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ЕКОЛОГИЧНА ФИЗИОЛОГИЯ НА РАСТЕНИЯТА

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ФИЗИОЛОГИЧЕСКАЯ РОЛЬ НЕКОТОРЫХ МИНЕРАЛЬНЫХ ЭЛЕМЕНТОВ В ОБРАЗОВАНИИ КЛУБЕНЬКОВ И ФИКСАЦИИ АТМОСФЕРНОГО АЗОТА У БОБОВЫХ РАСТЕНИЙ

И. СТАНЧЕВА, к. с.-х. н.*; М. ГЕНЕВА, М. ХРИСТОЗКОВА, Г. ЦВЕТКОВА, Г. ЗЕХИРОВ, Г. ГЕОРГИЕВ

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Исследовано влияние дефицита и повышенного содержания фосфора (P), бора (B) и молибдена (Mo) на образование клубеньков и активность фиксации азота у гороха и сои. Установлена непосредственная связь между содержанием фосфора и бора в питательной среде и количеством специфических флавоноидов в корневых эксудатах, играющих роль индукторов для образования клубеньков у бобовых. Отрицательное влияние отсутствия молибдена в питательной среде на образование клубеньков, активность азотфиксации и содержание аминокислот в растительных тканях можно избежать при введении питательных элементов через листья.

Симбиотическая азотфиксация включает комплекс взаимодействий бобовых растений с почвенными бактериями 5 различных родов: *Azorhizobium, Bradyrhizobium, Mesorhizibium, Sinorhizobium и Rhizobium*. В результате симбиотических взаимодействий образуется новый орган — клубеньки. Этапы образования клубеньков очень чувствительны к уровню минеральных элементов [1] как в условиях недостатка [17], так и их избытка [13].

После азота фосфор является одним из основных биогенных элементов, регулирующих рост и развитие растений. Фосфор оказывает влияние на симбиотическую фиксацию азота, так как стимулирует рост растения-хозяина, а также образование нормально функционирующих клубеньков [8]. Фосфорный дефицит оказывает влияние на процессы инфицирования, поскольку задерживает выделение специфических флавоноидов в корневых эксудатах, играющих роль индукторов при образовании клубеньков, а также прикрепление бактерий к корневым волоскам [12]. Бор в структуре клеточной стенки [7], фенолпропаноидном пути [15] и фитогормональном балансе [10] играет существенную роль в симбиотических отношениях между азотфиксирующими бактериями и растением-хозяином. Молибден является важным компонентом кофакторов ферментов, катализирующих восстановление или фиксацию азота [14]. При внесении восстановленного или органического азота потребность в молибдене уменьшается или исчезает вовсе. Одним из способов, позволяющим избегнуть подавляющий эффект неорганического азота на образование клубеньков [3], является подача минеральных элементов через листья. Листовая подкормка стимулирует выделение сахаров в корневых эксудатах, что, в свою очередь, положительно влияет на развитие клубеньков [9]. В настоящее время проведено недостаточно исследований влияния P, B и Mo на образование клубеньков и фиксацию атмосферного азота у бобовых.

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Root Ecology and its Practical Application, 3. ISRR Symp. Wien, Univ. Bodenkultur, 1991, L. Kutschera, E. Hübl, E. Lichtenegger, H. Persson, M. Sobotik (eds.) — 1992 — Verein für Wurzelforschung, A-9020 Klagenfurt.

EFFECT OF DIFFERENT LEVELS OF NITROGEN NUTRITION ON GROWTH, NODULATION AND NITROGEN FIXATION OF ACACIA PLANTS (Robinia pseudoacacia L.) INTERCROPPED WITH POPLAR PLANTS (Populus euroamericana L.)

G.I. Georgiev, K. Kalmuckov, E. Alexandrova, L. Tyankova
Ts. Tsanov

SUMMARY: The higher nitrogen mineral concentration (80 and 120 mgN/kg soil) exerts a stronger positive effect on the nodulation but decreases nitrogen fixation of acacia plants during the later stages of growth. Poplar plants successfully compete for the soil N with acacia plants on the low and middle ranges of concentration which contributes for the elevation of acacia nitrog fixation rate.

1 INTRODUCTION: Acacia plants can fix atmospheric N_2 after the successful inoculation with respective Rhizobium ssp. strains which allow them to play a significant role in maintaining the forest soils fertility and tree productivity (Atkins 1986). There is a very scare information about there ability to fix atmospheric N_2 under unfavourable environmental conditions of growth such as high soil nitrogen concentration and concurrent effect for soil N uptake with other fast growing tree species like poplar plants.

