ФИЗИОЛОГИЧЕН ОТГОВОР НА МЛАДИ РАСТЕНИЯ ОТ ФАСУЛ (*PHASEOLUS VULGARIS* L.) КЪМ НЕДОСТИГА НА НЯКОИ МИНЕРАЛНИ ЕЛЕМЕНТИ
PHYSIOLOGICAL RESPONSE OF YOUNG BEAN PLANTS (*PHASEOLUS VULGARIS L.*) TO SELECTED NUTRIENT DEFICIENCIES

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Резюме

Изследвано е влиянието на недостига на някои минерални елементи върху растежа и листния газообмен при фасула (*Phaseolus vulgaris* L., сорт "Borlotto rosso"). Доказано е, че е налице положителна корелация между липсата на хранителни елементи и нарушенията в растежа и водния статус на листата. Установени са и промени в газообмена (CO_2 асимилация и транспирация).

Abstract

The studies raise the problem of deficiency of selected mineral nutrients on the growth and leaf gas exchange in beans (*Phaseolus vulgaris* L. cv. 'Borlotto rosso'). It has been shown that there is a positive correlation between the lack of nutrients and disorders in the proper growth and water relations in the leaves (water balance) as well as changes in the gas exchange (including CO_2 assimilation and transpiration).

Ключови думи: *Phaseolus vulgaris* L., минерален дефицит, воден баланс, газообмен.
Key words: *Phaseolus vulgaris* L., mineral deficiency, water balance, gas exchange.

INTRODUCTION

The plants of genus Phaseolus include ornamental plants as *Phaseolus coccineus* L., fodder plants (*Phaseolus acutifolius*), and plants intended to consumption (*Phaseolus vulgaris* L., *Phaseolus lunatus*) (Łabuda, 2010). According to Podleśny (2005) common bean (*Phaseolus vulgaris* L.) is an economically important crop because of its high nutritional value. It is used primarily in the food industry, but also it can be used in medicine, as well as processing.

Production of common bean depends on availability of water, light, temperature and fertilization. Application of inappropriate fertilizer, often exclude one or more elements of mineral nutrition, what can lead to lowering yield quality, quantity or even crop diseases called physiological or non-infectious (Prusiński, 2006; Dordas, 2008; Wińska-Krysiak i Łata, 2007; Campos Bernardi et.al., 2010). Although nutrition stress can occur not only in agricultural but also in natural conditions (Dordas, 2008; Sharma, 2006; Zlatev et al., 2003).

The aim of the study was to determine characteristic symptoms of certain nutrients deficiency (N, P, K, Ca, Mg, S, Fe, and the effect of the lack of nutrients on some physiological parameters (water balance indicators, CO_2 assimilation, transpiration, stomatal conductance, CO_2 content in the intercellular expanses).

MATERIALS AND METHODS

Experience with common bean cv. 'Borlotto rosso' was conducted in April and May 2011, in the Department of Plant Physiology phytotron chamber, with controlled photoperiod 12h/12h (day/night) and light intensity of about 200 µmol $s^{-1}m^{-2}$. Initially, the plants were grown in universal soil 'Athena'. After the evaluation first pair of true leaves, plants were removed from the soil, carefully cleaned and transferred to containers with a capacity of 200 ml filled with the appropriate type of medium. The experiment was conducted in 3 replications, having regard to the full Hoagland medium - as control and without the various nutrients: medium without nitrogen, medium without phosphorus, medium without potassium, calcium free medium, medium without sulfur, medium without magnesium, and medium without iron.

Throughout the period of conducting the experiment, every-day the observations were made, all morphological changes were reported and photographic documentation was performed.

In order to determine RWC and WSD indicators, selected leaves (fresh plant material) were weighed on an analytical balance with an accuracy of 0.001 g. After that they were placed for 24 hours in a glass vessel filled with distilled water, next they were removed and dried with tissue paper. Leaves were weighed again to determine the weight of leaves at full saturation with water. Subsequently the plant material was dried in an oven to constant weight (at 80°C for 24 h), and weighted again. The obtained data were converted by patterns of two indicators (Bandurska, 1991; Kopcewicz, Lewak, 2002).

