

Effect of pH and Al³⁺ concentration on growth of spring brewer's barley

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Abstract. The aim of the study was to check reaction of spring brewer's barley seedlings to the pH and aluminium concentration of the growing medium. Seedlings of four cultivars of barley (Madonna, Orthega, Philadelphia and Rasbet) were grown at 4 levels of pH (3, 4, 5 and 6) and under 3 doses of Al³⁺ (0, 150 and 300 μmol dm⁻³). Significant differences in dry matter of roots and shoots were found for the studied cultivars and plants grown at different pH and concentrations of Al³⁺. Cv. Madonna had the highest tolerance to aluminium ions at low pH (3 and 4) of the medium and also the highest chlorophyll content in the leaves among those studied. With an increase of aluminium concentration, phosphorus content in dry matter of the leaves decreased from 0.66% in control plants to 0.52% under 300 μmol Al³⁺ dm⁻³ and the magnesium content decreased from 0.16% in control to 0.12% under 150 μmol Al³⁺ dm⁻³ and 0.10% under 300 μmol Al³⁺ dm⁻³.

Key words: malting barley, aluminium, pH, RGR, chlorophyll

INTRODUCTION

According to Marschner (1995) soil pH is one of the main factors determining soil fertility and the physicochemical state of the soil. Crop yields are much lower than expected when cultivated on acid soils, than one could expect taking into account their mineral content. Additionally, in acidic conditions, there is toxicity of aluminium and manganese ions and a deficit of phosphorus, magnesium and potassium (Anioł, 1981). Deficits of these macroelements inhibit some metabolic processes such as enzyme activity or synthesis of nucleic acids (Marschner, 1995).

The crust of the Earth contains about 7% aluminium (Darko et al., 2004). Aluminium is found in most rocks, excluding limestone and sandstone. At alkaline and neutral pH aluminium is not found in harmful oxides and aluminosilicates, but at pH < 5 it forms toxic Al³⁺ cations (Ma et al., 2001).

Plants can only take free aluminium ions from soils with pH below 5.0 (Borkowska, 1988). Even at that pH level all aluminium cannot be entirely available for plants because organic fraction of the soil can chelate metals. In this way Al³⁺ ions form complexes with galacturonic acid and humin acids.

Maslowski, (1997) observed that under high concentrations of aluminium in soil, plants have shortened root systems, impaired development of aboveground part and

lower yield. They exhibit changes of viscosity and permeability of cytoplasm and cell membranes. Epidermal cells lose their turgor and small cavities are formed. In resistant plants only the epiderma is damaged but, in sensitive plants, the cortex is almost entirely damaged as well (Wagatsuma et al., 1987).

In the presence of aluminium, necrosis on the leaves and dying of shoot tips can be observed, usually due to the effect of phosphorus and calcium deficit. Al^{3+} easily binds P, and unsolved phosphates are formed. Aluminium can form complexes with calmodulin, as well as with other compounds, and when present, can lead to the formation of phytochelatins.

Plants adapt differently to aluminium; differences are observed even in species belonging to the same genus or in cultivars of the same species (Simon et al., 1994; Nunes et al., 1995). For example, sugar beet and barley are sensitive to aluminium but maize is relatively resistant. Resistance occurs when plants limit aluminium uptake or by detoxifying it in the cells. Plants that increase pH level to above pH 5, near their root system, are resistant to aluminium, notably in some wheat, barley, rice, pea and maize cultivars (Foy et al., 1978). Taylor and Foy (1985) suggest that such changes of pH near root systems could be a result of higher uptake of NO_3^- than NH_4^+ . Plants are generally tolerant to aluminium when it is bound by citric acid or when they can uptake P and Ca in the presence of aluminium.

The aim of the work was to study growth, chlorophyll *a* and *b* content and magnesium and phosphorus concentration in the leaves of malting barley grown under different aluminium doses and at different pH levels (pH 3–6).

MATERIALS AND METHODS

Four spring barley cultivars: Madonna, Orthega, Philadelphia and Rasbet were used in the experiment. Four-day old seedlings (60 plants in one combination) were transferred into hydroponic culture with a nutrient solution. Two-thirds of the roots were submerged in the medium and 1/3 were above the medium. Seedlings were grown in liquid medium (0.4 mM CaCl_2 , 0.65 mM KNO_3 , 0.25 mM $\text{MgCl}_2 \times 6 \text{H}_2\text{O}$, 0.01 mM $(\text{NH}_4)_2\text{SO}_4$, 0.04 mM NH_4NO_3 and microelements in concentrations as in Hoagland solution) containing 0, 150 and 300 $\mu\text{mol Al}^{3+} \text{ dm}^{-3}$ ($\text{Al}_2(\text{SO}_4)_3 \times 18 \text{H}_2\text{O}$) and at pH 3, 4, 5 and 6 at 25°C. The nutrient solution was changed every two days. Daily pHs of the mediums were maintained with 1 M HCl or 1 M NaOH.

After 7, 14 and 21 days 18 plants from each combination were taken, dried, and dry matter was determined. Relative Growth Rate (RGR) was calculated using the following equation $(\ln W_2 - \ln W_1) / (t_2 - t_1)$. Results obtained were analyzed with ANOVA with $\alpha = 0.05$ in test of Tukey.