2 MATERIAL AND METHODS: The experiments were carried out as pot soil culture with one year old acacia (var. Svishtov) and poplar (var. T-I214) saplings. The plants were grown from root (acacia) and stem (poplar) cuttings. The pots contained 10 kg abs. dry low leached carbonate chernozem (pH=7,8; CO_3=0,89%; N=3,14%; P_2O_5=0.56%; K_2O=2,35%). N nutritions were at natural level of N (31,4 mg/kg), fertilized variants (80 mgN/kg & 120 mgN/kg). Soil moisture was at 60% FSWC. Inoculation of plants was done by adding soils

Table 1. N_2-fixing activity Of acacia nodules during growth period

Stage of growth	Soil nitrogen level								
	31,4 mgN/kg			80 mgN/kg			120 mgN/kg		
	ARA	Ureides per plant	Dwt	ARA	Ureides per plant	Dwt	ARA	Ureides per plant	Dwt
				Acacia as monoculture					
I	6,87a	0,0007a	0,02b	2,26a	0,0006a	0,01b	0,75c	0,005c	0,01b
II	99,19a	0,0040a	0,127b	29,40a	0,0067b	0,21a	1,52b	0,035a	0,55b
III	75,34b	0,143oa	0,800	14,30a	0,128a	1,01a	3,42a	0,135a	0,57b
				Acacia intercropped with poplar					
I	9,33b	0,0070b	0,090a	9,19c	0,004b	0,05	12,51a	o,003a	0,009b
II	19,52a	0.0033b	0,030a	0,81a	0,0009b	0,24a	0,62a	0,0009	0,03a
III	6,30a	0,074a	0,57a	0,62b	0,037b	0,81a	0,35a	0,013a	0,12a

Significant difference at level of 1% (a) and 5% (b) and non-significant (c) / Student t-test/.

P 6

EFFECT OF TOXIC Cu CONCENTRATION ON THE GROWTH, SYMBIOTIC N2-FIXATION AND ION EXCHANGE ACTIVITIES OF WILD STRAIN AND PLASMID-CURED DERIVATIVES OF *Rhizobium* sp. EFFECTIVELY NODULATING BLACK LOCUST /*Robinia pseudoacacia* L./

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Introduction The plasmid DNA found in the fast- growing rhizobial strains are responsible for determination of some physiological activities of cells like symbiotic N_2 fixation, stress tolerance reaction to different environmental disorders, cell membrane permeability determinants[6,8]. N_2 fixing properties of the *Rhizobium*-legume symbiotic relationships are very sensitive to the changes in the abiotic environment, but little information is available about the involvement of rhizobial plasmids in determination of the stress tolerance reaction of both, free-living and nodulating organisms[1,2].

The aim of this work was to study the plasmid profile of the indigenous isolate *Rhizobium sp.* DC-2, effectively nodulating *Robinia pseudoacacia* L. plants and to elucidate the role of these plasmids in the determination of the growth, ion exchange and symbiotic N_2 fixation of wild and plasmid-cured strains of *Rhizobium sp* DC-2 grown in the present of toxic Cu concentrations in the medium.

Material and methods Plant saplings of *Robinia pseudoacacia* L. clone Svishtov were grown from the root cuttings in the pots containing 15 kg of mixture /luvisol - sand - perlite = 1:1:1/ in the green house conditions [3]. The saplings were inoculated with bacterial suspension of *Rhizobium sp* DC-2 containing 10^8 cells per ml. The pots were supplied with the 200 ppm Cu during the stage of emergence. The plasmid contents of the wild type and cured derivatives of *Rhizobium sp.* isolate DC-2 were determined by a modified Erckhart method [5]. The plasmid curing and isolation of derivatives was performed by the method of Zurkowski and Lorkiewicz (1978). Mineral contents of plasmid-cured and wild strain of Cu stressed 48 h YEM broth culture of *Rhizobium sp.* DC-2 and the nodules of 90 days old plants were analysed by the atomic absorption spectrometry method [3]. The rate of acetylene reduction /ARA/ and ureide content of nodules were determined [3].

Results and Discussion. The wild strain *Rhizobium sp* DC-2 contained very large plasmid (> 1700 kb), as well two additional plasmids in size about 450 and 160 kb, respectively as shown in Fig1 (Fig.1, lanes 2,3 and 4). The derivatives of the

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НАСЛЕДЯВАНЕ ПРИЗНАКА СЪДЪРЖАНИЕ НА ВОДА В ЛИСТАТА НА РАСТЕНИЯТА ПРИ ХИБРИДИ ЗИМЕН ПИВОВАРЕН ЕЧЕМИК

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Резюме

Вълчева Д., Др. Вълчев, Г.Георгиев, 2006. Наследяване признака съдържание на вода в листата на растенията при хибриди зимен пивоварен ечемик.

В периода 2000-2003 година на сухоустойчиви хибриди зимен пивоварен ечемик е определено съдържанието на вода в листата, което е една от основните характеристики при определяне водния режим на ечемика. Чрез съотношението d/a е определен типа на наследяване на признака в хибридни комбинации. Проследена и установена е степента на изменчивост на показателя съдържание на вода в листата в зависимост от генотипа на родителите.

Ключови думи: Ечемик – Сухоустойчивост - Съдържание на вода в листата - Наследяване

Abstract

Valcheva D., Dr. Vulchev, G. Iv. Georgiev, 2006. Inheritance of water content in leaves in winter malting barley hybrids.

Water content in leaves of winter malting barley hybrids was determined; this is one of the basic characteristics in determining of barley water regime. By the ratio d/a, the type of inheritance in hybrid combination was specified. The rate of variability of water content in leaves according to the genotypes of the parental forms wa established.

