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ORIGINAL ARTICLE

Different functions of the histone acetyltransferase *HAC1* gene traced in the model species *Medicago truncatula*, *Lotus japonicus* and *Arabidopsis thaliana*

Irina Boycheva[1] · Valya Vassileva[2] · Miglena Revalska[1] · Grigor Zehirov[2] · Anelia Iantcheva[1]

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Abstract In eukaryotes, histone acetyltransferases regulate the acetylation of histones and transcription factors, affecting chromatin structural organization, transcriptional regulation, and gene activation. To assess the role of *HAC1*, a gene encoding for a histone acetyltransferase in *Medicago truncatula*, stable transgenic lines with modified *HAC1* expression in the model plants *M. truncatula*, *Lotus japonicus*, and *Arabidopsis thaliana* were generated by *Agrobacterium*-mediated transformation and used for functional analyses. Histochemical, transcriptional, flow cytometric, and morphological analyses demonstrated the involvement of *HAC1* in plant growth and development, responses to internal stimuli, and cell cycle progression. Expression patterns of a reporter gene encoding beta-glucuronidase (*GUS*) fused to the *HAC1* promoter sequence were associated with young tissues comprised of actively dividing cells in different plant organs. The green fluorescent protein (GFP) signal, driven by the *HAC1* promoter, was detected in the nuclei and cytoplasm of root cells. Transgenic lines with *HAC1* overexpression and knockdown showed a wide range of phenotypic deviations and developmental abnormalities, which provided lines of evidence for the role of *HAC1* in plant development. Synchronization of *A. thaliana* root tips in a line with *HAC1* knockdown showed the involvement of this gene in the acetylation of two core histones during S phase of the plant cell cycle.

Keywords Histone acetyltransferase · Model legumes · Plant growth and development · Transcriptional regulation · Replication · Gene activation

Handling Editor: Heiti Paves

Electronic supplementary material The online version of this article

Introduction

Posttranslational modifications of histones, such as acetylation, methylation, phosphorylation, ubiquitination, and glycosylation, are critical components in biological processes that affect almost all aspects of plant development. They can alter chromatin structure, thereby having a pivotal role for gene transcription or silencing. Posttranslational modifications are believed to act sequentially or in a combinatorial pattern termed a "histone code" that directs the chromatin to conformational changes, thus regulating the availability of genes to transcriptional machinery. Histone acetylation and deacetylation play important roles in regulating gene expression. Transcriptionally active genes are generally highly acetylated, whereas inactive genes are associated with hypoacetylated histones (Hebbes et al. 1988; Sterner and Berger 2000; Boycheva et al. 2014). Fundamental participants in histone acetylation are specialized enzymes, named histone acetyltransferases (HATs), which transfer acetyl groups

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ORIGINAL RESEARCH

Cyclin-like F-box protein plays a role in growth and development of the three model species *Medicago truncatula*, *Lotus japonicus*, and *Arabidopsis thaliana*

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Abstract: In eukaryotes, F-box proteins are one of the main components of the SCF complex that belongs to the family of ubiquitin E3 ligases, which catalyze protein ubiquitination and maintain the balance between protein synthesis and degradation. In the present study, we clarified the role and function of the gene encoding cyclin-like F-box protein from *Medicago truncatula* using transgenic plants of the model species *M. truncatula*, *Lotus japonicas*, and *Arabidopsis thaliana* generated by *Agrobacterium*-mediated transformation. Morphological and transcriptional analyses combined with flow cytometry and histochemistry demonstrated the participation of this protein in many aspects of plant growth and development, including processes of indirect somatic embryogenesis and symbiotic nodulation. The cyclin-like *F-box* gene showed expression in all plant organs and tissues comprised of actively dividing cells. The observed variations in root and hypocotyl growth, leaf and silique development, ploidy levels, and leaf parameters in the obtained transgenic lines demonstrated the effects of this gene on organ development. Furthermore, knockdown of cyclin-like F-box led to accumulation of higher levels of the G2/M transition-specific gene cyclin B1:1 (*CYCB1:1*), suggesting its possible role in cell cycle control. Together, the collected data suggest a similar role of the cyclin-like F-box protein in the three model species, providing evidence for the functional conservation of the studied gene.

Keywords: cyclin-like F-box, model legumes, *Arabidopsis thaliana*, plant growth, plant development, cell cycle

Introduction

J. Plant Nutr. Soil Sci. 2006, *169*, 247–254 **DOI:** 10.1002/jpln.200520560

Exogenous succinate increases resistance of maize plants to copper stress

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Accepted January 25, 2006 PNSS P05/60P

Summary

The effect of copper (Cu) excess (1.5, 4.7, 31, 78, 156 μM) and exogenously supplied succinate on plant growth, chlorophyll content, chlorophyll fluorescence, and isoenzym profiles of some antioxidant enzymes in maize plants was studied. Excessive Cu supply led to a reduction in the relative growth rate (RGR), tolerance index (TI), chlorophyll *a* and chlorophyll *b* contents, and the quantum yield of PSII electron transport in the light-adapted state (ΦPSII). Copper treatment induced several changes in the anionic and cationic peroxidases (PODs), as well as superoxide dismutase (SOD) isoenzyme profiles. After 8 d of 78 μM–Cu treatment, two new anionic and two new cationic peroxidase isoenzymes in the roots were registered. Copper applied at concentrations above 31 μM resulted in higher levels of manganese superoxide dismutase (Mn-SOD) in the roots and Cu,Zn-superoxide dismutase (Cu,Zn-SOD) in the leaves. However, the addition of Na-succinate (200 μM) to the root medium prior to Cu treatment increased the capacity of the plants to partially overcome Cu toxicity.