When the plants were 21 days old, chlorophyll was extracted with 80% acetone from the leaves of 9 plants from each combination and the chlorophyll content (mg Chl. g F.W.⁻¹) was measured. Optical density at 645 and 663 nm was measured with the use of spectrophotometer Lambda 11. Concentration of both chlorophylls was calculated using the following formulas: $\text{Chl. } a = (12.7D_{663} - 2.7D_{645})V/1000W$ and $\text{Chl. } b = (22.9D_{645} - 4.7D_{663})V/1000W$ where D_{645} and D_{663} optical density at 645 and 663 nm, V-volume of the solution (cm^3), W-fresh weight of the leaves sample (g). Obtained results were analyzed with ANOVA with $\alpha = 0.05$ in test of Tukey.

For mineralization of plant material, an 0.5 g sample was put into a 100 cm³ vial and 7 cm³ of concentrated sulfuric acid was added. Plant material was mineralized at 440°C for about 4 min, then 30% H₂O₂ was added (altogether 20 cm³). Finally, to dissolved sample distilled water was added to achieve the proper volume.

To determine phosphorus content using the vanado-molybdenum method, 10 cm³ (0.05 g of plant material) of mineralized plant material was transferred to a 50 cm³ vial. A mixture of 15 cm³ of vanado-molybdenum and distilled water was added to obtain a total volume of 50 cm³. Absorption of the solutions was measured at 470 nm after 30 min on spectrophotometer Lambda 11 (Parker Elmer) and phosphorus concentration was determined using a calibration curve.

To determine magnesium content, 20 cm³ (0.1 g of plant material) of mineralized plant material was transferred to 50 cm³ vial and 1.5 cm³ of hydroxylamine hydrochloride, 5 cm³ of starch solution (to 2 g of starch distilled water (100°C) was added and 100 cm³ of the starch solution was obtained), 1.5 cm³ of titan yellow and 5 cm³ 30% NaOH and distilled water to final 50 cm³ volume. Absorption of the analyzed solutions at 550 nm was measured with the spectrophotometer Lambda 11 (Parker Elmer) after 30 min from obtaining color; finally, magnesium concentration was determined using a calibration curve.

RESULTS AND DISCUSSION

Both doses of aluminium caused a decrease of dry matter of barley seedlings. During the entire plant growth period, the highest amount of dry matter of one seedling was found for control plants of cv. Philadelphia (6.1 mg) and the lowest for cv. Rasbet grown under 300 µmol Al³⁺ dm⁻³ (1.7 mg) (Table 1).

Table 1. Effect of aluminium treatments on the dry weight of barley (mg).

µM Al ³⁺	pH 3			pH 4			pH 5			pH 6		
	0	150	300	0	150	300	0	150	300	0	150	300
Cultivars												
Madonna after 7 days	2.7	2.3	2.2	3.3	3.0	2.6	3.0	2.8	2.6	3.6	3.2	3.0
Madonna after 14 days	2.8	2.1	2.2	2.9	2.5	2.3	8.5	7.0	6.3	8.0	6.9	5.5
Madonna after 21 days	8.1	5.2	5.4	6.1	5.7	5.2	10.6	8.7	5.9	8.8	8.6	6.9
Orthega after 7 days	2.1	2.0	2.0	2.9	2.4	2.6	2.8	2.5	1.0	3.6	2.9	3.2
Orthega after 14 days	2.1	2.1	1.5	3.7	3.6	2.6	4.3	2.3	2.4	5.0	3.9	4.1
Orthega after 21 days	2.7	2.5	2.2	3.7	3.6	2.6	4.3	2.3	2.4	6.3	4.1	3.6
Philadelphia after 7 days	3.5	2.8	2.6	2.6	2.8	2.8	3.1	3.2	2.9	4.1	4.0	3.5
Philadelphia after 14 days	6.6	4.8	4.7	5.8	4.6	4.7	4.4	4.2	4.0	10.5	10.1	9.2
Philadelphia after 21 days	5.7	5.3	5.0	7.1	5.0	3.8	5.8	5.4	5.1	14.1	12.6	11.6
Rasbet after 7 days	1.7	1.5	1.1	2.0	1.8	1.4	2.4	1.3	1.2	2.6	2.4	2.2
Rasbet after 14 days	1.9	1.8	1.5	2.7	2.1	1.7	3.2	2.4	1.2	3.6	3.1	2.7
Rasbet after 21 days	1.9	1.7	1.5	2.7	2.1	1.7	3.4	2.6	1.4	3.8	3.2	2.8

Table 2. Analysis of variance for dry matter.

Source of variation	Degrees of freedom	Mean squares
A-pH	3	0.009**
B-concentration Al ³⁺	2	0.003**
C-cultivars	3	0.0006**
A x B	6	0.001**
A x C	9	0.0001
B x C	6	0.0012**
Error	18	0.0002

**significant at $\alpha = 0.05$

Changes in root growth and development cause the decrease of nutrient uptake and a decrease of yielding (Smeck & Novak, 1994). These differences were significant for the following: pH, aluminium concentration, interaction of pH and aluminium concentration, and for interaction between aluminium concentration and dry matter of cultivars (Table 2).