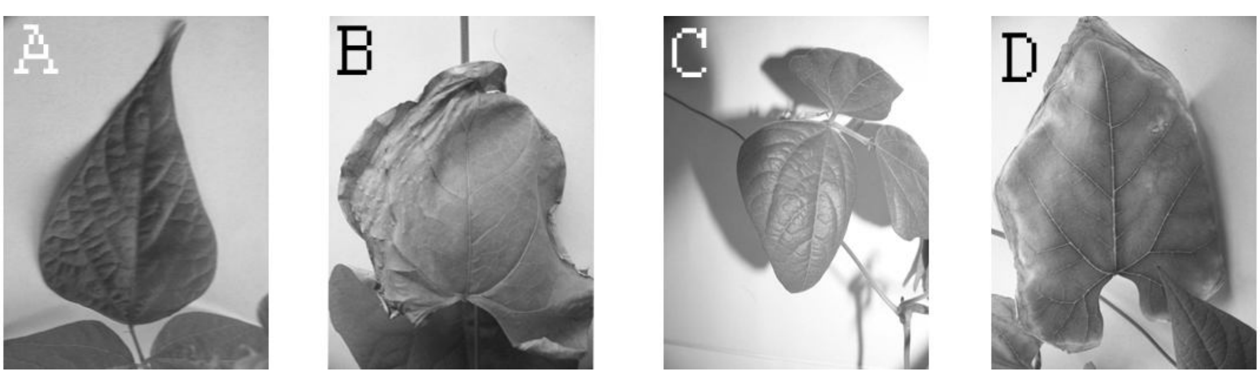
Measurements of CO_2 assimilation (μmol $m^{-2}s^{-1}$), leaf transpiration (mmol m^{-2} s^{-1}), stomatal conductance (mol m^{-2} s^{-1}) and CO_2 content in the intercellular expanses (μmol CO_2 mol^{-1}) were made using TPS-2 gas analyzer, produced by PP Systems company, working in an open system in which air was passing through the apparatus in a continuous manner. Apparatus was equipped with a leaf chamber with a lamp mounted on the light intensity 2053 μmol m^{-2} s^{-1}. The results were developed in the program "Statistica 9.1" produced by Statsoft. For statistical calculations univariate analysis of variance was used. The significance of differences between the variants of experiments were determined by Duncan test at significance level α = 0.05.

RESULTS AND DISCUSSION

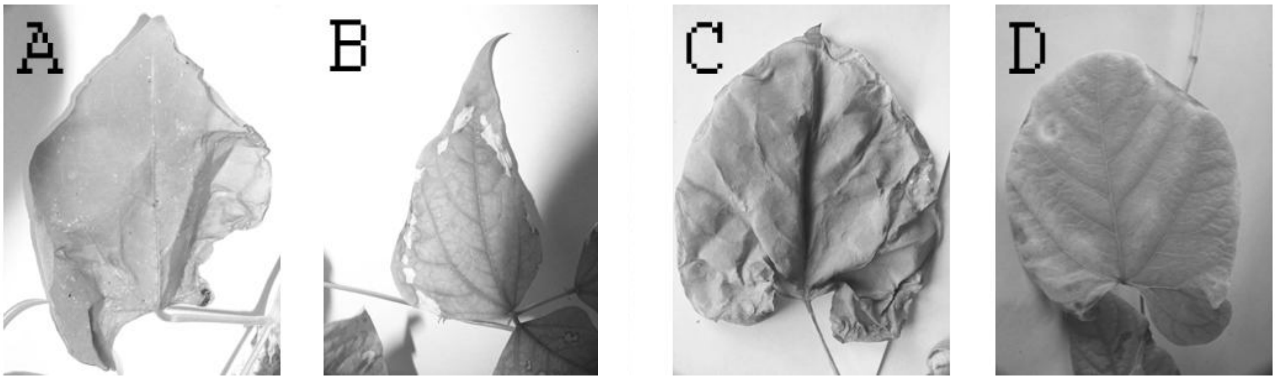
Deficiency of essential elements for plants in Hoagland nutrient solution resulted in morphological changes and the appearance of physiological diseases in common bean plants.

Nitrogen deficiency was characterized by bright color (chlorosis), on both older and younger leaves (Phot.1B). Plant growth was delayed, and the overall condition of the plant was poor. The lack of phosphorus caused the intense green color of leaves and stems. In the absence of phosphorus was also observed dull leaves and stiff habit of plants (Phot.1C). Other hand characteristic which is revealed in the samples with potassium deficiency was curling the edges of the leaf blade (especially in the top part). Plants conducted without access to potassium were significantly darker in color and the leaves were smaller. On older leaves appeared chlorotic boundary, leading to the death of leaf edges (Phot.1D).