Key words: Cu excess / Na-succinate / antioxidant enzymes / chlorophyll fluorescence

1 Introduction

antioxidants such as vitamin C and glutathione (*Babu* et al.,

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Effect of Soil Fertilizer, Foliar Fertilizer, and Growth Regulator Application on Milk Thistle Development, Seed Yield, and Silymarin Content

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Abstract: An important consideration for milk thistle (*Silybum marianum L.*) cultivation is regulating development to lengthen the reproductive stage and increase seed yield with high silymarin content. The treatment of milk thistle with foliar fertilizers and growth regulators—thidiazuron (Dropp®), 2,3,5-triiodobenzoic acid (Tiba®), mepiquat chloride (Pix®), and prohexadione-Ca (Regalis®)—resulted in an increase in the proportion of mature flower heads. Highest seed yield was observed in plants treated with Pix® and mineral soil fertilization, whereas in plants treated with foliar fertilizers, highest yields were observed with Pix® and Regalis®. The highest content of silymarin was found in plants treated with Dropp® and foliar fertilizer. Generally, treatment of milk thistle with plant-growth regulators in combination with soil or foliar mineral fertilizers increased the total amount of silymarin by increasing seed yield per hectare.

Keywords: Dropp®, Pix®, Regalis®, *Silybum marianum L.* silymarin, Tiba®

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PLANT TISSUE CULTURE

Tnt1 retrotransposon as an efficient tool for development of an insertional mutant collection of *Lotus japonicus*

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Abstract The *Tnt1* retrotransposon of tobacco (*Nicotiana tabacum*) has proven to be a very efficient mutagen for the model legume *Medicago truncatula* ecotype 108 and cultivar Jemalong 2HA and for economically important plants, such as soybean and potato. In this study, the activity of *Tnt1* in the model legume *Lotus japonicus* L. was tested. First, a new regeneration and transformation protocol was developed for *L. japonicus* that represents a new tool for legume mutagenesis and reverse genetics. Using this protocol, the *Tnt1* retrotransposon was introduced into *L. japonicus* by *Agrobacterium tumefaciens*-mediated transformation, and primary transgenic lines, named starter lines, were constructed. *In vitro* regeneration via indirect somatic embryogenesis using starter lines harboring two to eight copies of the transgene resulted in new *Tnt1* transposition events. The *Tnt1* retrotransposon remained inactive during plant growth and in the T_1 progeny, indicating that it is well suited for insertional mutagenesis in *L. japonicus*.

Keywords *Tnt1* retrotransposon · *Lotus japonicus* · Insertional mutagenesis · Starter lines · Regeneration · Embryogenesis

Introduction

Legume plants are a sustainable source of food and feed around the world. They establish beneficial symbiotic interactions with rhizobial bacteria, resulting in special structures, named root nodules. In these structures, rhizobia fix atmospheric nitrogen that plants can use, reducing the need for external fertilizers. Considering the importance of legumes, greater emphasis should be placed on the collection of genetic information for legume species.

Recent advances in "omics" approaches and studies performed on the two model legumes, *Medicago truncatula* Gaertn. (Cook 1999) and *Lotus japonicus* L. (Stougaard 2001), are adding to the understanding of legume biology. Both legume models have relatively small genomes, short life cycles, and can be easily regenerated and transformed. These advantages make it possible to analyze the phenotypic and

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The effect of inoculation of pea plants with mycorrhizal fungi and Rhizobium on nitrogen and phosphorus assimilation

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ABSTRACT

The study evaluated the response of pea (*Pisum sativum* cv. Avola) to arbuscular mycorrhizal fungi (AM) species *Glomus mosseae* and *Glomus intraradices* and *Rhizobium leguminosarum* bv. *viceae*, strain D 293, regarding the growth, photosynthesis, nodulation and nitrogen fixation activity. Pea plants were grown in a glasshouse until the flowering stage (35 days), in 4 kg plastic pots using leached cinnamonic forest soil (Chromic Luvisols – FAO) at P levels 13.2 (P1) and 39.8 (P2) mg P/kg soil. The obtained results demonstrated that the dual inoculation of pea plants significantly increased the plant biomass, photosynthetic rate, nodulation, and nitrogen fixation activity in comparison with single inoculation with *Rhizobium leguminosarum* bv. *viceae* strain D 293. On the other hand, coinoculation significantly increased the total phosphorus content in plant tissue, acid phosphatase activity and percentage of root colonization. The effectiveness of coinoculation with *Rhizobium leguminosarum* and *Glomus mosseae* was higher at the low phosphorus level while the coinoculation with *Glomus intraradices* appeared to be the most effective at higher phosphorus level.