The Relative Growth Rate of barley cultivars decreased independently of Al³⁺ concentration and pH during the experiment and was several fold higher at the beginning of growth than in the last period of the study (Fig. 1).

On average, the RGR was higher for control plants and decreased with aluminium doses in all studied periods of growth: e.g. in the first studied period it was 0.138 for control plants and 0.119 and 0.113 g g⁻¹ d⁻¹ for plants grown under 150 and 300 $\mu\text{mol Al}^{3+} \text{ dm}^{-3}$, respectively.

During the study, the RGR of cvs. Orthegea and Rasbet was the lowest (from 0 to 0.1 g g⁻¹ d⁻¹) in all growth periods and under all aluminium concentrations. Especially sensitive to Al³⁺ was cv. Rasbet (0–0.05 g g⁻¹ d⁻¹). The highest RGR (almost 0.25 g g⁻¹ d⁻¹) was found for cv. Philadelphia during the first period of growth without aluminium. The RGR of cv. Madonna grown with and without aluminium concentrations exhibited similar changes for a given pH; the RGR of this cultivar grown at pH 3 and 4 in the second growth period (from 7th to 14th day) decreased to about 0 g g⁻¹ d⁻¹, then, in the third growth period (from 14th to 21st day), increased to similar values as in the first growth period i.e. to about 0.1–0.15 g g⁻¹ d⁻¹ (Fig. 1). This cultivar may be able to adapt itself to low pHs, e.g. by production of the proper amount of organic acids which can detoxify aluminium ions in both the outer and inner environment of the plant (Ma et al., 2001). In other cases, a decrease of RGR was observed with growth time up to 0 g g⁻¹ d⁻¹.

Darko et al. (2004) also found that roots of sensitive and tolerant lines of wheat were shortest when growing at pH 4 and in the presence of 300 Al³⁺, longer when plants were grown without aluminium but at pH 4, and the longest, when plants were grown without aluminium at pH 5.2. Scholl et al. (2004) found that under increasing aluminium concentration, the root length and root and shoot dry matter of *Pinus sylvestris* and *Picea abies* decreased. Lower growth of barley seedlings in the presence of 150 and 300 $\mu\text{mol Al}^{3+} \text{ dm}^{-3}$ could be caused mainly by inhibition of root growth, especially of root tips. (Delheize & Ryan, 1995; Marschner, 1995). Under these conditions, roots are shorter, thicker and fragile, with fewer root hairs.