Key words: Barley - Water content - Drought resistance - Inheritance

УВОД

В резултат на обезводняването на тъканите през време на суша настъпват дълбоки промени в азотния метаболизъм на растенията (Hanson et al., 1979; Morgan, 1984; Sing et al, 1981). Установено е, че при засушаване в ечемиченото растение намалява количеството на общия азот, а нараства нивото на проламина, който влияе отрицателно върху състава и поведението на зърното при преработката му в малц и пиво (Вълчев, 1994, 1996, 1995, 1996, 2000). За характеристика на водния режим на растенията обикновено се използват следните показатели – оводненост на листата, относителна тургесцентност, "фракционен" състав на водата, воден дефицит и други (Станкова и др., 2002; Удовенко, 1970).

Съдържанието на вода в листата е важен показател за състоянието на водния режим и на органите и целите растения, който позволява да се характеризира влиянието на засушаването върху водния режим и обмяната на веществата. Някои изследователи предлагат този показател като един от критериите за сухоустойчивостта

Изследвания върху полските култури, 2004, Том I - 2
Field Crops Studies, 2004, Vol. I - 2

НАСЛЕДЯВАНЕ И ИЗМЕНЧИВОСТ НА ПРИЗНАКА ЕЛЕКТРООСМОЗА НА ЕЛЕКТРОЛИТИ ПРИ СУХОУСТОЙЧИВИ ФОРМИ ПИВОВАРЕН ЕЧЕМИК

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Резюме

Вълчев, Д., Д. Вълчева, Г. Георгиев, 2004. Наследяване и изменчивост на признака екзоосмоза на електролити при сухоустойчиви форми пивоварен ечемик.

Създадени са сухоустойчиви хибриди пивоварен ечемик. Определена е степента на екзоосмоза на електролити от клетките на листата, която е един от основните показатели, характеризиращ водния режим на ечемика. Съотношението d/a показва, че преобладава адитивно-доминантният тип на наследяване на признака. Установена е отрицателна трансгресивна изменчивост в системата, дължаща се вероятно на взаимодействието на доминантни гени за ниско ниво на екзоосмоза на електролити от родителските компоненти.

Ключови думи: Ечемик, Сухоустойчивост, Екзоосмоза на електролити, Наследяване, Трансгресия

Abstract

Vulchev, D., D. Valcheva, G. Georgiev , 2004. Inheritance and variability of outlet of electrolytes in drought resistant malting barley hybrids.

Drought resistance malting barley hybrids were developed. The type of inheritance outlet of electrolytes of malting barley F_1 - F_4 hybrids is determined by specific interaction of genotypes of the parental forms. The low level of the hybrids indicator is the cause for the negative transgression selective value of the hybrid population.

Key words: Barley, Drought resistance, Outlet of electrolytes , Inheritance, Transgression

УВОД

Степента на екзоосмоза на електролити от клетките на листата е един от основните показатели, характеризиращ водния режим на ечемика, и по него се съди за сухоустойчивостта на ечемика /Atsmon, 1973; Кожушко, 1988, Вълчев, 1994, Вълчев и др., 2003/. Признакът е с полимерен характер на детерминация, поради което се затруднява неговият генетичен анализ и е причина за получаване на противоречиви данни /Трофимовская и кол., 1971; Карамышев и кол., 1981;

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BIOLOGIE
Microbiologie

EXOCELLULAR POLYSACCHARIDES OF *RHIZOBIUM* SPP. STRAINS AS A FACTOR IN ION INTERACTIONS AND CELL GROWTH UNDER Cu^{++} STRESS

G. Iv. Georgiev, B. K. Atanasov

(Submitted by Corresponding Member K. Markov on June 14, 1996)

Up to now, information on the mechanisms of heavy metal resistance of *Rhizobium* cells is hardly available [6], due to the fact that since recently, the main object of such experiments were yeast cells [9,10]. There is not sufficient information about the toxic effect of copper ions on strain spectrum, vitality and symbiotic efficiency – factors strongly influencing nodule formation and symbiotic nitrogen fixation of *Rhizobium* population in soil [10]. Unlike higher organisms, bacterial cells neutralise the toxic effect of heavy metal ions by more intensive and effective mechanisms of ion and metabolite exchange with the medium [3,5]. A well known fact is, that bacterial capsule and its polysaccharides play the main role in symbiotic and parasitic interactions between micro- and macroorganisms [8]. At the same time they are very important for the relations of microbial cells with the surrounding medium as well [7]. Therefore, when investigating *Rhizobium* cell resistance to toxic levels of heavy metal ions, the ion exchange between cells and surrounding must be of major interest [5,11].

Following the above mentioned arguments, experiments for investigating the growth, Cu accumulation in bacterial cells and the role of exocellular polysaccharides (ECPS) of cell slime of cultivated *Rhizobium* spp. cells were carried out. The effect of toxic concentrations of Cu ions in the culture medium on K^+, Na^+, Ca^{++} and Mg^{++} ion accumulations on the cells of three *Rhizobium* species and in their polysaccharides was studied as well.

