REVIEW DOI: 10.2478/V10133-009-0010-X A&EB

DEVELOPMENT OF FUNCTIONAL GENOMIC PLATFORM FOR MODEL LEGUME *MEDICAGO TRUNCATULA* IN BULGARIA

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ABSTRACT

Legumes, because of the high protein content of their seedsin grain legumes, and leaves in forage legumes, are major crop plants used in human food and animal feed. They have the unique capacity among plants to associate with soil bacteria of the genus Rhizobium to form nitrogen-fixing nodules, thereby limiting the need for exogenous nitrate. The use of the model legume Medicago truncatula over the last 10 years has dramatically improved our understanding of genomic structure and gene function for legumes in general. Nevertheless, the development of new molecular and genetic tools is essential in order to optimise the exploitation of this model plant. For example, insertional mutagenesis is necessary to construct the large-scale mutant collections which will help to identify both key symbiotic and developmental genes, as well as genes of agronomical importance. Although large-scale insertional mutagenesis using T-DNA is not feasible in legumes, the Tnt1 tobacco retrotransposon can be used as a very efficient mutagen in the M. truncatula 2HA Jemalong. This mini review comments utility and challenges of exploring forward and reverse genetic tools for functional genomic studies in M. truncatula and particular attention is paid on the use of tobacco Tnt1 retrotransposon as a tool for insertional mutagenesis in this model legume species. Current and future research activities of the AgroBioInstitute that concern this model species and the exploitation of Tnt1 insertional mutant collection will carry on in frame of the NSF funded DO02-105 "Centre for sustainable development of plant and animal genomics" project.

Keywords: Functional genomics, *Medicago truncatula, Tnt*1 mutant collection

Biotechnol. & Biotechnol. Eq. 2009, **23**(4), 1440-1443

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Bimodular auxin response controls organogenesis in *Arabidopsis*

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Contributed by Marc C. E. Van Montagu, December 30, 2009 (sent for review September 17, 2009)

Like animals, the mature plant body develops via successive sets of instructions that determine cell fate, patterning, and organogenesis. In the coordination of various developmental programs, several plant hormones play decisive roles, among which auxin is the best-documented hormonal signal. Despite the broad range of processes influenced by auxin, how such a single signaling molecule can be translated into a multitude of distinct responses remains unclear. In *Arabidopsis thaliana*, lateral root development is a classic example of a developmental process that is controlled by auxin at multiple stages. Therefore, we used lateral root formation as a model system to gain insight into the multifunctionality of auxin. We were able to demonstrate the complementary and sequential action of two discrete auxin response modules, the previously described SOLITARY ROOT/INDOLE-3-ACETIC ACID (IAA)14-AUXIN REPONSE FACTOR (ARF)7-ARF19–dependent lateral root initiation module and the successive BODENLOS/IAA12-MONOPTEROS/ARF5–dependent module, both of which are required for proper organogenesis. The genetic framework in which two successive auxin response modules control early steps of a developmental process adds an extra dimension to the complexity of auxin's action.

AUXIN/INDOLE-3-ACETIC ACID | AUXIN RESPONSE FACTOR | cell cycle | lateral root

PLANT BIOLOGY

Current Biology 20, 1697–1706, October 12, 2010 ©2010 Elsevier Ltd All rights reserved DOI 10.1016/j.cub.2010.09.007

Article

A Novel Aux/IAA28 Signaling Cascade Activates GATA23-Dependent Specification of Lateral Root Founder Cell Identity

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Summary

Background: Lateral roots are formed at regular intervals along the main root by recurrent specification of founder cells. To date, the mechanism by which branching of the root system is controlled and founder cells become specified remains unknown.

Results: Our study reports the identification of the auxin regulatory components and their target gene, *GATA23*, which control lateral root founder cell specification. Initially, a meta-analysis of lateral root-related transcriptomic data identified the GATA23 transcription factor. *GATA23* is expressed specifically in xylem pole pericycle cells before the first asymmetric division and is correlated with oscillating auxin signaling maxima in the basal meristem. Also, functional studies revealed that GATA23 controls lateral root founder cell identity. Finally, we show that an Aux/IAA28-dependent auxin signaling mechanism in the basal meristem controls *GATA23* expression.

