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# ANAIS

# VIII Simpósio Brasileiro de Genética Molecular de PLANTAS

30 de maio a 2 de junho de 2023

Costão do Santinho Resort & Spa ▪ Praia do Santinho - Florianópolis - SC

SBG SOCIEDADE BRASILEIRA DE GENÉTICA

BAYER

CNPq Conselho Nacional de Desenvolvimento Científico e Tecnológico

# Apresentação

O Simpósio Brasileiro de Genética Molecular de Plantas é um evento de frequência bianual, realizado desde 2007. Iniciativa de pesquisadores da área vegetal de diversas regiões do Brasil, conta com o apoio pela Sociedade Brasileira de Genética (SBG).

O encontro presencial, com a interação direta entre pesquisadores e estudantes, sempre será um fator diferencial que não pode deixar de existir. O SBGMP é uma grande oportunidade, tanto para pesquisadores quanto para estudantes de pós-graduação e de graduação atualizarem seus conhecimentos e interagirem com os cientistas que estão produzindo conhecimento e modificando a visão e conceitos na área. Apesar de ser um evento nacional, o SBGMP tem alcance internacional e por isso é totalmente realizado em inglês.

O VIII SBGMP foi realizado em maio de 2023, no Costão do Santinho, na ilha de Florianópolis, em Santa Catarina e contou com 28 palestras de expoentes da pesquisa molecular vegetal no Brasil e no exterior, convidados em função de seus excelentes trabalhos em temas inovadores e disruptivos. Nosso encontro foi composto de 7 conferências temáticas e 9 simpósios, além de sessões de pôsteres, premiações e atividades de confraternização na abertura e encerramento.

Contamos com sua adesão e participação nos próximos simpósios para mantermos este evento como um ponto alto de encontro e discussão da comunidade molecular de plantas.

# Comitês

## COMITÊ ORGANIZADOR

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Marcio de Castro Silva Filho, *USP*
Elizabeth Pacheco Batista Fontes, *UFV*
Maria Fatima Grossi de Sa, *EMBRAPA*

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Juliane Karine Ishida, *UFMG*
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Marcia Margis-Pinheiro, *UFRGS*
Marcio de Castro Silva Filho, *USP*
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Rogério Margis, *UFRGS*

# Apoio

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# Patrocínio

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# Scientific program



## Tuesday - May 30

**18:30 - 19:00** — **Opening and Welcome Session**

**19:00 - 20:00** — **Plenary Lecture 1**

### The history of plant molecular genetics in Brazil

**Speaker:** Paulo Arruda, UNICAMP, Campinas, SP, Brazil

**Chair:** Rogerio Margis, UFRGS, Porto Alegre, RS, Brazil

# Wednesday - May 31

## 09:00 - 10:00 Plenary Lecture 2

### Characterisation of ALOG genes controlling rice inflorescence development

**Speaker:** Martin Kater, University of Milan, Milan, Italy

**Chair:** Maria Helena S. Goldman, FFCLRP/USP, Ribeirão Preto, SP, Brazil

## 10:30 - 12:00 Symposium 1

### Plant, Pathogen & Insect Interaction

**Chair:** Marcio de Castro Silva Filho, ESALQ/USP, Piracicaba, SP, Brazil

Mycovirus infection in insect pathogenic fungi

Ioly Kotta-Loizou, Imperial College London, UK

Making agriculture a more suitable niche for microbiome functioning

Fernando Dini Andreote, ESALQ/USP, Piracicaba, SP, Brazil

Molecular mechanisms in plant-multitrophic interactions

Márcio de Castro Silva Filho, USP, Piracicaba, SP, Brazil

## 14:00 - 15:30 Symposium 2

### Plant growth and development

**Chair:** Maria Helena S. Goldman, FFCLRP/USP, Ribeirão Preto, SP, Brazil

The role of Vacuolar Protein Sorting-Associated 13 (VPS13) in sexual and asexual plant reproduction

Lucia Colombo, University of Milan, Italy

The balance of holding on and letting it grow: uncovering new branches in the plant TOR network