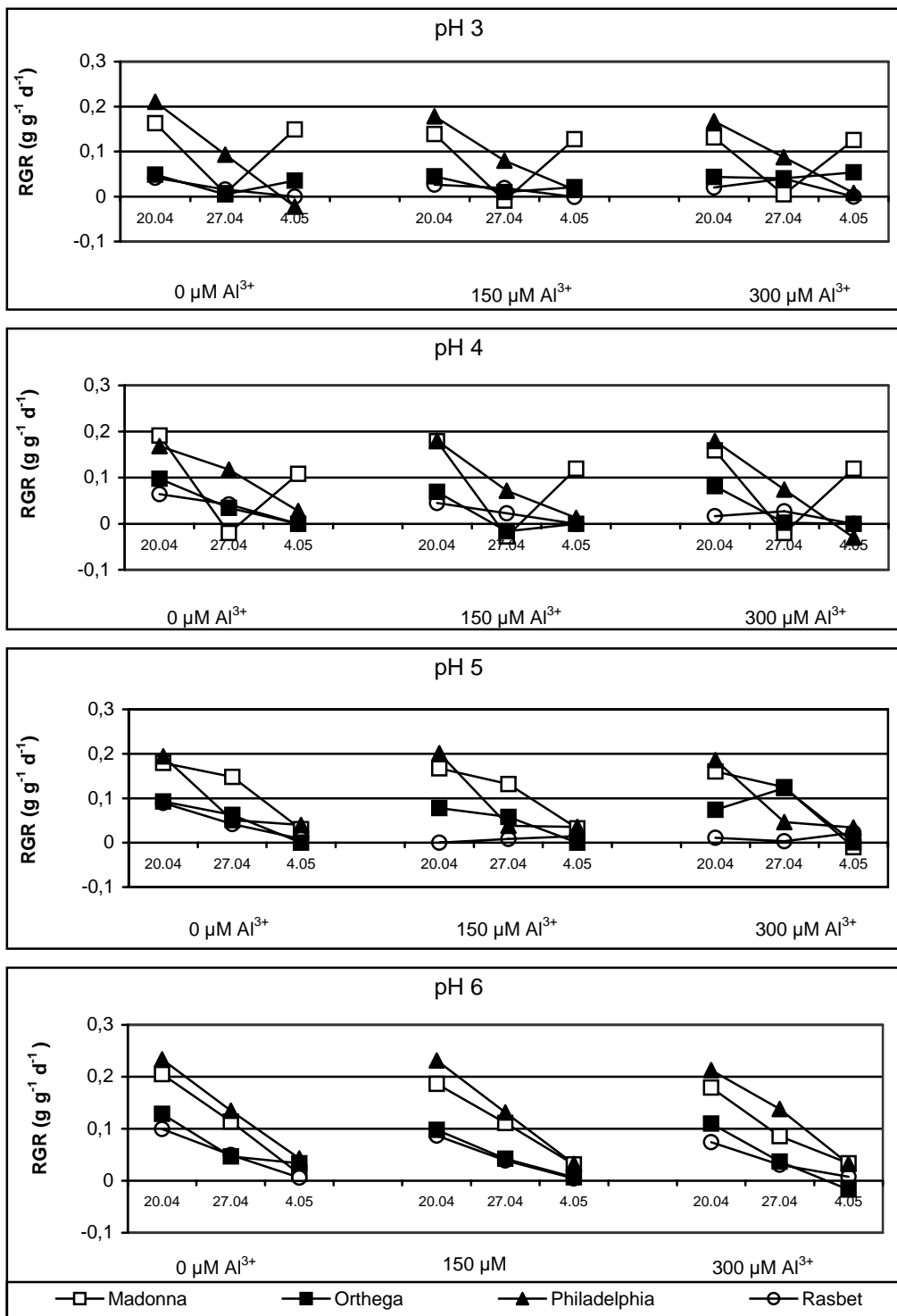


Fig. 1. Relative growth rate (RGR in g D.M. g D.M.⁻¹ d⁻¹) of barley seedlings grown at different pH (3, 4, 5 and 6) and Al³⁺ concentration (0, 150, 300 $\mu\text{M Al}^{3+}$).

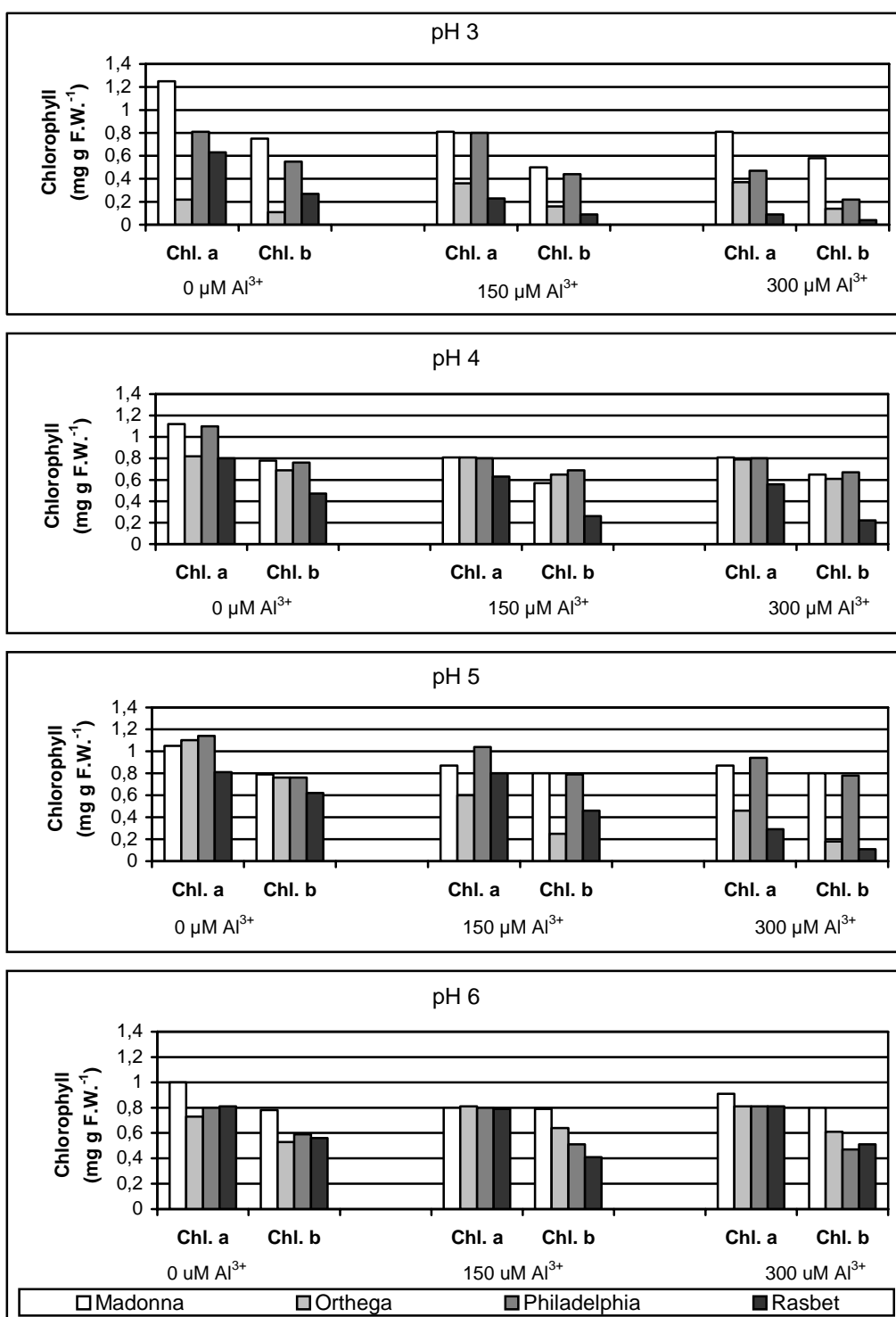


Fig. 2. Influence of pH (3, 4, 5 and 6) and Al³⁺ concentration (0, 150, 300 μM Al³⁺) on leaves' chlorophyll content (mg Chl. g F.W.⁻¹).