Conclusions: We have identified the first molecular components that control lateral root founder cell identity in the *Arabidopsis* root. These include an IAA28-dependent auxin signaling module in the basal meristem region that regulates *GATA23* expression and thereby lateral root founder cell specification and root branching patterns.

ARTICLE DOI: 10.2478/V10133-010-0086-3 A&EB

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.

J Plant Res (2011) 124:147–154
DOI 10.1007/s10265-010-0340-7

REGULAR PAPER

Genotypic variation in drought stress response and subsequent recovery of wheat (*Triticum aestivum* L.)

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Received: 22 December 2009 / Accepted: 8 March 2010 / Published online: 26 May 2010

Abstract Three wheat (*Triticum aestivum* L.) genotypes, Sadovo, Katya and Prelom, with different tolerance to drought were comparatively evaluated in terms of leaf respiratory responses to progressing dehydration and consecutive rewatering. Under drought stress, the respiration of all varieties gradually decreased, as the drought-tolerant Katya showed the most pronounced decline at earlier stages of dehydration. When water stress intensified, this genotype gave relatively stable respiration rates compared with the drought-sensitive varieties. Additionally, dehydrated Katya leaves displayed lower stomatal conductance and higher photosynthesis values, which resulted in greater water use efficiency during the dehydration period. Combination of drought stress and short-term changes in leaf temperature also induced genotype-specific response that differed from the response to drought only. Over the whole temperature range, the leaves of Katya exposed to dehydration for 14 days, showed higher respiration rates compared to the drought-sensitive varieties. The sensitive varieties maintained higher respiration rates under control conditions and mild dehydration, and very low rates under severe drought. In Katya, respiration and photosynthesis were fully restored from the stress within the first day of rewatering. The drought-sensitive genotypes displayed a considerably slower recovering capacity. The results are discussed in terms of possible physiological mechanisms underlying plant tolerance to drought.

Keywords Drought tolerance · Leaf respiration · Stomatal conductance · Wheat variety

Abbreviations

Q_{10}	Change in respiration during a 10°C increase in temperature
WUE	Water use efficiency

Current Genomics, 2011, *12*, 147-152

Recent Progress in Development of *Tnt1* Functional Genomics Platform for *Medicago truncatula* and *Lotus japonicus* in Bulgaria

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Abstract: Legumes, as protein-rich crops, are widely used for human food, animal feed and vegetable oil production. Over the past decade, two legume species, *Medicago truncatula* and *Lotus japonicus*, have been adopted as model legumes for genomics and physiological studies. The tobacco transposable element, *Tnt1*, is a powerful tool for insertional mutagenesis and gene inactivation in plants. A large collection of *Tnt1*-tagged lines of *M. truncatula* cv. Jemalong was generated during the course of the project 'GLIP': Grain Legumes Integrated Project, funded by the European Union (www.eugrainlegumes.org). In the project 'IFCOSMO': Integrated Functional and COmparative genomics Studies on the MOdel Legumes *Medicago truncatula* and *Lotus japonicus*, supported by a grant from the Ministry of Education, Youth and Science, Bulgaria, these lines are used for development of functional genomics platform of legumes in Bulgaria. This review presents recent advances in the evaluation of the *M. truncatula Tnt1* mutant collection and outlines the steps that are taken in using the *Tnt1*-tagging for generation of a mutant collection of the second model legume *L. japonicus*. Both collections will provide a number of legume-specific mutants and serve as a resource for functional and comparative genomics research on legumes. Genomics technologies are expected to advance genetics and breeding of important legume crops (pea, faba bean, alfalfa and clover) in Bulgaria and worldwide.

Keywords: Insertional mutagenesis, legume genomics, *Medicago truncatula*, *Lotus japonicus*, phenotyping, *Tnt1* mutants.