Keywords: *Pisum sativum*; *Glomus mosseae*; *Glomus intraradices*; *Rhizobium leguminosarum*

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EMBRYO CULTURE

Agrobacterium-mediated transformation of *Medicago truncatula* cell suspension culture provides a system for functional analysis

Anelia Iantcheva · Miglena Revalska · Grigor Zehirov · Valya Vassileva

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Abstract Over the past decade, *Medicago truncatula* has been adopted as a model legume species for a range of "omic" studies. The availability of different transformation techniques has greatly advanced functional genomic studies in this species. In the present work, an efficient procedure for *Agrobacterium*-mediated transformation of *M. truncatula* cv. "Jemalong 2HA" through a cell suspension culture was developed. This procedure resulted in transformed single cells or cell clusters, giving rise to stable transgenic plants within 4 months. Transformation experiments were performed with a vector carrying two marker genes: β-glucuronidase (GUS) and green fluorescent protein (GFP) under the control of endogenous gene promoters from *LIKE AUX1 3* (*LAX3*) and GRAS transcription factor (named after GD3BERELLIC *ACID INSENSITIVE* [*GA1*], *REPRESSOR OF GA1* [*RGA*], and *SCARECROW* [*SCR*]), as well as with a binary destination vector for overexpressing the cyclin-like F-box gene fused to GFP. Maximum transformation efficiency was achieved under the following experimental conditions: acetosyringone at a concentration of 25 μM, bacterial suspension with an optical density of 0.3 at 600 nm, inoculation under agitation at 100 rpm for 24 h, co-cultivation periods of 48 h, and an uninterrupted selection with 50 mg/L kanamycin. Selection of positive transformation events was imposed early in the regeneration stage (after 48 h co-cultivation), following a large-scale screening for GFP activity. Histochemical GUS and GFP reporter activity was detected in single cells, embryogenic zones, emerging embryos, in vitro plantlets, and T_1 progeny seedlings. The transgenic nature of transformed plants was further confirmed by *nptII*-specific PCR amplification of T_0 and T_1 plant lines. The transgenic plants grown under standard greenhouse conditions displayed a wild-type phenotype and the obtained progeny segregated in a classical Mendelian manner. The fundamental steps in the transformation procedure are outlined and discussed.

Keywords Cell suspension culture · *Agrobacterium* transformation · Transcriptional reporters · β-Glucuronidase · Green fluorescent protein · Transformation efficiency

Introduction

Unlike the model flowering plant *Arabidopsis thaliana*, the legume *Medicago truncatula* establishes symbiotic relationships with nitrogen-fixing bacteria (rhizobia). This feature, together with the small, diploid and almost sequenced genome, self-fertility, short life cycle, and high levels of natural diversity makes it an excellent model for legume "omic" studies (Young et al. 2011). Furthermore, the genome of its microsymbiont *Sinorhizobium meliloti* has been sequenced and well studied (Galibert et al. 2001). In addition, *M. truncatula* is phylogenetically closely related to economically valued legumes: clover, alfalfa, pea, faba bean, chickpea, and lentils, which facilitates ready transfer of knowledge and research tools from the model

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http://wwwsoc.nii.ac.jp/jsme2/ doi:10.1264/jsme2.ME11104

Characteristics of Bacteroids in Indeterminate Nodules of the Leguminous Tree *Leucaena glauca*

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Rhizobia establish symbiosis with legumes. Bacteroids in indeterminate nodules of Inverted Repeat Lacking Clade (IRLC) legumes undergo terminal differentiation caused by Nodule-specific Cysteine-Rich peptides (NCRs). Microscopic observations of bacteroids and the detection of NCRs in indeterminate nodules of the non-IRLC legume *Leucaena glauca* were performed. A portion of the bacteroids showed moderate cell elongation, loss of membrane integrity, and multiple nucleoids. The symbiosome contained multiple bacteroids and NCR-like peptides were not detectable. These results indicate that bacteroid differentiation in *L. glauca* is different from that in IRLC legumes although both hosts form indeterminate nodules.