Material and methods. S t r a i n s. The following bacterial strains were used in our experiments : *Rhizobium leguminosarum biovar phaseoli* 127K 105 (Lipha Tech, USA), *Rhizobium leguminosarum biovar viciae* 128C 5b (Lipha Tech, USA) and *Rhizobium* sp. *(Robinia)* – isolated from effective root nodules of black locust plants (*Robinia pseudoacacia* L.) (Vardim population N6, Svishtov region).

M e d i a. The basic medium used in our cultivation experiments was yeast extract-manitol medium (YEM) [12], containing in g/l: K_2HPO_4 – 0.5, $MgSO_4.7H_20$ – 0.2, NaCL – 0.1, yeast extract (Fluka) – 1, manitol – 10 [7,8]. The same medium was solidified with Bacto agar to 1.5% for strain maintenance. Sterilization was achieved at 120°C for 20 min. For copper resistance experiments aliquots of sterile $CuS0_4.5H_20$ solution were added to YEM medium to final concentration as follows: 2.5 ppm (0.05 mmol); 12.5 ppm (50 μmol); 25 ppm (100 μmol); 62.5 ppm (250 μmol) and 125 ppm (500 μmol).

C u l t i v a t i o n. Cells of studied strains were cultivated in 500 ml Erlenmeyer flasks with 100 ml of appropriate medium, inoculated with 50 μl of cell suspension (about 10^8 cells/ml) on a gyrotary shaker (200 rpm) at 28°C until late exponential phase (72 hours) [12]. The biomass was

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BIOLOGIE
Physiologie des plantes

EFFECT OF BORON DEFICIENCY ON THE CONTENTS OF LIGNIN, SOLUBLE PHENOLS, SUGARS AND WATER RELATED TO NODULATION AND N_2 FIXATION OF SOYBEAN PLANTS

G. T. Zehirov, G. I. Georgiev

(Submitted by Corresponding Member E. Karanov on January 28, 2000)

Boron (B) is an essential micronutrient for plant growth and regulation of phenolpropanoid pathway (PPP), but its relation to N_2 fixation activity of leguminous plants has not been elucidated thoroughly. Boron, acting in plants as boric anion, forms stable mono- or diesters with cis diols, like sugar alcohols, uronic acids, mannuronic acids, polyphenols, etc. These complexes of B usually influence the synthesis and stabilize certain cell wall constituents, including plasma membrane [1]. It explains why most of B is usually firmly attached to cell wall. LEE and ARONOFF [2] proposed a hypothesis for the participation of B in phenolic metabolism. It is based on the suggestion that B forms stable 6-phospho-gluconate-borate complexes and thus restricts the hexosomonophosphate flux in pentosophosphate pathway and inhibits the synthesis of phenols [3]. Another important PPP plant product is lignin – the second most abundant aromatic polymer in plants and constituent of secondary cell wall and vascular tissues [4]. Except mechanical strength, it provides certain barrier against invading harmful microorganisms and probably facilitates the partnership relations between symbiotic bacteria Rhizobium and host legume plant. Little is known about the role of lignin in the processes of attachment and development of symbiotic structures like nodules. On the other hand, certain flavonoids produced by PPP are known as signal molecules involved in Rhizobium-host legume recognition [5]. The structure of lignin is defined of polymeric material arising after polymerization of monolignols - metabolic precursors derived from PPP [4]. These precursors form β-0-4-alkylaryl ether bonds, the most common linkage lignin subunits (40 to 60%), but the proportion of subunits and character of linkage can considerably differ depending on species, tissues or stage of development. Thus, the changes in cell wall lignin quantity and structure could influence the regulation of ion and water uptake by plant cells which would reflect on plant growth rate.

The aim was to study the effects of B deficiency on the quantity and structure of lignin, total soluble phenols and reducing sugars in nodulated soybean plants, and the relationships of these changes with the plant fresh and dry matter accumulation, nodulation rate and N_2-fixation, water and potassium contents.

Material and methods. Germinating seeds of soybean plant cv. Beesson were inoculated with bacterial suspension of 10^8 cell/ml of *Bradyrhizobium japonicum* strain 639 cultured on liquid YEM medium [6]. Four-day old seedlings were transferred to

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BIOLOGIE
Physiologie des plantes

CONDUCTOMETRIC STUDY OF ION LEAKAGE FROM PLANT TISSUES

K. V. Kocheva, G. I. Georgiev

(*Submitted by Academician E. Karanov on April 20, 2005*)

Abstract

The exchange of ions between plant tissues and the external solution in vitro exhibits prominent biphasic kinetics. This is generally ascribed to the different contribution of the two compartments, apoplast and symplast, involved in the process. In this regard a semi-empirical diffusion model of the electrolyte leakage is proposed in the paper. Based on the balance of fluxes through the plasmalemma and the cell wall a function describing the ion concentration in the outer solution is derived. The behaviour of the function's parameters obtained by fitting with experimental data correlates adequately with water deficit conditions of the samples. Thus these parameters may be used to characterize the physiological status of the investigated plants.

Key words: diffusion, ions efflux, conductometry, water deficit, plant stress

Introduction. Efflux of substances from plant tissues soaked in a solution is widely used as a measure of the membrane permeability in investigations of various stress conditions, factors of growth and development, genotype specificity, etc. In many cases it is determined by analysis of the conductivity of the solution in which the tissues are incubated. Thus, distilled water with samples in it gradually increases its conductivity owing to the leakage of ions from the internal cellular space under disturbed membrane permeability.