Camila Caldana, Max Planck Institute of Molecular Plant Physiology, Germany

Genes involved in flower development and plant growth

Maria Helena S. Goldman, FFCLRP/USP, Ribeirão Preto, SP, Brazil

## 15:30 - 16:30 Plenary Lecture 3

### Sugar and brassinosteroids promote plant growth through overlapping networks of protein O-glycosylation and phosphorylation

**Speaker:** Zhiyong Wang, Carnegie Institut, CA, USA

**Chair:** Marcia Margis-Pinheiro, UFRGS, Porto Alegre, RS, Brazil

**17:00 - 18:30** **Symposium 3**

## Plant nutrition and ionomics

**Chair:** Felipe Klein Ricachenevsky, UFRGS, Porto Alegre, RS, Brazil

### The roots of nutrient-efficient plants

Ricardo Giehl, Leibniz Institute of Plant Genetics and Plant Crop Research, Germany

### The subcellular distribution of iron in seeds, a specific character in plant evolution?

Hannetz Roschzttardtz, Pontificia Universidad Catolica de Chile, Chile

### The wild side of the ionome: natural variation in elemental composition in plants

Felipe Klein Ricachenevsky, UFRGS, Porto Alegre, RS, Brazil

**18:30 - 19:30** **Enabling Solutions in Agriculture**

**Speaker:** Augusto Crivellari, Bayer

**Chair:** Rogerio Margis, UFRGS, Porto Alegre, RS, Brazil

**19:30 - 21:00** **Poster Session** (Letter A)

# Thursday - June 1st

## 09:00 - 10:00 Plenary Lecture 4

### Translational research focused on HD-Zip I transcription factors. From a model plant grown in a culture chamber to crops in field conditions and back to fundamental science

**Speaker:** Raquel Chan, Centro Científico Tecnológico CONICET Santa Fe, Argentina

**Chair:** Franceli Kulcheski, UFSC, Florianópolis, SC, Brasil

## 10:30 - 12:00 Symposium 4

### Plant Evolution and Systems Biology

**Chair:** Juliane Karine Ishida, UFMG, Belo Horizonte, MG, Brazil

Unfolded protein response in *Arabidopsis thaliana*

Federica Brandizzi, Michigan State University, USA

Rock with me: Tropical outcrops as laboratories for plant evolution and climate change adaptation

Suzana de Fátima Alcantara, UFSC, Florianópolis, SC, Brazil

Parasitic Plants: Uncovering the Evolutionary Mysteries of Nature's Ingenious Survivors

Juliane Karine Ishida, UFMG, Belo Horizonte, MG, Brazil

## 14:00 - 15:30 Symposium 5

### Plant microorganims interactions

**Chair:** Maité Vaslin de Freitas Silva, UFRJ, Rio de Janeiro, RJ, Brazil

*Citrus* leprosis: unraveling plant and viral factors underlying host-pathogen interaction

Gabriella Dias Arena, ULR em Biologia Molecular Aplicada, Instituto Biológico, São Paulo, SP, Brazil

Signaling between plant growth controls and benefical diazotrophic bacteria

Adriana Silva Hemerly, UFRJ, Rio de Janeiro, RJ, Brazil

Plant: virus interaction: how a member of N-degradon pathway can impact virus resistance in plants

Maité Vaslin de Freitas Silva, UFRJ, Rio de Janeiro, RJ, Brazil

**15:30 - 16:30** **Plenary Lecture 5**

## When the beginning determines the end: How N-terminal acetylation controls protein fate

**Speaker:** Markus Wirtz, University of Heidelberg, Germany

**Chair:** Rogerio Margis, UFRGS, Porto Alegre, RS, Brazil

**17:00 - 18:30** **Symposium 6**

## Plant Evolution and Systems Biology

**Chair:** Elizabeth P. B. Fontes, UFV, Viçosa, MG, Brazil

TIR domains function in cell death and immune pathways across the tree of life

Marc Nishimura, Colorado State University, USA

Nonhost plants as a source of resistance genes against *Xanthomonas citri* subsp. *Citri*