Key words: *Leucaena glauca*, *Bradyrhizobium*, bacteroid, nodule

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Agricultural Academy

ANALYSING THE FUNCTION AND THE EXPRESSION PATTERN OF AUXIN RESPONSE FACTOR B3 FROM *MEDICAGO TRUNCATULA* IN THE MODEL PLANT *LOTUS JAPONICUS*

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Abstract

REVALSKA, M., V. VASSILEVA, G. ZEHIROV and A. IANTCHEVA, 2016. Analysing the function and the expression pattern of Auxin response factor B3 from *Medicago truncatula* in the model plant *Lotus japonicus*. *Bulg. J. Agric. Sci.*, 22: 253–261

In plants, Auxin Response Factors (ARFs) regulate gene expression in response to auxin and may act as a transcriptional activators or repressors. ARF proteins bind to auxin response elements (AuxREs) in auxin-responsive gene promoters. Auxin Response Factor B3 from *Medicago truncatula* (*MtARF-B3*) was heterologously expressed in the model legume *Lotus japonicus*. Stable transgenic plants, overexpressing *MtARF-B3* and transcriptional reporters were created. In addition, *MtARF-B3* ortholog gene of *L. japonicus* was downregulated and knockdown plants were constructed. Phenotypic and morphological evaluation, quantitative real-time polymerase chain reaction (qRT-PCR) and histochemical GUS assay were used to study the function and expression pattern of *MtARF-B3* in the process of somatic embryogenesis and development of tissues and organs. A complex analysis of the obtained results suggests that *MtARF-B3* play role in root architecture and in fertility of the model legume *L. japonicus*.

Key words: Auxin Response Factor B3; gene expression; *Lotus japonicus*; plant growth; plant development; fertility
Abbreviations: MtARF-B3 – Auxin Response Factor B3 from *Medicago truncatula;* OE – overexpression; RNAi – RNA interference; WT – wild type

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Agricultural Academy

EVALUATION OF THE FUNCTION AND EXPRESSION PATTERN OF *MEDICAGO TRUNCATULA AUXIN RESPONSE FACTOR B3* AFTER HETEROLOGOUS EXPRESSION IN *ARABIDOPSIS THALIANA*

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Abstract

REVALSKA, M., V. VASSILEVA, G. ZEHIROV and A. IANTCHEVA, 2016. Evaluation of the function and expression pattern of *Medicago truncatula Auxin Response Factor B3* after heterologous expression in *Arabidopsis thaliana*. *Bulg. J. Agric. Sci.*, 22: 783–793

The phytohormone auxin plays a vital role in almost every aspect of plant growth and development. Expression of auxin-responsive genes is controlled by a family of Auxin Response Factor (ARF) transcription factor family. This study examined the function and expression pattern of a gene encoding Auxin Response Factor B3 from *Medicago truncatula* (*MtARF-B3*) after its heterologous expression in the model plant *Arabidopsis thaliana*. Stable transgenic plants with *ARF-B3* overexpression, downregulation and transcriptional reporters were constructed. Transcriptional and histochemical assays revealed a stable *MtARF-B3* expression in various stages of somatic embryogenesis and during the postembryonic development of *A. thaliana*. Morphological analysis and morphometric measurements confirmed the important role of *MtARF-B3* in general plant growth and development, root growth and seed production.

Key words: Arabidopsis thaliana; gene expression; *Medicago truncatula* Auxin Response Factor B3 (*MtARF-B3*); plant development; plant growth
Abbreviations: ARF – Auxin Response Factor; GFP – green fluorescent protein; GUS – β-glucuronidase; NLS – nuclear localization signal; OE – overexpression; RNAi – RNA interference; TF – transcription factor; WT – wild type

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ARTICLE; SYSTEMS BIOLOGY

Is the auxin influx carrier *LAX3* essential for plant growth and development in the model plants *Medicago truncatula, Lotus japonicus* and *Arabidopsis thaliana*?

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(*Received 27 February 2015; accepted 17 March 2015*)

The phytohormone auxin is transported by two distinct pathways in plants. Indole-3-acetic acid is mainly transported throughout the plant by an unregulated bulk flow in the mature phloem. The major auxin distribution is regulated via direct transport from cell to cell, known as polar auxin transport (PAT). PAT is maintained by the coordinated action of efflux (*PIN*) and auxin influx (*AUX/LAX*) carrier proteins. In this study, we examine, compare and localize the expression of a gene encoding an auxin influx carrier (*MtLAX3*) from *Medicago truncatula* in the model plants *M. truncatula*, *Lotus japonicus* and *Arabidopsis thaliana*. Transgenic plants with overexpression and down-regulation of *MtLAX3*, as well as with expressed *promMtLAX3* transcriptional reporters, were constructed for the three model species, using *Agrobacterium*-mediated transformation. Histochemical and transcriptional analyses revealed the expression of *MtLAX3* during various stages of somatic embryogenesis and plant development, as well as during formation of symbiotic nodules. The alteration of the *MtLAX3* expression, as well as its overexpression in the analysed model species, results in various abnormal phenotypes and disturbance of leaf and root development. The reported results show that *MtLAX3* plays an important role in proper plant growth and development, modelling of the root system and the number of formed nodules and seeds.