Auxin-Dependent Cell Cycle Reactivation through Transcriptional Regulation of *Arabidopsis E2Fa* by Lateral Organ Boundary Proteins [W]

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Multicellular organisms depend on cell production, cell fate specification, and correct patterning to shape their adult body. In plants, auxin plays a prominent role in the timely coordination of these different cellular processes. A well-studied example is lateral root initiation, in which auxin triggers founder cell specification and cell cycle activation of xylem pole–positioned pericycle cells. Here, we report that the E2Fa transcription factor of *Arabidopsis thaliana* is an essential component that regulates the asymmetric cell division marking lateral root initiation. Moreover, we demonstrate that *E2Fa* expression is regulated by the LATERAL ORGAN BOUNDARY DOMAIN18/LATERAL ORGAN BOUNDARY DOMAIN33 (LBD18/LBD33) dimer that is, in turn, regulated by the auxin signaling pathway. LBD18/LBD33 mediates lateral root organogenesis through *E2Fa* transcriptional activation, whereas *E2Fa* expression under control of the *LBD18* promoter eliminates the need for LBD18. Besides lateral root initiation, vascular patterning is disrupted in *E2Fa* knockout plants, similarly as it is affected in auxin signaling and *lbd* mutants, indicating that the transcriptional induction of *E2Fa* through LBDs represents a general mechanism for auxin-dependent cell cycle activation. Our data illustrate how a conserved mechanism driving cell cycle entry has been adapted evolutionarily to connect auxin signaling with control of processes determining plant architecture.

Journal of Agronomy and Crop Science

J. Agronomy & Crop Science (2012) ISSN 0931-2250

DROUGHT STRESS

Long-Term Field Drought Affects Leaf Protein Pattern and Chloroplast Ultrastructure of Winter Wheat in a Cultivar-Specific Manner

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Keywords
drought-responsive proteins; soil drought; ultrastructure; wheat; yield

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Accepted September 8, 2011

doi:10.1111/j.1439-037X.2011.00492.x

Abstract

Recurrent drought periods of varying duration often cause extensive crop damage and affect wheat production in Southern Europe. This study compares biochemical and ultrastructural responses of four wheat (*Triticum aestivum* L.) cultivars to long-term field drought, and their contribution to final grain yield. Gel electrophoresis and immunoblotting analyses combined with transmission electron microscopy and grain yield evaluation were employed to assess drought susceptibility of the wheat cultivars. Two of them behaved as drought-tolerant, the other two presented as drought sensitive. Enhanced degradation of Rubisco large subunit (RLS), Rubisco small subunit (RSS) and Rubisco activase (RA) accompanied by an increased protease activity and reduced levels of heat shock proteins (HSP70) and dehydrins (DHNs) were associated with drought sensitivity. Drought tolerance coincided with relatively stable or increased HSP70 and DHN contents, and unchanged/higher levels of RLS, RSS and RA. Sensitive cultivars were more vulnerable to ultrastructural damages, showing obvious degradation of chloroplast membrane systems and depletion of leaf starch reserves. These drought responses affected yield potential, as tolerant cultivars gave higher yield under intense drought. Thus, our results provide additional insights into the complexity of plant drought responses, identifying multiple interacting traits that may serve as indirect selection criteria for wheat drought tolerance.

Journal of Plant Interactions
Vol. 7, No. 3, September 2012, 204–213

Taylor & Francis
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RESEARCH ARTICLE

Drought, high temperature, and their combination affect ultrastructure of chloroplasts and mitochondria in wheat (*Triticum aestivum* L.) leaves

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(*Received 16 November 2011; final version received 29 December 2011*)

Plants experience a number of limiting factors, as drought and heat, which are often coinciding stress factors in natural environment. This study evaluated the changes in mesophyll cell ultrastructure in the leaves of two varieties of winter wheat (*Triticum aestivum* L.), differing in their drought tolerance, under individual or combined drought and heat treatment. Although the individual stress factors affected leaf ultrastructure, the damaging effect of the combined drought and heat was more pronounced and manifested certain differences between genotypes. Chloroplasts and mitochondria were affected in a variety-specific manner under all adverse treatments. The organelles of the drought-tolerant Katya were better preserved than those in the sensitive variety Sadovo. Leaf ultrastructure can be considered as one of the important characteristics in the evaluation of the drought susceptibility of different wheat varieties.