Paulo José Pereira Lima Teixeira, Esalq/USP, Piracicaba, SP, Brazil

The NIK1/RPL10/LIMYB signaling module in growth control and plant immunity

Elizabeth P. B. Fontes, UFV, Viçosa, MG, Brazil

**18:30 - 19:30** **Technical Conference**

Agilent solution for an Efficient and Reliable Nucleic Acid Fragment Analysis

Solange Borg, Ph.D., Product Manager, Automated Electrophoresis, Agilent Technologies

**19:30 - 21:00** **Poster Session** (Letter B)

# Friday - June 2

## 09:00 - 10:30 Oral presentations (Selected from submitted abstracts)

Fighting back herbivory: Transcriptional responses of cotton (*Gossypium hirsutum*) upon cotton boll weevil (*Anthonomus grandis*) infestation
Ana Luiza Atella de Freitas, UFRJ, Rio de Janeiro, RJ

The Ribosome associated Quality Control (RQC) in plants: how do plants deal with unfinished proteins at the ribosome?
Andréia Dias Santino da Silva, UFRJ, Rio de Janeiro, RJ

CRISPR/Cas9-mediated loss-of-function of slsbp13 increases the size of the shoot apical meristem in tomato *Solanum lycopersicum*
Carlos Hernan Barrera Rojas, ESALQ/USP, Piracicaba, SP

QTL mapping and identification of SNP-haplotypes affecting black pod resistance of *Theobroma cacao* L.
Dandara Bispo Oliveira, UESC, Itabuna, BA

LIMYB induces lignin accumulation: would this affect the defense against bacterial pathogen?
Fellipe Ramos Sampaio, UFV, Viçosa, MG

OsbHLH035: a transcription factor involved in rice anther development and flag leaf senescence
Francieli Ortolan, UFGRGS, Porto Alegre, RS

## 11:00 - 12:30 Symposium 7

### Plant signal integration

**Chair:** Marcia Margis-Pinheiro, UFRGS, Porto Alegre, RS, Brazil

Transmission and integration of redox signals in plants
Andreas Meyer, Univesitat Bonn, Germany

Xylem-borne sulfate acts as the long-distance signal for stomata closure during soil-drying
Markus Wirtz, University of Heidelberg, Germany

Going beyond antioxidant defenses: a peroxidase involved in etioplast differentiation
Marcia Margis-Pinheiro, UFRGS, Porto Alegre, RS, Brazil

**14:30 - 16:00** — **Symposium 8**

## Genome editing

**Chair:** Felipe dos Santos Maraschin, UFRGS, Porto Alegre, RS, Brazil

CRISPR/Cas genome editing for rice functional genomics and precision breeding

Yinong Yang, Pennsylvania State University, USA

Soybean genome editing via CRISPR/Cas for improved nutricional quality and drought tolerance

Liliane Marcia Mertz-Henning, EMBRAPA Soja, Londrina, PR, Brazil

Gene edition towards iron and zinc biofortification in rice

Felipe dos Santos Maraschin, UFRGS, Porto Alegre, RS, Brazil

**16:00 - 17:30** — **Symposium 9**

## Applied Plant Biotechnology

**Chair:** Maria de Fatima Grossi de Sá, EMBRAPA, Brasília, DF, Brazil

Gene discovery and editing for the improvement of stress tolerance in maize

Ricardo Augusto Dante, Genomics for Climate Change Research Center (GCCRC), UNICAMP, SP- Brazil

CRISPR technology modulation of DCD-NRP pathway elements (bip/nac30 genes) in soybean for drought tolerance

Carolina Vianna Morgante, Embrapa Semi-Arid, EMBRAPA, Brasília, DF, Brazil

GM cotton control of Cotton Boll Weevil by Multiple Gene Silencing

Daniel D. N. Vasquez, Embrapa Genetic Resources and Biotechnology, Brasilia, DF, Brazil