Table 3. Analysis of variance for chlorophyll *a* and *b*.

Source of variation	Degrees of freedom	Mean squares	
		Chlorophyll <i>a</i>	Chlorophyll <i>b</i>
A-pH	3	0.33**	1.81**
B-concentration Al ³⁺	2	0.39**	0.44**
C-cultivars	3	0.11**	0.38**
A B	6	0.07**	0.15**
A C	9	0.06**	0.08**
B C	6	0.13**	0.58**
A B C	18	0.07**	0.13**
Error	96	0.00045	0.00011

**significant at $\alpha = 0.05$

Aluminium inhibits cell divisions in tips of roots and shoots by binding to nuclear DNA; it also lowers respiration and in this way interferes with energy metabolism of the plants (Bennet & Breen, 1993). Lower dry matter production and lower RGR could be also an effect of lower shoot growth (Masłowski, 1997), but according to Zawada et al. (2003) aluminium has a greater inhibitory effect on growth of roots than on shoots.

Aluminium could lower the photosynthetic rate of barley seedlings through damage of thylakoid membranes and inhibition of electron transport. For example, it caused a decrease in the ratio of variable fluorescence to fluorescence origin in lemon seedlings (F_v/F_o) (Pereira et al., 2000) and it could an explanation of the lower growth and lower RGR of barley seedlings grown in the presence of both doses of aluminium.

In most cases higher RGR was observed for plants grown at higher pH (5 and 6) than for those grown at lower ones (3 and 4). For example, in the first growth period, the RGR for plants grown at pH 5 and 6 was 0.119 and 0.154 but for plants grown at pH 3 and 4 it was 0.101 and 0.119 g g⁻¹ d⁻¹, respectively. In the second growth period, it was 0.070 and 0.080 versus 0.033 and 0.026 g g⁻¹ d⁻¹, respectively. That suggests lower uptake of different nutrients at lower pH levels (Marschner, 1995).

The average highest chlorophyll content was found in plants which were grown without aluminium (0.89 and 0.61 mg g F.W.⁻¹ of chlorophyll *a* and *b* respectively) and decreased significantly when both doses of aluminium were used (to 0.73 and 0.66 mg g F.W.⁻¹ for chlorophyll *a* and to 0.50 and 0.46 mg g F.W.⁻¹ for chlorophyll *b* for plants grown under 150 and 300 $\mu\text{mol Al}^{3+} \text{dm}^{-3}$, respectively) (Fig. 2, Table 3).

Aluminium influenced chlorophyll content especially in plants grown at pHs 3–5, but not when plants were grown at pH 6; then chlorophyll *a* content was in the range 0.80–0.83 and chlorophyll *b* in the range 0.59–0.61 mg g F.W.⁻¹.

Studied concentrations of aluminium significantly decreased the chlorophyll content of all studied cultivars, but had the greatest influence on the maximum content of both chlorophylls in cv. Rasbet (0.76, 0.61 and 0.44 of chlorophyll *a* and 0.48, 0.30 and 0.22 mg g F.W.⁻¹ for plants grown under 0, 150 and 300 $\mu\text{mol Al}^{3+} \text{dm}^{-3}$, respectively) (Fig. 2).

In cv. Rasbet, the lowest chlorophyll content was found in the leaves of barley grown at pH 3 and 300 $\mu\text{mol Al}^{3+} \text{dm}^{-3}$ and chlorophyll *a* and *b* content was 0.09 and 0.04 mg g F.W.⁻¹, respectively. In the plants grown under the highest aluminium dose and at the lowest pH, chlorophyll *a* and *b* content were the highest in cv. Madonna

(0.81 and 0.58 mg g F.W.⁻¹, respectively) versus 0.09–0.47 and 0.04–0.22 mg g F.W.⁻¹, respectively in other cultivars.

On average, chlorophyll content in the leaves of plants grown at pH 3 was much lower (0.57 and 0.32 mg g F.W.⁻¹ for chlorophyll *a* and *b*, respectively) than for plants grown at other pHs (0.82–0.83 mg g F.W.⁻¹ for chlorophyll *a* and 0.59–0.60 mg g F.W.⁻¹ for chlorophyll *b*) (Fig. 2).

Big differences in average chlorophyll *a* and *b* content were observed among cultivars grown at pH 3; cv. Madonna had the highest chlorophyll *a* and *b* content (0.96 and 0.61 mg g F.W.⁻¹, respectively), followed by cv. Philadelphia (0.69 and 0.41 mg g F.W.⁻¹, respectively). Other cultivars had much lower content of both chlorophyll types (0.32 and 0.13–0.14 mg g F.W.⁻¹, respectively).