First symptoms of calcium deficiency could be observed after only 4 days after placement of plants in nutrient solution. Initially, bean leaves were dull and shineless (Phot.2A). Leaves were characterized by withered habit and the occurrence of numerous chlorosis, leading to necrosis, covering the entire surface of leaves. After that the youngest leaves and shoot tip were twisted and died off. For plants grown in nutrient solution without magnesium were characteristic assimilative tissue discoloration (chlorosis) between veins of leaves. Then chlorosis passed in necrosis (assimilation tissue dying)(Phot. 2B). There was a typical marbling of the leaves of beans. Throughout the experiment necrosis covered approximately 15% of the leaf lamina. After two weeks, leaves become brittle and fragile, and the roots began to die, as it was seen by its browning. Absence of sulfur in the composition of the medium caused inhibition of new leaves development, greatly weakening its condition by inhibiting the shoot growth in thickness, which was characterized by a wispy plant habit. It was also found to reduce leaf turgor and cause chlorosis spots (Phot. 2C). The only external symptom of iron deficiency in beans, were chlorosis covering the whole leaf blades. This



Снимка 1. *Листа от фасул, отглеждан в различни хранителни среди (A - контрола, B – без азот, C – без фосфор, D – без калий)*
Photography 1 ABCD. *Leaves of common bean depending on type of medium (A – control, B – nitrogen-free medium, C – phosphorus-free medium, D - potassium-free medium)*



Снимка 2. *Листа от фасул, отглеждан в различни хранителни среди (А – без калций, В – без магнезий, С - без сяра, D - без желязо)*
Photography 2 ABCD. *Leaves of common bean depending on type of medium (A – calcium-free medium, B – magnesium-free medium, C – sulfur-free medium, D - iron-free medium)*

disease was evident on both the young and old leaves, on young leaves, however, it was much more intense (Phot. 2D).

Analysis of variance revealed significant effects of selected nutrient deficiencies on water balance indicators of *Phaseolus vulgaris*.

The highest values of relative water content in the leaf (RWC) was observed in plants grown on potassium-free medium (which represented 121% of control), while the lowest were characterized by nitrogen-free trial (89% of control). The lowest value of WSD indicator was recorded in the sample deprived of potassium (75% of control), while the highest value was characterized by an attempt of nitrogen-free (113% of control) (Fig.1).

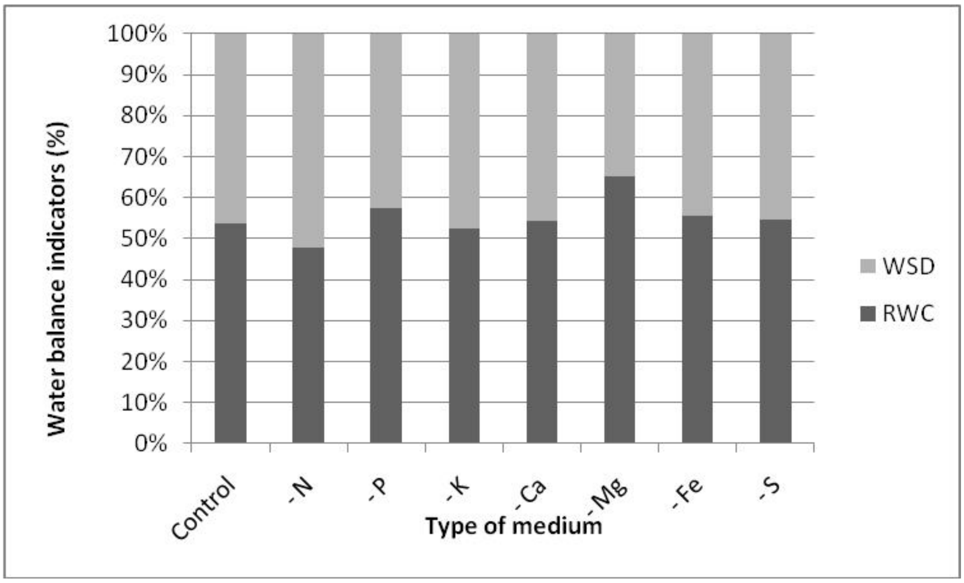
Significant influence of the lack of certain nutrients on the intensity of gas exchange processes, as well as stomatal conductance and CO_2 concentration in the intercellular expanses was found. The lowest values of the intensity of the process of assimilation were noted in the plants grown under nitrogen deficiency (0,05 μmol CO_2 $m^{-2} s^{-1}$), while the highest in the absence of potassium (Table 1.). Plants growing in phosphorus-free medium were characterized by significantly lowest transpiration intensity (0,34 mmol H_2O $m^{-2} s^{-1}$). Significantly the highest value of stomatal conductance was reported in plants growing without access to the sulfur (52,50 mol $m^{-2} s^{-1}$), while the highest content of CO_2 in the intercellular expanses was found in plants grown in control medium (Table 1).