Keywords: auxin influx carrier *LAX3*; model legumes; nodule development; plant growth and development

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Assessment of the function and expression pattern of auxin response factor B3 in the model legume plant *Medicago truncatula*

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Abstract: The phytohormone auxin is a critical signal molecule, regulating fundamental processes in plant growth and development, such as shaping the root and shoot architecture, organ patterning, and nodulation. Auxin regulates plant gene expression mainly through auxin response factors (ARFs), which bind to auxin response elements in the promoter, upstream of auxin-activated genes. Here we examine and assess the function and expression pattern of a gene described as an auxin response factor, containing a DNA-binding pseudobarrel and B3 DNA-binding domains, from *Medicago truncatula* (*MtARF-B3*). For the model legume species *M. truncatula*, stable transgenic plants with *MtARF-B3* overexpression, downregulation, and transcriptional reporters were constructed. Phenotypic and morphological evaluation of the obtained transgenic plants confirmed the important role of *MtARF-B3* in general plant growth and development, modeling of root architecture, and development of seeds. Detailed histochemical and transcriptional analysis revealed expression of the gene in various stages of somatic embryogenesis, during formation of plant organs and tissues, and symbiotic nodulation. The fact that *MtARF-B3* was strongly expressed in stamens and pollen grains in *M. truncatula* suggests that this gene could play a role in the fertility of this model legume.

Key words: Auxin response factor B3, gene expression, model legume, plant growth, plant development

GENETICS AND PLANT PHYSIOLOGY – 2016, VOLUME 6(3–4), PP. 116–134

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OPEN ACCESS

RESEARCH ARTICLE

AMINOPEPTIDASE ACTIVITIES IN ROOTS AND LEAVES OF DROUGHT STRESSED WINTER WHEAT SEEDLINGS

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Received: *02 December 2016* Accepted: *13 December 2016*

Summary: In order to evaluate the role of aminopeptidases (APs) in drought response and their potential as protein markers to distinguish between stress tolerant and sensitive varieties, various AP activities were studied in roots and leaves of winter wheat seedlings, subjected to severe but recoverable soil drought stress. Two varieties with contrasting drought tolerance – Yantar (drought tolerant) and Miziya (sensitive) were compared. Activity changes under severe water stress and subsequent recovery were related to changes in the pools of the major redox buffers ascorbate and glutathione, changes in protein profiles and total proteolysis in roots and leaves. Glutathione was responsive to drought both in roots and leaves, with increased total pool and transient rise in the oxidized form; stronger response in the roots of Yantar was observed. The sensitive variety had higher ascorbate content in leaves under stress. Severe drought led to reversible changes in protein profiles and increase in major protease bands in leaves but not in roots. AP activities were partly independent from the predominant endoprotease activities. Highest activities in roots were detected with substrates releasing terminal leucine, lysine and metionine. In stressed leaves AP activities toward most of the substrates increased under drought, without clear differences comparing varieties. Activities tested with Gly-pNA were raised in leaves only in recovery from stress. In roots, the tolerant variety Yantar presented increased AP activities under stress with most of the substrates used except Leu-pNA and Phe-pNA, whereas the sensitive variety Miziya had almost unchanged AP activities. Based on activity profile changes, at least two different AP enzymes should exist in wheat. It remains to be established which activities towards different substrates reflect distinct aminopeptidases.

Keywords: Aminopeptidase; drought; recovery; ascorbate; glutathione; *Triticum aestivum L.*

PROCEEDINGS OF THE BALKAN SCIENTIFIC CONFERENCE OF BIOLOGY
IN PLOVDIV (BULGARIA) FROM 19TH TILL 21ST OF MAY 2005
(EDS B. GRUEV, M. NIKOLOVA AND A. DONEV), 2005 (P. 343–348)

EFFECTS OF FOLIAR FERTILIZER CONCENTRATION ON THE BIOMASS ACCUMULATION AND NITRATE ASSIMILATION RATE OF MILK THISTLE (SILYBUM MARIANUM L)

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ABSTRACT . The milk thistle plants (Silybum marianum L.) were grown for 21 days under glasshouse conditions in a 0,8 plastic pot (4 plants/pot) contained ½ strength Hellriegel's solution at the following variants: (1) control plants, without application of foliar fertilizer; (2) plants, grown with application of 0.3% foliar fertilizer; (3) plants, grown with application of 0.5% foliar fertilizer. The plants grown with addition of foliar nutrition have shown increased leaf number and dry biomass accumulation, reduced rosette diameter, enhanced rates of nitrogen assimilatory enzymes. A favorable effect of foliar feeding on the protein content, tissue nitrogen and potassium concentration have been also established.

KEY WORDS: Milk thistle (Silybum marianum L.), foliar fertilizer, dry biomass, nitrate reductase activity, glutamine synthetase activity.

EFFECTS OF COMBINED INOCULATION OF PEA PLANTS WITH ARBUSCULAR MYCORRHIZAL FUNGI AND *RHIZOBIUM* ON NODULE FORMATION AND NITROGEN FIXING ACTIVITY

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Summary. The response of pea (*Pisum sativum* cv. Avola) to arbuscular micorrizal fungi (AM) species *Glomus mosseae* and *Glomus intraradices* and *Rhizobium leguminosarum* bv. *Viciae*, strain D293 regarding growth, nodulation and nitrogen fixing activity was studied. Pea plants (*Pisum sativum* cv. Avola) were grown in a glasshouse until flowering stage (35 days) in 4 kg plastic pots using leached cinnamonic forest soil at low phosphorus level (60mg P_2O_5 kg $soil^{-1}$). The obtained results demonstrated that dual inoculation of pea plants increased plant biomass, nodulation parameters, N_2 fixation activity at varying levels compared to plants submitted to single inoculation with *Rhizobium leguminosarum*, strain D293, and depended on AM fungi species. Coinoculation increased significantly total P content in plant tissues and percentage of root colonization. Coinoculation efficiency of *Rhizobium* bacteria and *Glomus mosseae* was higher compared with *Glomus intraradices* regarding biological N_2 fixation and AM colonization at the tested P concentration.