Keywords: combined drought and heat stress; wheat; ultrastructure; mitochondria; chloroplasts; plastoglobules

TÜBİTAK

Turkish Journal of Botany

http://journals.tubitak.gov.tr/botany/

Research Article

Turk J Bot
(2013) 37: 698-707
© TÜBİTAK
doi:10.3906/bot-1209-8

Cadmium-induced structural disturbances in *Pisum sativum* leaves are alleviated by nitric oxide

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Received: 10.09.2012 • **Accepted:** 14.03.2013 • **Published Online:** 02.07.2013 • **Printed:** 02.08.2013

Abstract: In the present study, the protective effect of nitric oxide (NO) against Cd-induced structural disturbances in pea (*Pisum sativum*) leaves was investigated. Cadmium treatment resulted in a decreased leaf size and thickness of the lamina, reduced intercellular spaces in the mesophyll, small pavement cells, and a high density of stomata. These abnormalities were partially or fully reversed by a simultaneous application of Cd and the NO donor, sodium nitroprusside (SNP). The concentration of 1000 μM SNP was very effective in counteracting the adverse effects of Cd and resulted in leaf structural parameters close to those of the control leaves. These findings suggest that exogenous NO can effectively facilitate structural adjustments in pea leaves under Cd stress, which could improve stress tolerance at the whole-plant level.

Key words: Cadmium, leaves, guard cells, nitric oxide, pavement cells, *Pisum sativum*

Bulgarian Journal of Agricultural Science, 19 (No 4) 2013, 706-713
Agricultural Academy

HUMAN LACTOFERRIN CHANGES LEAF MORPHOLOGY AND PATHOGEN RESISTANCE OF *MEDICAGO SATIVA* L.

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Abstract

STEFANOVA, G., V. VASSILEVA and M. VLAHOVA, 2013. Human lactoferrin changes leaf morphology and pathogen resistance of *Medicago sativa* L. *Bulg. J. Agric. Sci.,* 19: 706-713

Human lactoferrin (hLf) is an iron-binding glycoprotein having antimicrobial activity, which is known to be involved in iron absorption and cell growth and proliferation. This study aimed investigation of the effect of hLf expression on leaf epidermal cell morphology of transgenic alfalfa plants (*Medicago sativa* L.), which exhibited enhanced resistance to bacterial pathogens. Leaf epidermal parameters were measured and a clear tendency of increasing size and decreasing number of pavement cells was found, which seemed to be related to hLf-cell cycle inhibition and compensatory cell enlargement. Furthermore, stomatal density was lower on both leaf surfaces of transgenic plants, probably due to Lf-induced inhibition of stomatal development as well. In addition, transgenic plants exhibited some characteristics, such as significant elongation of leaf epidermal cells, reduction in overall leaf size, and occasionally visible reduction of leaf chlorophyll content, which are usually related to a condition of iron deficiency, and in our case might due to iron chelation properties of hLf. Based on our observations, we assume that hLf expression changed leaf morphology, which partially contributed to the improved pathogen resistance of alfalfa in addition to the direct antimicrobial effect of the recombinant protein.

Key words: human lactoferrin, alfalfa, expression, leaf morphology

Abbreviations: hLf – human lactoferrin

In Vitro Cell.Dev.Biol.—Plant (2014) 50:149–157
DOI 10.1007/s11627-013-9554-4

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

Received: 18 March 2013 / Accepted: 19 August 2013 / Published online: 31 August 2013 / Editor: J. Forster

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 mo. 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* [*GAI*], *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

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28 *Current Genomics,* **2014,** *15,* **28-37**

Histone Acetyltransferases in Plant Development and Plasticity

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Abstract: In eukaryotes, transcriptional regulation is determined by dynamic and reversible chromatin modifications, such as acetylation, methylation, phosphorylation, ubiquitination, glycosylation, that are essential for the processes of DNA replication, DNA-repair, recombination and gene transcription. The reversible and rapid changes in histone acetylation induce genome-wide and specific alterations in gene expression and play a key role in chromatin modification. Because of their sessile lifestyle, plants cannot escape environmental stress, and hence have evolved a number of adaptations to survive in stress surroundings. Chromatin modifications play a major role in regulating plant gene expression following abiotic and biotic stress. Plants are also able to respond to signals that affect the maintaince of genome integrity. All these factors are associated with changes in gene expression levels through modification of histone acetylation. This review focuses on the major types of genes encoding for histone acetyltransferases, their structure, function, interaction with other genes, and participation in plant responses to environmental stimuli, as well as their role in cell cycle progression. We also bring together the most recent findings on the study of the histone acetyltransferase HAC1 in the model legumes *Medicago truncatula* and *Lotus japonicus.*