**17:30** — **Closing Ceremony**

SOCIEDADE BRASILEIRA DE GENÉTICA

# VIII Simpósio Brasileiro de Genética Molecular de PLANTAS

- Agricultura Tropical
- Biodiversidade e Evolução de Plantas
- Biologia de RNAs (miRNA, siRNAs, ncRNAs, circRNAs)
- Desenvolvimento Vegetal
- Estresse Abiótico
- Estresse Biótico - Interação Planta - Micro-organismos
- Estrutura e Função de Proteínas
- Genômica Aplicada de Plantas
- Transdução de Sinais em Plantas

## Agricultura Tropical ..... 21



## Biodiversidade e Evolução de Plantas ..... 32

## Estresse Biótico - interação planta-micro-organismos ..... 91

## Genômica Aplicada de Plantas ..... 126



## Transdução de Sinais em Plantas ..... 139

SOCIEDADE BRASILEIRA DE GENÉTICA

# VIII Simpósio Brasileiro de Genética Molecular de PLANTAS

# Agricultura Tropical

# A GENÉTICA EXPLICA A DIFERENÇA NA QUALIDADE DO ALHO ROXO CATARINENSE?

**Dalvan Carlos Beise [1]; Ana Kelly de Carvalho [1]; Suelen Gutteres [1]; Andressa Hilha [1]; Valdir Marcos Stefenon [2]**

[1]Bolsista . Rodovia Admar Gonzaga, 1346 Bairro Itacorubi 88.034-001 - Florianópolis - SC. Programa de Pós-Graduação em Recursos Genéticos Vegetais. Universidade Federal de Santa Catarina - UFSC. ; [2]Docente. Rodovia Admar Gonzaga, 1346 Bairro Itacorubi 88.034-001 - Florianópolis - SC. Programa de Pós-Graduação em Recursos Genéticos Vegetais. Universidade Federal de Santa Catarina - UFSC.

**Abstract:**
Garlic (Allium sativum) is a plant that has been cultivated for millennia, due to its medicinal properties and its use in cooking by several societies. Some varieties stand out in the economic sector for having improved characteristics for an increasingly demanding consumer market. This is the case of purple garlic, also known as noble, cultivated in different regions of Brazil. The Plateau of Santa Catarina is renowned for ts tradition in the production purple garlic, promoting the South region of Brazil, as well as the Midwest and Southeast regions. Although garlic-farming methods are similar in different regions, it is possible to notice differences between the final products. In fact, studies showed that there are differences in the chemical composition, with greater intensity in the purple coloration of the skin of the bulbs in the samples from the South. Therefore, in order to understand whether this difference can be explained genetically, an analysis was carried out using molecular markers. First, genomic DNA was isolated using the 2% CTAB method, and a set of 13 garlic specific SSR markers were amplified via PCR. The amplification product was separated by capillary electrophoresis, and later some diversity and genetic distance indices were analyzed. These analyzes demonstrated that there is no significant difference between garlic samples produced in different regions. The genotypic and allelic patterns of the evaluated microsatellite regions are equivalent, resulting in no genetic character differentiating the samples according to the region. Thus, it is suggested that the differences observed in relation to color and chemical composition are related to edaphoclimatic factors (temperature, altitude, rainfall), and above all, human interference from the type of management used. This demonstrates that the noble purple garlic produced in the Santa Catarina plateau presents a significant uniqueness, which should be valued to secure greater benefits and recognition for producers.
**Key-words:** genetic; garlic; DNA; SSR;

# OBTENÇÃO DE PLANTAS DE MACIEIRA (MALUS × DOMESTICA) COM SUPRESSÃO DE DORMÊNCIA ATRAVÉS DE EDIÇÃO GENÔMICA

**Felipe dos Santos Maraschin [1]; Stefano Piazza [2]; Mickael Malnoy [2]; Luis Fernando Revers [3]**
[1]Docente. Porto Alegre, RS. Brazil. Intituto de Biociências - Departamento de Botânica, UFRGS; [2]Pesquisador. San Michele all'Adige, TN. Italy. Fondazione Edmund Mach; [3]Pesquisador. Bento Gonçalves, RS. Brazil. Embrapa Uva e Vinho