Keywords: *Glomus intraradices, Glomus mosseae, Pisum sativum, Rhizobium leguminusarum*

Plant Peptides Govern Terminal Differentiation of Bacteria in Symbiosis 16

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Legume plants host nitrogen-fixing endosymbiotic *Rhizobium* bacteria in root nodules. In *Medicago truncatula*, the bacteria undergo an irreversible (terminal) differentiation mediated by hitherto unidentified plant factors. We demonstrated that these factors are nodule-specific cysteine-rich (NCR) peptides that are targeted to the bacteria and enter the bacterial membrane and cytosol. Obstruction of NCR transport in the *dnf1-1* signal peptidase mutant correlated with the absence of terminal bacterial differentiation. On the contrary, ectopic expression of NCRs in legumes devoid of NCRs or challenge of cultured rhizobia with peptides provoked symptoms of terminal differentiation. Because NCRs resemble antimicrobial peptides, our findings reveal a previously unknown innovation of the host plant, which adopts effectors of the innate immune system for symbiosis to manipulate the cell fate of endosymbiotic bacteria.

Symbiotic nitrogen fixation by legumes is a major contributor to the combined nitrogen pool in the biosphere. It takes place in specialized root organs called nodules (*1*). The symbiotic nodule cells are large polyploid cells (*2*) housing thousands of bacteroids. Bacteroids are differentiated *Rhizobium* bacteria with specialized metabolic activity, capable of reducing atmospheric nitrogen and supplying the plant with ammonium as a nitrogen source (*3*). In addition to this metabolic adaptation, the endosymbionts of *Medicago truncatula* and related legumes undergo striking morphological changes such as cell elongation coupled to genome amplification, membrane modifications, and the loss of reproductive capacity (*4*–*6*). The polyploid state of bacteroids and the induction of bacteroid-like cells by genetic interference with the rhizobial cell cycle (*7*–*10*) suggest that terminal bacteroid differentiation is a cell cycle–related process.

This terminal bacteroid differentiation is specific for legumes belonging to the inverted repeat–lacking clade (IRLC) such as *Medicago*, *Pisum*, or *Trifolium*, whereas bacteroids in the non-IRLC legumes, such as *Lotus japonicus*, show no sign of terminal differentiation as they maintain their normal bacterial size, genome content, and reproductive capacity (*6*). The same *Rhizobium* strains that form symbiosis with both IRLC and non-IRLC legumes have different bacteroid differentiation fates in the two legume types. Therefore, it was concluded that terminal bacteroid differentiation is determined by unknown host factors that are produced by the IRLC legumes and do not exist in the non-IRLC legumes (*6*). The nodule-specific cysteine-rich (NCR) peptides were likely candidates for these factors (*11*, *12*). *NCR* genes were found only in

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Transcript Profiling of Serine- and Cysteine Protease Inhibitors in *Triticum aestivum* Varieties with Different Drought Tolerance

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A high number of protease inhibitors (PI) have been identified in diverse plant species but information about their role in plant stress responses is still fragmentary. Transcript profiling of six published serine and cysteine protease inhibitor sequences in water-deprived plants from four winter wheat *(Triticum aestivum)* varieties with varying tolerance was performed in order to outline PIs predominantly accumulating under drought. Expression was analyzed by real time RT-qPCR. Considerable transcript accumulation of Bowman–Birk type PI WALI3 (BBPI) was detected in drought stressed leaves suggesting an important regulatory role of BBPI in adjustment of protein metabolism in leaves under dehydration. Serpin transcripts were less represented in water-deprived plants. Transient accumulation of cystatin transcripts revealed organ-specificity. Under drought cystatin and serpin expression in the leaves of the most drought tolerant variety "Katya" tended to preserve relatively stable levels close to the controls. This preliminary data will serve for future detailed study of regulation of proteolysis in winter wheat subjected to unfavorable environmental factors for development of molecular-based strategies for selection of tolerant varieties.