Received on: July 15, 2013- Revised on: October 15, 2013- Accepted on: October 21, 2013

Keywords: Histone acetyltransferases, Gene interaction, Cell cycle progression, Transcriptional regulation, Plant development, Model legumes.

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In: Advances in Environmental Research. Volume 39. ISBN: 978-1-63482-366-1
Editor: Justin A. Daniels

Chapter 9

CHANGES IN 2-DE PROTEIN PROFILE OF WHITE AND RED CLOVER LEAVES IN RESPONSE TO WATERLOGGING STRESS AND RECOVERY

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ABSTRACT

Legumes are widely used forage crops often grown in flooding prone areas but data about proteome changes in waterlogged plants are scarce. In the present study leaf 2-DE protein profiles of white (*Trifolium repens* L. cv. Haifa) and red (*Trifolium pratense* L. cv. Start) clovers, differing in waterlogging tolerance, were compared. Flooding was imposed on 21-day-old plants for a period of 14 days, followed by a 21 days of recovery. Plant physiological status was assessed by changes in leaf area, water content, photosynthetic parameters and total soluble protein. Maximum (Fv/Fm) and the actual (Φ_{PSII}) PSII efficiency were not significantly affected in both cultivars. However, non-photochemical quenching (NPQ) increased substantially in waterlogged white clover. Following 2-DE separation (pI 5-8 and 12% SDS-PAGE), 90 variable protein spots were identified using MALDI-TOF/TOF MS, resulting in reliable hits for 22 individual proteins in red and 26 - in white clover, 17 of them being common for both clovers. In both varieties a strong diminution under stress was observed in Rubisco subunits, ATP synthase subunits α and β, oxygen-evolving enhancer protein and other chloroplastic proteins. Cytochrome b6-f complex iron-sulfur subunit exhibited opposite trends under waterlogging stress - decrease in red and increase in white clover. Several proteins

manifested post-recovery over-accumulation. Results give some insight about the biochemical basis for the higher adaptation potential of *T. repens* under waterlogging stress.

Keywords: clover (*Trifolium*); flooding stress; photosynthesis, protein profiles; recovery; two-dimensional gel electrophoresis

ABBREVIATIONS

CHAPS	3-[(3-cholamidopropyl)dimethylammonio]-1-propane sulfonate
2-DE PAGE	two dimensional polyacrylamide gel electrophoresis
DTT	dithiothreitol
EDTA	ethylenediaminetetraacetic acid
F_0, F_m, F_v	initial, maximum and variable chlorophyll fluorescence in dark adapted leaves
F_0', F_m'	initial and maximum fluorescence in light adapted leaves
FW	fresh weight
IEF	isoelectric focusing
MALDI-TOF/TOF MS	matrix-assisted laser desorption/ionization time-of-flight/ time-of-flight mass spectrometry
NPQ	non-photochemical quenching
pI	isoelectric point
PSII	Photosystem II
RA	Rubisco activase
RBP	Rubisco binding protein
RLS	Rubisco large subunit
RSS	Rubisco small subunit
Rubisco	ribulose-1,5-bisphosphate carboxylase/oxygenase
RT	room temperature
TCA	trichloroacetic acid
BSA	bovine serum albumin
Φ_{PSII}	effective quantum yield.