Among studied cultivars, on average the highest chlorophyll content was found for cv. Madonna (0.93 and 0.72 mg g F.W.⁻¹, respectively), a bit lower for cv. Philadelphia (0.86 and 0.60 mg g F.W.⁻¹, respectively) and the lowest for cv. Rasbet (0.60 and 0.33 mg g F.W.⁻¹, respectively) (Fig. 2). Chlorosis of the leaves of both cultivars was then observed.

In most cases the ratio of chlorophyll *a* to chlorophyll *b* was relatively low (usually below 2).

Aluminium similarly lowered chlorophyll *a* and chlorophyll *b* content, what caused that this ratio was independent of aluminium in the medium (1.43–1.47) (Fig. 2). Thus, aluminium decreases chlorophyll content in the leaves and in this way can be responsible for inhibiting photosynthesis (Okhi, 1986).

The highest ratio of chlorophyll *a* to chlorophyll *b*, on average, was for plants grown at pH 3 (1.78) and it was shown to decrease with increasing pH; for plants grown at pH 4–6, it was 1.37–1.40.

The highest chlorophyll *a* to chlorophyll *b* ratio, independent of aluminium dose and pH, was observed for cv. Rasbet (1.81) and the lowest for cv. Madonna (1.29).

Toxicity of Al³⁺ and deficit of different elements observed in the presence of Al³⁺ cause inhibition of many metabolic processes including synthesis of nucleic acids and enzymic activity which, for example, can lead in turn to a decrease of chlorophyll content in the leaves (Anioł, 1981; Marschner, 1995). According to Foy (1983) symptoms of aluminium toxicity on aboveground parts of plants are rather secondary and are an effect of water and nutrient deficit.

Aluminium concentration in the medium significantly influenced phosphorus and magnesium content in barley seedlings. Phosphorus content was the highest in plants grown without aluminium (0.66%). It decreased with an aluminium dose: it was 0.59% for plants grown under 150 μmol Al³⁺ dm⁻³ and 0.52% for those grown under 300 μmol Al³⁺ dm⁻³ (Fig. 3, Table 4).

Phosphorus content was similarly lowered by applying both aluminium doses in plants which were grown at all studied pHs, from 0.86 to 0.57 at pH 3 and from 0.57 to 0.42% at pH 6 and under 0 and 300 μmol Al³⁺ dm⁻³, respectively.

Neither dose of aluminium influenced the phosphorus content in the leaves of the barley seedlings of cvs. Madonna and Philadelphia but did affect cv. Rasbet (0.98, 0.88 and 0.71% for 0, 150 and 300 μmol Al³⁺ dm⁻³, respectively, and cv. Orthega (0.72, 0.68 and 0.57% for 0, 150 and 300 μmol Al³⁺ dm⁻³, respectively) (Fig. 3).