Keywords: Bowman–Birk protease inhibitors, cystatin, drought, serpin, wheat

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SEMI-QUANTITATIVE RT-PCR ANALYSIS OF SELECTED PROTEASE INHIBITORS IN DROUGHT-STRESSED *TRITICUM AESTIVUM*

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Summary: Proteases and their specific inhibitors are ubiquitously distributed and play a key regulatory role in many biological processes. Gene expression and activity of certain proteases has been shown to increase in *Triticum aestivum* L. leaves under drought, with a major contribution of cysteine proteases, especially in sensitive wheat varieties. However, little is known about the stress response of protease inhibitors (PIs) and their role in the regulation of intracellular proteolysis. In this study the changes in transcript abundance of some protease inhibitors (belonging to cystatin and serpin classes) were evaluated by semi-quantitative RT-PCR in leaves and roots of winter wheat seedlings from two varieties with differing tolerance. The expression of two cysteine proteases in the same samples was also assessed. The expression of the studied genes was compared in the tolerant variety "Katya" and the more susceptible to water deprivation variety "Sadovo", applying severe but recoverable soil drought. Growth inhibition and stress related parameters confirmed the relatively higher drought sensitivity of variety "Sadovo". Serpin transcript abundance in control roots was higher than in the leaves. An opposite trend was documented for cystatins – the level of their expression was stronger in the non-treated leaves compared to roots. Drought stress inhibited PI expression in roots, while varying effects on the transcript levels were detected in the leaves of water deprived plants. The levels of the two cysteine protease transcripts under drought exhibited organ-specific response – they declined in roots, and increased in leaves. Further detailed studies using more sensitive methods are necessary to evaluate the potential of protease inhibitors as biochemical markers for drought tolerance.

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Keywords : Cystatin; drought; leaves; roots; serpin; wheat.

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VARIABLE LEAF EPIDERMAL MORPHOLOGY IN *TNT1* INSERTIONAL MUTANTS OF THE MODEL LEGUME *MEDICAGO TRUNCATULA*

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ABSTRACT

In this report some typical leaf morphological characteristics of M. truncatula mutants generated by a Tnt1 retrotransposon insertion mutagenesis were evaluated and summarized. It was found that all the examined leaf epidermal parameters were strongly influenced in the Tnt1 mutant lines. Epidermal cells varied in shape and size, and diversified in the patterns of cell walls. Although the leaves of all mutant plants were amphistomatic, stomata were more abundant at the lower (abaxial) leaf surfaces than the upper (adaxial) leaf surfaces. On the other hand, the number of stomata on both leaf surfaces varied widely among different Tnt1 lines. Based on these observations, we conclude that most of the observed mutant phenotypes were caused by the Tnt1 insertions. In addition, the evaluated leaf epidermal features can be reliably applied for phenotypic profiling of M. truncatula mutant lines. Morphological variables in leaf epidermis in all the screened mutants demonstrated that Tnt1 is a very efficient mutagen, confirming that Tnt1 gene tagging strategy is one of the most valuable systems for legume functional genomics.

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and non-LTR retrotransposons, that together account for

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CHANGES IN β-GLUCOSIDASE ACTIVITY RELATED TO THE PHENOLIC CONTENT IN APOPLAST OF BORON DEFICIENT SOYBEAN ROOTS DURING INFECTION WITH *BRADYRHIZOBIUM JAPONICUM*

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(*Submitted by Corresponding Member E. Karanov on May 28, 2003*)

Abstract

The activity of β-glucosidase and the content of soluble phenols in germinating boron (B) deficient soybean plants were studied. The results show that β-glucosidase activity was low during the initial stage of seed germination and no effect of B withdrawal was observed. Further, some increase of enzyme activity of B deficient root cytozol was found between day 3 and 5 of seedling growth. At the same time, apoplastic enzyme activity was also measured. It was higher than cytozol activity of the enzyme extracted from the roots of B deficient plants. The content of soluble phenolics analysed in exudate and cytozol of roots showed some decrease during the first 10 days of growth following B deficiency treatment. The observed changes were discussed in view to elucidate the probable relationships between β-glucosidase activity and glycosylation of phenolics exuded by roots under B deficiency.

Key words: boron deficiency, β-glucosidase, phenol exudation, soybean, nodulation

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EFFECTS OF BORON STARVATION ON THE APOPLASTIC AND TOTAL SOLUTE CONCENTRATIONS INFLUENCING NODULE GROWTH AND ACETYLENE REDUCTION RATE

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Summary. The effect of boron (B) deficiency on cell permeability and connected with that, changes in soluble sugars, amino acids and ureides partitioning between root and nodule apoplast and symplast of N_2 fixing soybean plants grown in water culture were studied. Exposure of a transient 10 -day B deficiency stress was found to inhibit nodule number and to increase nodule dry weight. The formation of larger nodules in B deficient plants was found to coincide with the deterioration of solute exchange between root and nodule apoplast and symplast. These results are discussed as induced by B deficiency in cell wall and membrane permeability changes.

Key words: Boron deficiency, symplast, apoplast, nitrogen fixation, soybean

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EFFECTS OF BORON STARVATION ON LIGNIN CONTENT AND MINERAL COMPOSITION OF N_2-FIXING SOYBEAN PLANTS (GLYCINE MAX L. MERR).

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ABSTRACT. In a greenhouse experiment, symbiotic system soybean (Glycine max L. merr)-Bradyrhizobium japonicum was grown as liquid culture without boron in the nutrient solution. The dry weight of nodules and leaves was reduced drastically in boron- starved plants. The lignin concentration in nodules and leaves was increased but decreased in the roots. That changes were accompanied by increased concentration of Ca and Mg in the nodules and decreased in the leaves of boron-starved plants. In the nodules of boron- starved plants were found increased concentration of soluble phenols.