The conductometric assessment of the ionic content of liquid media offers some clear advantages, such as non-expensive and easy maintainable apparatus, possibilities for routine analysis in outdoor conditions, etc. Furthermore, as most of the electrochemical methods, this technique is accurate and sensitive enough and allows high reproducibility of the results. The use of conductometry for these purposes has not lost its actuality despite the time elapsed from the first reported works [1]. This is revealed by the literature in which a permanent tendency toward improvement of the method is observed [2,3].

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Tome 55, No 5, 2002

BIOLOGIE
Physiologie des plantes

HOST-SPECIFICITY OF ATTACHMENT OF *BRADYRHIZOBIUM JAPONICUM* 639 CELLS TO THE ROOTS OF BORON DEFICIENT SOYBEAN (*GLYCINE MAX* L. MERR.) PLANTS RELATED TO ROOT CELL WALL STRUCTURE

G. T. Zehirov, G. Iv. Georgiev

(*Submitted by Corresponding Member E. Karanov on December 20, 2001*)

Abstract

The effect of root exudates (RE) released from soybean plants grown on the Boron (−B) deficient and Boron (+B) sufficient media on the attachment ability of pretreated with RE bacterial cells of *Bradyrhizobium japonicum* 639 prior to infection was studied. The pretreatment of bacteria with RE from – B deficient plants inhibited the attachment ability of cells to roots. But RE – B plants influenced positively the content of firmly attached cells. This fraction of cells correlated positively with the quantity of formed thereafter nodules. The results were discussed in view of explaining the role of altered pectin and hemicellulose contents under B-deficiency and connected with them changes of the bound to the cell wall Ca and Mg ions on the first steps of symbiotic relationships.

Key words: attachment, boron deficiency, *Bradyrhizobium japonicum* 639, root exudates, soybean (*Glycine max* L. Merr. cv. Beeson)

The development of nitrogen-fixing nodules of leguminous plants as a result of specific rhizobium infection is a complex multistaged process. One of the earliest steps in nodule formation is the binding of rhizobial cells to root hairs [9]. The molecular basis for cell-to-cell recognition mechanisms has been a matter of interest. There have been several models explaining the attachment of symbiotic bacteria to its host plant. Some of the experiments provided evidence that rhizobia attach nonspecifically to their host plants but other researchers considered this step specific [10]. The involvement of specific molecules, ions or other substances all of which are known to be in the root exudate (RE) was evidenced [8]. WISNIEWSKI and DELMOTE [10] reported the effect of root exudates influencing the competency for nodulation of the respective Rhizobium strain. The mechanism of binding has also been considered to involve some purely physicochemical interactions between the cell walls which were found to be very sensitive to the changes of environmental conditions [1, 9]. As the mineral nutrition is a significant factor of the environment its influence on cell permeability and cell wall properties can be suggested. All these plant functions can alter the root exudate properties [1, 10]. The content of root exudates depends strongly on the nutrient availability

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EFFECT OF INCREASED PHOSPHORUS NUTRITION ON WATER RELATIONS, PHOTOSYNTHESIS AND N_2 FIXATION OF NODULATED BLACK LOCUST PLANTS (*ROBINIA PSEUDOACACIA* L.) SUBJECTED TO DROUGHT STRESS

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(Submitted by Corresponding Member E. Karanov on June 11, 1997)

Drought affects many physiological and biochemical processes and thus can reduce plant growith [10,12]. These effects can be magnified by the absence of soil nutrients as well. It can happen when the plants grow in soils suffering not only moisture shortage but the nutrients deficits as well [10,11]. Such processes can be stopped by planting on these soils the appropriate perennial vegetation which can efficiently grow and use the soil fertility [1]. Black locust is a tree legume which can fix atmospheric nitrogen symbiotically with the appropriate *Phizobium* sp. in its root nodules [5]. It was found, for fixing successfully N_2 black locust plants need except the appropriate *Rhizobium* sp. in soil some other growth factors like sufficient supply of phosphorus (P) [11]. Drought reduces photosynthesis and effects negatively the processes of assimilate partitioning [5]. It can decrease the bacterial vitality and population in soil as well, thus reflecting negatively nodule formation and functioning [12]. Mineral nutrition is a powerful tool for improving plant growth under drought stress conditions [10]. However, the effects of fertiliser supply, especially P, on symbiotic relationships between *Rhizobium* sp. and black locust under water stress conditions are poorly understood.

So, the aim of this study was to investigate the relations between plant photosynthesis, water state and nitrogen fixation of nodulated black locust subjected to drought during the intensive growth period.