Cramer et al. (2009) showed in their study a strong correlation between the availability of nitrogen for plants, and water content in tissues, with special attention to the shortage of this element in the substrate, and decrease of water saturation in leaf. Conducting research on the cultivation of corn, Niedziółka et al. (2004) determined the impact and role of some nutrients in the plant. The authors have indicated that calcium deficiency has not only a significant effect on carbohydrate metabolism, but also on water balance, strongly reducing the water content in the leaves. Grzesiuk and Koczowska (1991) give as a specific symptom for sulfur deficiency in plants, poor condition induced by water deficit. In this study, similar symptoms were observed in the case of nitrogen deficiency. Plants grown in media without sulfur, as well as in media deprived of iron were characterized by a insignificantly larger value of RWC index.

Yeh et al. (2000) and Starck (2008) in their studies on reaction of plants to nitrogen deficiency show significantly reduction of chlorophyll content in leaves as also as strong suspension of growth. Moreover, not only the lack of nitrogen, but also it's excess significantly affects reduction of intensity of photosynthesis and transpiration in plants. Olszewska et al. (2008) suggest that it is associated with a decrease in stomatal conductance.

The reduction of all indicators of gas exchange (except insignificant increase in stomatal conductance) has also been noted in case of own research. Lack of nitrogen significantly influenced the increase in the value of biometric identifiers, which might suggest accelerating the development of plants as a defense mechanism against adverse conditions (Dickson, 2000; Dordas, 2008).

Lack of access to the calcium limits the plant growth and weakens the immune system of plant organisms to adverse environmental conditions. The content of this element determines many characteristics of the plant that is: the permeability of membranes, cell signaling and membrane coherence (Dickson, 2000; Wińska-Krysiak M., patch B., 2007; Wrobel et al., 2005). What could be causing a significant reduction in the content of CO_2 in the intercellular expanses of plants grown in media without calcium.



Фиг. 1. *Воден баланс (%) при фасула в зависимост от вида на хранителната среда*
Figure 1. *Common bean indicators of water balance (%) depending on the type of medium*

Таблица 1. Средни стойности на показателите за газообмен при фасула в зависимост от хранителната среда
Table 1. Average values of gas exchange indicators of common bean depending on the type of medium used (h.g. = homogenous groups)

Хранителна среда/ Type of medium	CO_2 асимилация/ CO_2 assimilation		Транспирация/ Transpiration		Устична проводимост/ Stomatal conductance		Концентрация на CO_2 в междуклетъчното пространство Concentration of CO_2 in intercellular expanses	
	(μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$)	h.g.	(mmol $H_2O \cdot m^{-2} \cdot s^{-1}$)	h.g.	(mol$\cdot m^{-2} \cdot s^{-1}$)	h.g.	(μmol $CO_2 \cdot$ mol^{-1})	h.g.
Control	0,22	d	0,52	abc	31,17	b	80,67	b
- N	0,05	a	0,44	a	31,67	b	60,17	a
- P	0,05	a	0,34	d	23,17	a	72,33	ab
- K	0,20	d	0,45	ab	33,33	b	53,67	a
- Ca	0,13	b	0,56	c	36,17	b	56,83	a
- Mg	0,10	ab	0,41	a	28,67	b	61,17	a
- S	0,15	c	0,77	e	52,50	d	63,17	a
- Fe	0,17	cd	0,54	bc	44,83	c	52,83	ab

Magnesium deficiency, in turn, strongly reduces the intensity of photosynthesis and transpiration, and has a significant impact on the decline in yields. Magnesium is essential for the synthesis of chlorophyll, and the lack of this element in the substrate is synonymous with the decline in leaf pigment content and a strong reduction of photosynthesis (Olszewska, 2005). The results obtained show, that magnesium deficiency can be stimulating for root system development. It can be connected with mechanism for seeking essential nutrients.

Research has shown that the reaction mechanisms of nutrient deficiencies are highly diverse and they should not be regarded as a schematic.

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