**Abstract:**
Apple (*Malus x domestica*) is a temperate fruit tree with great economic importance in Brazil and in the world. Among the main abiotic factors that limit the productivity of Brazilian orchards is the need for enough chilling exposure to break bud dormancy. The main dormancy-maintaining genes are the MADS-box-type transcription factors called DAM "Dormancy-associated MADS-box". In peach (*Prunus persica*), *EVERGROWING "EVG"* mutants show total absence of dormancy due to deletion of *DAM* genes. This trait allowed the understanding, in an analogous way, of the dormancy control mechanisms in apple. Thus, in order to obtain apple varieties with suppressed dormancy we employed a CRISPR/Cas9-mediated gene editing approach to generate combinations of site- specific deletions in the DAM genes loci. Eight sgRNAs were designed with the purpose of generating combined deletions in *DAM* gene loci (*DAM1-2-4-b DAM1-4, DAM1-b and DAM2-b*), which have the potential to generate dormancy-suppression phenotypes (attenuation or absence of dormancy). We have stablished the *in vitro* culture of the new Purple Gala genotype and optimized a genetic transformation protocol. In a first trial, one thousand explants were transformed with a combination of sgRNA targeting *DAM1-2-4-b* . Selection and genotyping of potential edited events are in progress. Obtaining of this kind of genetic variation is expected to reduce the dormancy period, representing a potential biotechnological innovation for the sustainability of the apple tree production chain, within the context of the climate change impacts in the main cultivation regions in the South of Brazil.
**Key-words:** DAM; CRISPR/CAS; DORMANCY; ENVIRONMENTAL STRESS; FRUITCULTURE

## Acknowledgement

CNPq, FAPERGS, CAPES

# ISOLAMENTO E CARACTERIZAÇÃO DE PROMOTORES DE ALGODÃO (*GOSSYPIUM HIRSUTUM*) COM ALTA ATIVIDADE EM TECIDOS REPRODUTIVOS

**Gabriel Oliveira Ferreira [1]; Ana Luiza Atella [1]; Marcio Alves Ferreira [1]; Luiz Mors Cabral [2]**
[1]. . Universidade Federal do Rio de Janeiro; [2]. . Universidade Federal Fluminense

**Abstract:**
The discovery of the properties of cotton fibers by humans directed the domestication processes resulting in a legacy where the plant cotton (*Gossypium hirsutum*) is the main source of natural fiber for the textile industry as well as a source of seed oil. However, cotton culture is strongly affected by animals recognized as pests, especially the cotton boll weevil (*Anthonomus grandis*), a coleopteran that feeds and oviposits in the reproductive tissues of cotton. The control of the cotton boll weevil through strategies such as pesticides is not satisfactory, so the use of biotechnological strategies such as BT strategy (expression of cry toxic protein harmful to different insect groups) is an interesting alternative. The expression of these proteins in cotton depends on a regulatory sequence (known as a promoter). Constitutive promoters are commonly used because of their high and regular expression, but they demand a lot of energy and can cause damage to insects that are not harmful to cotton. As the cotton boll weevil attacks mainly reproductive tissues, our aim is to identify and characterize promoters with high and specific expression in cotton tissues. Previous results of our group pointed to two candidate genes for isolation of promoter region: PC1343 and PC0727. The genes were mined from public RNAseq projects available in NCBI and the expression pattern was validated by qPCR using different sets of *G. hirsutum* cDNA samples. The gene PC1343 presents a more specific expression profile to reproductive tissues at different stages, while the gene PC0727 presents an expression profile more similar to constitutive expression. In silico analyses were performed to characterize their regulatory sequences and were found six cis-elements repeats related to reproductive tissues in pPC1343 and five cis-elements repeats in pPC0727. Fragments of approximately 400 and 800bp of these sequences were fused to the *uidA* (GUS) and GFP reporter genes. *Arabidopsis thaliana* bearing the constructs pPC1343-400:GUS/GFP, pPC1343-800:GUS/GFP, pPC0727-400:GUS/GFP, and pPC0727-800:GUS/GFP were obtained and preliminary analysis of pPC1343-800:GUS/GFP showed high activity of GUS in floral buds, fruits, branches and the apical portion of the siliques. On the other hand, pPC0727-800:GUS/GFP plants showed peak activity in leaf and branch and more moderate levels of expression in fruit. GUS activity was also detected in leaf tissues and the apical portion of the siliques. Currently, we are selecting homozygous lines for all constructs above mentioned to better understand the activity of these promoter fragments and evaluate their biotechnological potential to direct CryA expression in cotton plants.
**Key-words:** *Gossypium hirsutum*; Cotton; *Anthonomus grandis*; Cotton boll weevil; tissue-specifc promoters