Material and methods. One root cutting of *Robinia pseudoacacia* L. (Vardim population N6, Svishtov) was planted per pot, everyone of which contained mixture of 5.5 kg dry alluvial meadow soil and 5.5 kg washed dry sand [1,8]. The plants were grown in naturally lit and heated greenhouse (day/night t°C – 20/32°). The analysis of soil shows 27.7 mg N, 18 mg P and 150 mg K per mg absolutely dry soil. Sand contained 7.4 mg N and 2.4 mg P per kg dry sand [1,2]. The soil-sand mixture was supplied with the appropriate salts (NH_4NO_3, K_2SO_4 and $CaHPO_3$) to obtain the nutrients concentrations as follows: 1. Non-fertilized (control) – $N_1P_1K_1$ – 40 mg N/kg, 18 mg P/kg and 150 mg K/kg soil; 2. Fertilized – $N_1P_3K_1$ – 40 mg N/kg, 56 mg P/mg and 150 kg K/kg soil mixture [1,5]. After emergency the saplings were inoculated with the appropriate *Rhizobium* sp. bacterial suspension (10^8 cells/ml) obtained after laboratory cultivation on YEM broth [1,5]. Soil moisture in the pots was kept at the level of 70% of full soil moisture capacity (FSMC) up to the 75 days after planting (DAP). Part of the pots were transferred to the moisture level of

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ALTERATIONS IN CELL PERMEABILITY, APOPLASTIC AND TISSUE COMPARTMENTATIONS OF SOLUTES IN THE ROOT END NODULES OF BORON DEFICIENT N_2 FIXING SOYBEAN PLANTS

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(*Submitted by Corresponding Member E. Karanov on July 12, 2000*)

Boron (B) deficiency causes many physiological and biochemical disorders usually connected with changes in the structure and function of cell wall and membranes of crop plants [3,11,1]. The absence of B in the medium effects regulation of structure and function of primary and secondary cell wall which leads to significant alterations in transport of nutrients and metabolites between the cell cytozol and vascular system of root. Studying these changes is especially important for elucidating the reasons influencing the efficiency of N_2 fixing relations of Rhizobium-Legume symbiosis. B deficiency induces changes in root and nodule formation of *Bradyrhizobium japonicum* – soybean symbiosis [11]. 30-days-old B-deficient soybean plant inoculated with *Bradyrhizobium japonicum* strain 639 has formed smaller but increased number of nodules which, however, have showed significantly lowered nitrogenous activity [11]. These changes have been studied in view to elucidate the effects of B on cell wall formation and related effects on the uptake of nutrients, water and assimilates during the period of nodule formation and functioning. The role of this trace element in the regulation of cell integrity of roots and nodules and its effects on the assimilate partitioning between apoplastic and symplastic spaces of cells has not been studied thoroughly [6]. The aim of this study was to describe the effects of B deficiency on cell permeability and connected with that changes in the soluble sugars, amino acid and amide and ureide partitioning between root and nodules apoplast and symplast of N_2 fixing soybean plants.

Material and methods. Germinating seeds of soybean cv. Beesson were inoculated with bacterial suspension of 10^8 cells/ml of *Bradyrhizobium japonicum* strain 639 cultured on liquid YEM medium [11]. Plants were grown in greenhouse as liquid culture in pots containing 1.2 L of nutrient solution with required macro- and microelements in which inorganic nitrogen (N) was omitted [11]. Boron was supplied as H_3BO_3 to control plants as 44 μg B/L. In the experimental variants no boron (B) was added in culture medium. All other conditions of growth were as described previously [11]. 35-days-old plants were harvested and analysed for soluble sugars by phenol-sulphuric method [2], free amino acids and amides were determined with ninhydrine reagent [10], ureides [9] and free soluble phenols were analysed colorimetrically [7]. Apoplastic concentrations of studied metabolites were analysed after infiltration studies of samples with 50% methanol. Recovering of the received rinses was as water solutions which were used for analysis of free space or "apoplastic" exudates [8]. The total extracts of nodules and roots were obtained from the rinsed samples which were ground and reextracted with 80% ethanol, evaporated under vacuum and solved additionally in water [5,8]. The received solutions were called total nodule and root "symplast" extracts [8]. The release of electrolytes from tissue samples were performed as described [1]. The relative leakage

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EFFECT OF Cu CONCENTRATIONS ON NITROGEN FIXATION AND Cu ACCUMULATION IN BLACK LOCUST PLANT *(ROBINIA PSEUDOACACIA* L.*)* AND ITS SYMBIOTIC *RHIZOBIUM* SP.

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(Submitted by Corresponding Member E. Karanov on June 11, 1996)

Many physiological and biochemical disturbances in micro- and macroflora were found in the soils harbouring excess of heavy metals [6,7]. Cleaning up and restoring the fertility of such poluted soils is an expensive and time consuming operation. Introduction of resistant vegetation which can grow successfully and thus can improve soil fertility is one of the promising and relatively inexpensive ways[1]. Symbiotic N_2 fixing perennual plants are the organisms which are capable of using efficiently soil fertility of such disturbed soils suffering very often from nitrogen deficiency[11]. Black locust plants can fix successfully athmospheric nitrogen when grown in soils with low fertility [1,2,11]. Data about stress tolerance of symbiotically grown trees are very scarce[1].

The aim of this study was to elucidate the effect of soil Cu excess on copper accumulation in nodulated black locust plants and in the cells of the related symbiotic bacteria *Rhizobium* sp. Some special features of N_2 fixing relationships between black locust and *Rhizobium* sp. were studied as well.

