago sativa*.

Słowa kluczowe: koniczyna biała, kostrzewa łąkowa, kultura hydroponiczna, kupkówka pospolita, lucerna, odczyn pożywki

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