Biotechnology & Biotechnological Equipment, 2015
Vol. 29, No. 4, 786–797, http://dx.doi.org/10.1080/13102818.2015.1031698

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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

Journal of Experimental Botany, Vol. 66, No. 17 pp. 5245–5256, 2015
doi:10.1093/jxb/erv329 Advance Access publication 10 July 2015
This paper is available online free of all access charges (see http://jxb.oxfordjournals.org/open_access.html for further details)

RESEARCH PAPER

The GLV6/RGF8/CLEL2 peptide regulates early pericycle divisions during lateral root initiation

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Received 9 March 2015; Revised 18 May 2015; Accepted 3 June 2015

Editor: Ruediger Simon

Abstract

Small peptides of the *Arabidopsis* GLV/RGF/CLEL family are involved in different developmental programmes, including meristem maintenance and gravitropic responses. In addition, our previous report suggested that they also participate in the formation of lateral roots. Specifically, *GLV6* is transcribed during the first stages of primordium development and *GLV6* overexpression results in a strong reduction of emerged lateral roots. To investigate the cause of this phenotype we analysed primordium development in gain-of-function (*gof*) mutants and found that *GLV6* induces supernumerary pericycle divisions, hindering the formation of a dome-shaped primordium, a prerequisite for successful emergence. The *GLV6* phenotype could be reproduced by ectopic expression of the gene only in xylem-pole pericycle cells. Furthermore, GLV6 seems to function at the very beginning of lateral root initiation because GLV6 excess—either gene overexpression or peptide treatment—disrupts the first asymmetric cell divisions required for proper primordium formation. Our results suggest that GLV6 acts during lateral root initiation controlling the patterning of the first pericycle divisions.

Key words: Asymmetric division, *Arabidopsis thaliana*, CLE-like, GOLVEN, lateral root development, primordium initiation, root growth factors, secreted peptides.

Abbreviations: dag, days-after-germination; ELR, emerged lateral root; gof, gain-of-function; LRFC, lateral root founder cell; LRP, lateral root primordium; ORF, open reading frame; RAM, root apical meristem; SP, signal peptide; UAS, Upstream Activating Sequence; VR, variable region; XPP, xylem pole pericycle.

nature COMMUNICATIONS

17

ARTICLE

Received 3 Jan 2015 | Accepted 7 Oct 2015 | Published 18 Nov 2015

DOI: 10.1038/ncomms9821 OPEN

A coherent transcriptional feed-forward motif model for mediating auxin-sensitive *PIN3* expression during lateral root development

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Multiple plant developmental processes, such as lateral root development, depend on auxin distribution patterns that are in part generated by the PIN-formed family of auxin-efflux transporters. Here we propose that AUXIN RESPONSE FACTOR7 (ARF7) and the ARF7-regulated FOUR LIPS/MYB124 (FLP) transcription factors jointly form a coherent feed-forward motif that mediates the auxin-responsive *PIN3* transcription *in planta* to steer the early steps of lateral root formation. This regulatory mechanism might endow the *PIN3* circuitry with a temporal 'memory' of auxin stimuli, potentially maintaining and enhancing the robustness of the auxin flux directionality during lateral root development. The cooperative action between canonical auxin signalling and other transcription factors might constitute a general mechanism by which transcriptional auxin-sensitivity can be regulated at a tissue-specific level.

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Research and Reports in Biology

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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*

This article was published in the following Dove Press journal:
Research and Reports in Biology
4 August 2015
Number of times this article has been viewed

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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

In Vitro Cell.Dev.Biol.—Plant (2016) 52:338–347
DOI 10.1007/s11627-016-9768-3

PLANT TISSUE CULTURE

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

Anelia Iantcheva[1] · Miglena Revalska[1] · Grigor Zehirov[2] · Irina Boycheva[1] · Kevin Magne[3] · Mariana Radkova[1] · Pascal Ratet[3] · Valya Vassileva[2]

Received: 20 October 2015 / Accepted: 18 May 2016 / Published online: 6 June 2016 / Editor: Mark Jordan

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

Bulgarian Journal of Agricultural Science, 22 (No 2) 2016, 253–261
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

Protoplasma
DOI 10.1007/s00709-016-0983-x

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]

Received: 3 December 2015 / Accepted: 6 May 2016

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

Published online: 14 May 2016

TÜBİTAK

Turkish Journal of Biology

http://journals.tubitak.gov.tr/biology/

Research Article

Turk J Biol
(2016) 40:
© TÜBİTAK
doi:10.3906/biy-1602-36

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

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Received: 09.02.2016 • **Accepted/Published Online:** 29.05.2016 • **Final Version:** 00.00.2016

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

agriculture

MDPI

Review

Selection and Breeding of Suitable Crop Genotypes for Drought and Heat Periods in a Changing Climate: Which Morphological and Physiological Properties Should Be Considered?