Material and methods. Plant saplings of black locust (*Robinia pseudoacacia* L.) were grown in pots in green house conditions. Pots contained 5.5 kg absolutely dry alluvial medow soil and 5.5 kg washed dry sand [1,2].To a part of the pots were added different quantities of Cu ions as $CuSO_4.5H_2O$ (2.5, 50, 200, 400 ppm Cu). One root cutting (clone 6, Svishtov) was planted per pot and was innoculated with laboratory cultivated bacterial suspension (10^8cells/ml) of symbiotic isolate of *Rhizobium* sp. [1,2]. The growth of free-living *Rhizobium* sp. cells under excess of copper in the medium YEM (2.5, 12.5, 25, 62.5, 125 ppm Cu) was studied [5]. During the late exponential phase (48 hrs) of cultivation, cells and exopolysaccharide slim (ECPS) were harvested as described previously and Cu contents were analyzed by atomic absorption spectrometry [4,10]. 90 days after the planting (DAP) the plants were harvested, dried, milled and analyzed for total N by the Kehldal method [4]. Acetylene reduction assay as a measure of nitrogenase (EC.1.17.99.2) activity of detached nodules was performed [8]. Ureides content (allantoic acid and allantoin) was analyzed after extraction with 80% ethanol, collorimetrically with phenylhydrazine [1,2]. Leaf nitrate reductase (EC.1.7.13.1) activity was assayed by "in vivo" method [9]. Leaf photosynthetic rate was measured with the leaf chamber and IR gas-analyser "Infralit-4" (Germany) connected differentially [2]. The data were processed statistically[3].

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BIOLOGIE
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GROWTH, CHEMOTAXIS AND NODULATION TO HOST ROOTS OF *BRADYRHIZOBIUM JAPONICUM* 639 IN RESPONSE TO THE EFFECT OF ROOT EXUDATES RELEASED FROM BORON DEFICIENT SOYBEAN PLANTS

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(*Submitted by Corresponding Member E. Karanov on December 5, 2001*)

Abstract

The effect of preincubation with root exudates released from boron deficient soybean roots on the growth, chemotaxis and nodulation activities of *Bradyrhizobium japonicum* 639 cells was investigated. The results show that the absence of boron in the nutritional medium of plants caused some changes in the content of released by the root exudates substances. The in vitro treatment of bacterial cells with root exudates from boron deficient plants before inoculation of plants leads to some positive changes in the growth and motility of cells which resulted in the formation of increased number of nodules after 2 week of growth. The observed changes in the symbiotic properties of Bradyrhizobium cells was discussed in view of connecting the effects of boron in the phenol-propanoid metabolism and cell permeability regulations.

Key words: boron deficiency, *Bradyrhizobium japonicum* growth and motility, root exudates

Introduction. Symbiotic interactions between rhizobia, and legume host plants result in the formation of nodular structure on the root, in which bacteria fix atmospheric nitrogen in exchange for organic carbon. The formation of legume nodules is divided into several steps which are highly sensitive to conditions of the environment [1]. Especially sensitive are the steps of preinfection and infection of the host root by rhizobial cells. These steps involve several bacterial functions which are closely related to the processes of partner recognition. The most important is the chemotaxis activity to gradients of some chemicals in the root rhizosphera [2]. Some of these compounds which act as attractants for Rhizobium are produced by the host root and are common metabolites typical of root exudates such as amino acids, organic acids or sugars, but some of them are not typical like the flavonoids. The intensive study carried out recently on the nature and function of flavonoids has shown that some of them act in the bacterial cells as inductors of specific genes recognized as *nod* genes [3]. A special group of *nod* gene cluster called *nod* D ABC is responsible for induction of cortical cell division and root hair curling in the host as response to the flavonoid action and is believed that it has primary effect on the signal exchange between symbionts. The chemotaxis activity to host root exudates is related to the growth of rhizobia as well

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ASSESSMENT OF SOLUTE ACCUMULATION IN THE LEAVES OF BARLEY SEEDLINGS UNDER DEHYDRATION AND REHYDRATION

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(*Submitted by Academician E. Karanov on December 22, 2004*)

Abstract

The physiological status of two barley (*Hordeum vulgare* L.) cultivars subjected to polyethylene glycol-induced dehydration is evaluated in the work. Relative water content and the levels of free proline, total amino acids and reducing sugars are compared as indicative characteristics in these conditions. The results suggest that the exposure to 48 h of 25% polyethylene glycol 8000 (−1,2 MPa) cannot be referred to as severe osmotic stress. Moreover, under subsequent rehydration the chosen parameters are restored almost to their initial values. All this is in accordance with the concepts regarding water deficit up to 30% as a mild stress.

Key words: barley, osmotic stress, polyethylene glycol 8000, proline, relative water content

Introduction. Osmotic adjustment is a distinguished mechanism for avoiding dehydration in many plant species experiencing water insufficiency. It represents net increase in intracellular solutes at lower water potential in order to sustain cell turgor. This is especially important for crop species where to maintain productivity while growing in adverse conditions is a major goal [1]. A well-established way of tolerating abiotic stress is the accumulation of 'compatible' solutes. Some of them like sugars, polyamines, proline, polyols and quaternary ammonium compounds are known to be effective as osmoprotectants [2]. Free proline accumulation is one of the most immediate responses to water deficit in a wide range of organisms. In some plant tissues its levels increase as much as 100-fold [3]. This does not sound strange taking into account the exclusive role of stress-induced proline in plant adaptation to the changing surroundings [4].