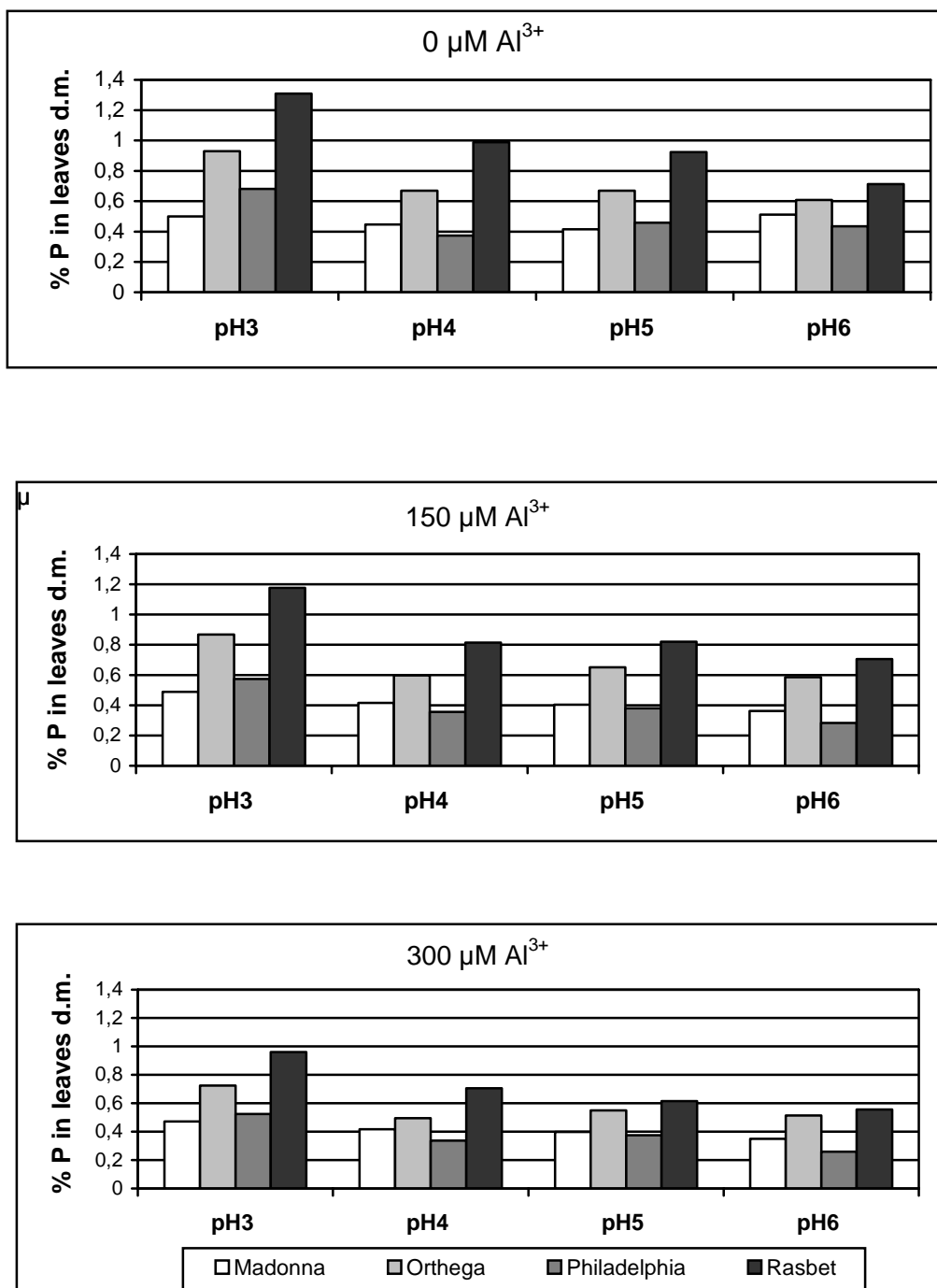


Fig. 3. Influence of pH (3, 4, 5 and 6) and Al^{3+} concentration (0, 150, 300 $\mu\text{M Al}^{3+}$) on phosphorus content in leaves of spring brewer's barley (% P in leaves D.M.).