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Academic Editor: Annelie Holzkämper

Received: 19 April 2016; Accepted: 25 May 2016; Published: 1 June 2016

Abstract: Selection and breeding of genotypes with improved drought/heat tolerance become key issues in the course of global change with predicted increased frequency of droughts or heat waves. Several morphological and physiological plant traits must be considered. Rooting depth, root branching, nutrient acquisition, mycorrhization, nodulation in legumes and the release of nutrients, assimilates or phytohormones to the shoot are relevant in root systems. Xylem embolism and its repair after a drought, development of axillary buds and solute channeling via xylem (acropetal) and phloem (basipetal and acropetal) are key processes in the stem. The photosynthetically active biomass depends on leaf expansion and senescence. Cuticle thickness and properties, epicuticular waxes, stomatal regulation including responses to phytohormones, stomatal plugs and mesophyll resistance are involved in optimizing leaf water relations. Aquaporins, dehydrins, enzymes involved in the metabolism of compatible solutes (e.g., proline) and Rubisco activase are examples for proteins involved in heat or drought susceptibility. Assimilate redistribution from leaves to maturing fruits via the phloem influences yield quantity and quality. Proteomic analyses allow a deeper insight into the network of stress responses and may serve as a basis to identify suitable genotypes, although improved stress tolerance will have its price (often lowered productivity under optimal conditions).

Keywords: drought; heat; climate change; crop genotypes; morphology; physiology; stress susceptibility; assimilate allocation; yield

Genetics and Plant Physiology – 2016, Volume 6(1–2), pp. 03–13

Available online at http://www.ifrg-bg.com

LEAF EPIDERMAL PROFILING AS A PHENOTYPING TOOL FOR DNA METHYLATION MUTANTS

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Received: *09 March 2016* Accepted: *06 April 2016*

Summary: Phenotypic evaluation of epigenetic mutants is mainly based on the analysis of plant growth and morphological features. However, there are cellular level changes that are not visible to the naked eye and require analysis with higher resolution techniques.
In this study, we carried out a phenotypic characterisation of several *Arabidopsis thaliana* hypomethylation mutants by quantitative image analysis combined with flow cytometry. This phenotyping approach permitted identification of abnormalities at the cellular level in mutants with wild-type morphology at the organ level. Morphometry of adaxial leaf epidermis revealed variations in the size and number of pavement cells, and the density and distribution of stomata in the analysed second rosette leaves from the mutants studied. A direct correlation between DNA ploidy status and leaf pavement cell size in wild type and mutant leaves was observed. Recognition of hidden phenotypic variations could facilitate the identification of key genetic loci underlying the phenotypes caused by modifications of DNA methylation. Thus, this study outlines an easy and fast phenotyping strategy that can be used as a reliable tool for characterisation of epigenetic mutants at the cellular level.

Citation: Vassileva V., E. Hollwey, D. Todorov, P. Meyer, 2016. Leaf epidermal profiling as a phenotyping tool for DNA methylation mutants. *Genetics and Plant Physiology*, 6(1–2): 03–13.

Keywords: *Arabidopsis*; DNA methylation; DNA ploidy; pavement cells; hypomethylation mutants; leaf morphology.

Abbreviations: CMT2 – CHROMOMETHYLASE 2; CMT3 – CHROMOMETHYLASE 3; DDM1 - DECREASE IN DNA METHYLATION; *DIC* - differential interference contrast; DRM1 - DOMAINS REARRANGED METHYLTRANSFERASES 1; DRM2 - DOMAINS REARRANGED METHYLTRANSFERASES 2; MET1 - METHYLTRANSFERASE 1; RdDM - RNA-directed DNA methylation.

Bulgarian Journal of Agricultural Science, 22 (No 5) 2016, 783–793
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