In the present work we study the effect of short-term water stress and the contribution of sugars, amino acids and particularly proline to osmotic adjustment in the leaves of young barley plants. The use of osmotically active substances like polyethylene glycols (PEGs) for investigation of plant water relations allows the osmotic potential of the medium to be controlled [5]. PEG 8000 is non-toxic for plants and it is generally accepted that polymers with this size usually do not penetrate into the cells.

Materials and methods. Two barley (*Hordeum vulgare* L.) cultivars, Odesskii and Houters, were used in the experiments, differing in their sensitivity to dehydration

Contribution of mineral nutrition to the response of barley seedlings to polyethylene glycol–induced mild water stress

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Summary

The effect of polyethylene glycol–induced osmotic stress on the activity of nitrate reductase, glutamine synthetase, and glycolate oxidase in leaves of young barley plants grown under two nutrient-supply regimes was studied. The activity of nitrate reductase gradually decreased after polyethylene glycol (PEG) application, while glutamine synthetase and glycolate oxidase were increased. It is speculated that the enhanced glutamine synthetase and glycolate oxidase activities are due to increased flux of metabolites through the photorespiratory cycle. Prominent increase in concentrations of free proline, reducing sugars, and free amino acids was observed. The possible contribution of these cellular solutes to the process of osmotic adjustment and the role of mineral supply is discussed. It is suggested that low N supply in combination with stress conditions switched the preferred osmolyte type from amino acids (N-containing) to sugars (C-containing).

Key words: glycolate oxidase / nitrate assimilation / osmolites / proline /water deficit

1 Introduction

Water and nitrogen (N) availability are among the major factors limiting plant growth and development. Drought may restrain nitrate acquisition by roots as well as restrict the ability of plants to reduce and assimilate nitrogen (*Frechilla* et al., 2000). Nitrate reductase (NR) is the first and rate-limiting enzyme in the nitrate-assimilation pathway (*Lillo* et al., 1996). Its activity was shown to decline in water-stressed leaves in a wide range of plant species (*Bandurska*, 1993; *Flores* et al., 2000; *Correia* et al., 2005). The regulation of this particular enzyme is related to several internal and external factors (*Vincentz* et al., 1993; *Lillo* et al., 1996). Nitrate is the primary signal inducing the transcription of NR genes. At post-translational level, NR activity is enhanced by sugars and repressed by glutamine and closely related end-products of N assimilation (*Li* et al., 1995; *Yaneva* et al., 2000; *Lillo* et al., 2004).

Glutamine synthetase (GS) is an important enzyme in higher plants involved in the re-assimilation of ammonium (NH_4^+) from photorespiration. Glutamine synthetase activity is indirectly related to osmotic adjustment since the synthesis of proline depends on glutamine (*Brugiere* et al., 1999; *Medici* et al., 2003/4). Glycolate oxidase (GO) is a key enzyme in the glycolate pathway of photorespiration that is enhanced in conditions of water deficiency, and this process represents an intersection of N and carbon (C) turnover (*Haupt-Herting* and *Fock*, 2002).

Physiological and biochemical strategies of plants to tolerate dehydration are objectives of interest in recent studies (*Bajji* et al., 2000; *Ghisi* et al., 2002). The appropriate provision with carbohydrates and amino acids in the required amounts must be achieved by efficient communication, regulation, and coordination of N and C metabolism (*Foyer* et al., 1998). The foliar concentrations of major cellular osmolytes such as free amino acids and sugars have been measured in plants grown under different N supply in order to gain insight into their possible action as signaling compounds (*Man* et al., 1999; *Correia* et al., 2005). In the present study, water deficit was imposed to plants grown under two mineral-supply levels to evaluate whether the relationships between N nutrition and the quantities of C- and N-containing compatible solutes were merely correlative or could be varied by causes other than dehydration. The aim was to determine the effects of mineral nutrition on the response of young barley plants to moderate water stress caused by PEG 8000. Polyethylene glycols (PEGs) are a family of neutral, osmotically active polymers often used in plant physiology to induce water deficit by lowering the osmotic potential of the nutrient solution and thus limit water availability for the plant (*Steuter* et al., 1981). The use of these osmotica gives very precise and reproducible results, which is more difficult to accomplish using soil cultures. The degree of stress was assessed by monitoring some aspects of the process of N assimilation, accumulation of solutes, and the development of water deficit.

2 Materials and methods

2.1 Plant material and imposition of stress

Barley (*Hordeum vulgare*, L.) plants from cultivar Odesskii, known for its adaptation to water-stress conditions in the field, were used. After surface sterilization, seeds were germinated for 48 h on wet filter paper in Petri dishes in a thermostat at 25°C in the dark. The seedlings were transferred to either 25% (reduced) or 100% (full strength) Knop nutrient solution for the experiments, the respective N levels being 2 and 8 mM N. Macronutrient composition at full strength was: 3.0 mM

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