All these changes suggested that negative effect of boron starvation depended not only by a factor but complex of factors, which finally negatively affected nitrogenase activity (measured as ARA) and nodulation of symbiotic soybean plants.

KEY WORDS: boron starvation, lignin, mineral nutrition, N_2-fixation.

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Relationships between cell membrane stability, exudate content and infectivity of *Bradyrhizobium japonicum* strain 639 to boron starved soybean plants

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Key words: attachment ability, boron starvation, *Bradyrhizobium*, chemotaxis, N_2-fixation, root exudates, soybean

Abstract

The influence of boron starvation on the root exudates content in soybean seedlings (*Glycine max*. L. Merr.) and the effect of exudates pretreatment on the pre-infection processes in symbiotic system *Br. japonicum* strain 636 and soybean were investigated. Root cell membrane stability of boron starved soybean plants (-B) decreased compared to the control. The concentrations of all analyzed metabolites (reducing sugars, free amino acids, organic acids, soluble phenols and total flavonoids) from root exudates of –B plants were lower than the control concentrations. Analysis of polyphenols after HPLC chromatography of root exudates showed significant difference of peak numbers between chromatograms of exudates obtained from boron starved and from control plants.

Bacterial culture treatment with root exudates from –B plants showed decreased growth, chemotaxis and attachment ability toward the host root compared to the control exudate treatments. These changes were accompanied by decreased nodulation and acetylene reduction activity of boron starved soybean plants.

Introduction

The main function of boron under physiological conditions is to stabilize the pectic network of plant cell wall by forming di-esters with the polysaccharide moiety of rhamnogalacturonan–II (Matoh and Kobayashi 1998). Other physiological effects of boron in plants are connected with the formation of conjugated transport products with polyhydric alcohols in the phloem or stabilization of cell membrane structure and function by influencing the activity of membrane- associated proteins (Shkolnik 1984). The role of boron in nodulation and nitrogen fixation of legume plants is also well documented (Bolaños *et al.* 1994, Yamagishi and Yamamoto 1994).

Omission of boron from the nutrient solution is followed by rapid negative changes in growth and membrane transport processes of plant cells. The latter was described as loss of membrane integrity resulting in increased solute leakage into the cell apoplast (Cakmak *et al.* 1995, Pfeffer *et al.* 1998). Analysis of the received exudates showed in-

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Comparison of the Effects of Different Site of Supply (root or foliar) of Phosphate and Nitrate on the Growth and Nitrate Assimilation Enzymes Activity in Milk thistle (*Silybum marianum L.*) Plants

Abstract

The Milk thistle plants (*Silybum marianum L.*) were grown for 21 days under glasshouse conditions in a 0.8 L plastic pot (4 plants/pot) contained Heilriegel's solution at the following variants: 1) control - ½ strength solution in the growth medium; 2) ½ strength solution with the exception of NO_3 (reduced to ¼ strength solution - 1.5 mM) and $PO4^{-3}$ (increased to full strength solution - 1.0 mM) in the growth medium; 3) ¼ strength solution in the growth medium with adding 0.3 % of foliar fertilizer Agroleaf in order to make level equal to the control variant nutrients.

Increased P supply did not influence leaf number towards the control, but resulted in higher values of rosette diameter, which corresponded to the higher shoot dry weight. The plants with foliar fertilization had the lower leaf number towards the control but bigger rosette diameter, which suggests its larger leaf area. The plants grown with addition of foliar fertilizer showed the highest activities of the enzymes connected with primary N assimilation - nitrate reductase (NR: NADH, EC 1.6.6.2) and glutamine synthetase (GS: EC 6.3.1.2). A correspondence between root N content and S:R dry weight ratio was found.

Keywords: foliar fertilizer, nitrate reductase, glutamine synthetase, shoot and root dry weight, shoot and root total P and total N content

tions requirements, because the plants grow naturally in lands without management. In several pot and field experiments Zheljazkov and Nikolov (1996) have established the effects of some heavy metals on milk thistle productivity and silymarin content. In our previous study low nitrogen requirements of milk thistle seedlings was shown (Stancheva et al., 2004a).

Regularly supply of all macronutrients required by plants (N, P, S, K, Mg and Ca) was limiting factor for the rate of plant growth. Nitrogen is required for the synthesis of proteins, protoplasm, chlorophyll and enzymes. Nitrates also serves as an important signal for growth as plants respond to nitrate by altering their metabolism and by including genes in the nitrate assimilation pathway (Crawford and Glass, 1998). P is involved in the transfer of energy from the leaves to the active growing points. P is also essential for the development of cell nuclei, cell membranes and cell subdivision, all vital functions in the early stages of plant and root growth. There is general agreement that shoot to root dry weight ratio (S:R) decreases when growth is limited by N supply (Andrews

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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.