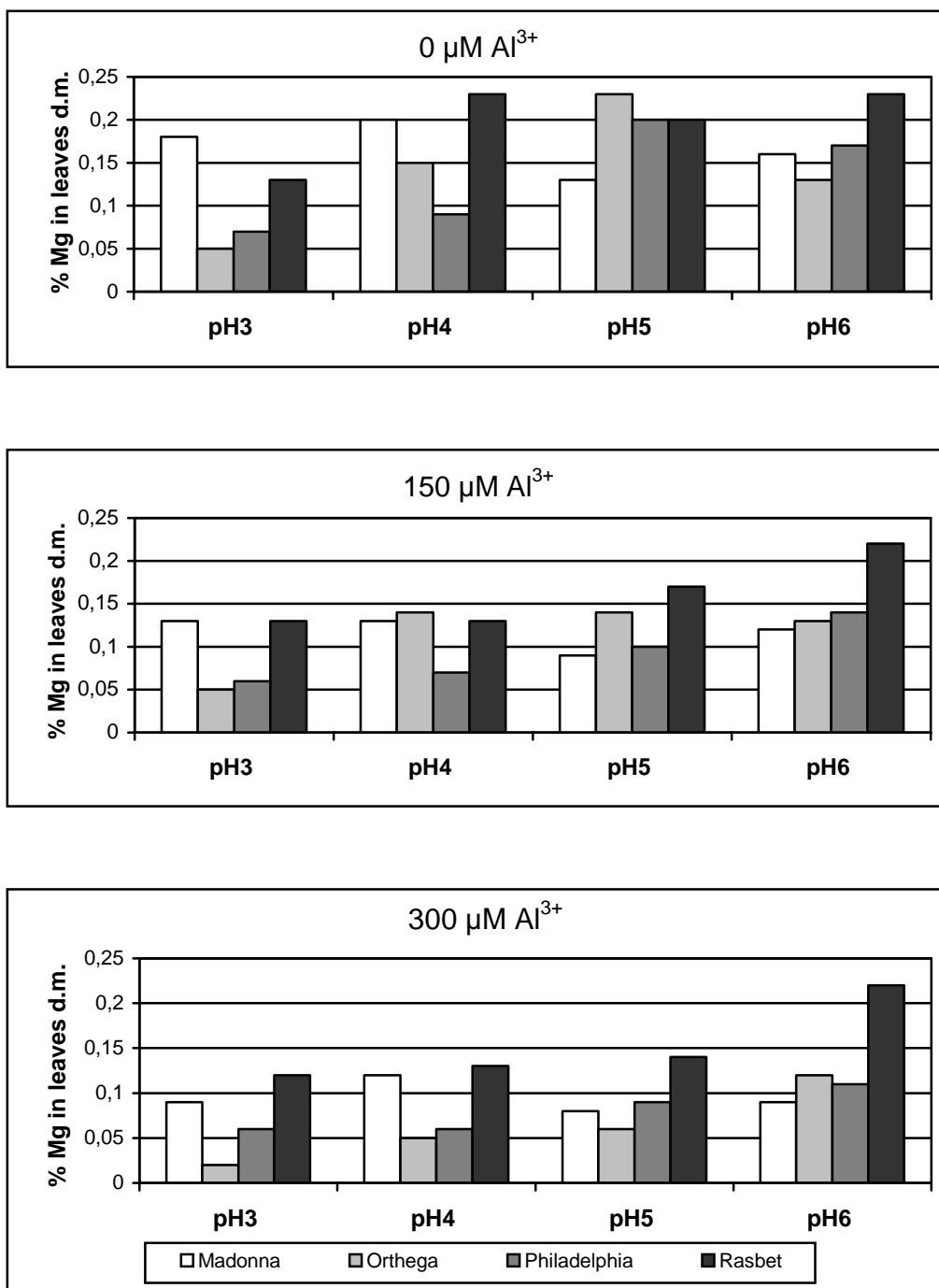


Fig. 4. Influence of pH (3, 4, 5 and 6) and Al^{3+} concentration (0, 150, 300 μM Al^{3+}) on magnesium content in leaves of spring brewer's barley (% Mg in leaves D.M.).

Table 4. Analysis of variance for magnesium and phosphorus.

Source of variation	Degrees of freedom	Mean squares	
		Magnesium	Phosphorus
A-pH	3	0.012**	0.52**
B-concentration Al ³⁺	2	0.0034**	0.25**
C-cultivars	3	0.0036**	0.019**
A B	6	0.0034**	0.027**
A C	9	0.0013*	0.0014
B C	6	0.0073**	0.025**
Error	18	0.00036	0.0034

**significant at $\alpha = 0.05$

Phosphorus content in the leaves was the highest for all studied cultivars at pH 3 (0.78%), but was similar for other pHs (0.50–0.56%) (Fig. 3). Phosphorus content in cv. Madonna was only slightly influenced by pH (0.41–0.49%), but it was influenced in all others cultivars, e.g. in cv. Rasbet (0.66–1.15%) or in cv. Orthega (0.60–0.90%).

The highest average phosphorus content was observed for cv. Rasbet (0.86 %), followed by cv. Orthega (0.66%) and much lower for both other cultivars (0.42–0.43%) (Fig. 3). Phosphorus in the plant influences activity of different enzymes, e.g. NAD kinase (ATP-NAD-2-phosphotransferase), which phosphorylates NAD in the presence of ATP (Pettersson et al., 1988). It is probable that the formation of different soluble compounds of phosphorus with aluminium on the surface of the roots and inside the roots leads to phosphorus deficit in the plants (Blamey et al., 1983).

Magnesium content in the leaves of studied seedlings decreased with an increase of aluminium concentration in the medium. For all cultivars and pHs on average in control plants was 0.14; it was 0.12 in plants grown under 150 $\mu\text{mol Al}^{3+} \text{ dm}^{-3}$ and 0.10% Mg in plants grown under 300 $\mu\text{mol Al}^{3+} \text{ dm}^{-3}$ (Fig. 4).

The effect of aluminium on magnesium content was the lowest when plants were grown at pH 6 (0.17 and 0.13% Mg under 0 and 300 $\mu\text{mol Al}^{3+} \text{ dm}^{-3}$, respectively) and higher at other pHs, e.g. at pH 4 (0.17 and 0.09% Mg under 0 and 300 $\mu\text{mol Al}^{3+} \text{ dm}^{-3}$, respectively).

The biggest decrease of magnesium content between control plants and plants grown under 300 $\mu\text{mol Al}^{3+} \text{ dm}^{-3}$ was found for cv. Madonna (0.09%) and was much lower for other cultivars (0.01–0.04%).

Magnesium content was lower at pH 3 (0.09 %) than at other pH values, i.e. at pH 4 (0.12 %), at pH 5 (0.14 %) and at pH 6 (0.15 %).

For cv. Madonna there were no differences in magnesium content when plants were grown at pH 3 and pH 6 (0.13%) but other cultivars had about 0.08–0.10% greater magnesium content when grown at pH 6 than at pH 3.

On average the highest magnesium content was found for cv. Rasbet (0.18%) and the lowest for cv. Orthega (0.08%) (Fig. 4).

Kinraide et al. (1992) arranged several cations according to their abilities to compete with aluminium ions. Also Grauer and Horst (1990) ranged four cations as follows: $\text{H}^+ \gg \text{Ca}^{+2} > \text{Mg}^{+2} \gg \text{K}^+$; maximal removing of these elements by aluminium is at $\text{pH } 4 \pm 0.4$. At lower pH there is protonization of external carboxylic groups, limiting uptake of other cations by roots.

CONCLUSIONS

1. Aluminium in concentrations 150 and 300 $\mu\text{mol dm}^{-3}$ lowers RGR of spring barley seedlings.
2. Among studied cultivars cv. Madonna is the most resistant to low pHs (3 and 4), proved by highest chlorophyll content and an increase of RGR and during the last growth period.
3. Usually chlorophyll content is the lowest at pH 3; aluminium significantly decreases chlorophyll *a* and *b* content at lower pHs.
4. The presence of aluminium in the medium and low pHs cause a decrease in phosphorus and magnesium content in the aboveground parts of studied spring barley seedlings.

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