# PRODUÇÃO DE PLANTAS TRANSGÊNICAS DE ARROZ QUE SUPEREXPRESSAM UM GENE DE AQUAPORINA DE BROMÉLIA E SEUS EFEITOS NO DESENVOLVIMENTO VEGETAL E NO METABOLISMO DO NITROGÊNIO

**Ítalo Vinícius Cantanhêde Santos [1]; Bruno Silvestre Lira [1]; Maria Magdalena Rossi [1]; Cristiane Paula Gomes Calixto [1]; Lucas Roani Ponte [2]; Angie Geraldine Sierra Rativa [2]; Paloma Koprovski Menguer [2]; Marcia Pinheiro Margis [2]; Rogerio Margis [2]; Felipe dos Santos Maraschin [2]; Felipe Klein Ricachenevsky [2]; Helenice Mercier [1]**

[1]. Butanta, São Paulo - SP. Universidade de São Paulo; [2]. Av. Bento Gonçalves - Agronomia, Porto Alegre - RS. Universidade Federal do Rio Grande do Sul

**Abstract:**

Rice is one of the most important crop plants to the human species, serving as staple food for more than half of the world's population. Its cultivation demands a high use of nitrogen fertilizers, which leads to several environmental impacts and a high production cost. Thus, the development of cultivars with greater efficiency in the use of nitrogen (N) is of great importance. Therefore, the aim of the present work was to develop transgenic rice plants overexpressing an aquaporin gene that transports both water and ammonium, from the epiphytic bromeliad *Vriesea gigantea*, and evaluate its effects on the transgenic rice plants development. For this, rice plants (*Oryza sativa* L. ssp. *japonica* cv. Nipponbare) were transformed with the coding sequence of *VgPIP1;2*. First, the gene was cloned into a pENTR/D-TOPO entry vector, then the clones containing the stop codons were subcloned into a pANIC6A binary vector using the Gateway® system, and the construct was introduced into *Agrobacterium tumefaciens*. After infection and formation of embryogenic calli, the transformed ones were selected and regenerated. Plants from 3 transformed lines of the T2 generation and the wild type (N=10) were cultivated in modified Yoshida nutrient solution (1976), with 3 decreasing concentrations of $(NH?)_2SO_4$ (1.44 mM, 0.72 mM and 0.14 mM) as the sole nitrogen source for 3 weeks. At the end of 21 d of treatment, growth parameters were measured, and samples were collected for physiological analyses. A significant difference was observed between the plants from transgenic lines compared to the wild type regarding plant development, with the former having a greater number of leaves and tillers, greater dry and fresh weight, as well as greater root and shoot area. These results were more marked when the nitrogen concentrations of the nutrient solution were decreased, especially with 0.14 mM of $(NH?)_2SO_4$. Considering these results, a RNAseq analysis was performed using samples of roots from transgenics and wild-type plants (n=4), which were grown in complete Yoshida medium for 40 days. RNA extraction was performed with Direct-zol RNA MiniPrep Plus. Four libraries per genotype were assembled, which were then sequenced using the Ilumina paired end technology, with a read size of 100pb. Bioinformatics analyses showed different gene expression profiles between genotypes in relation to genes that participate in N metabolism. Transgenic plants showed an overexpression of genes that encode N transporters, as well as enzymes that participate in inorganic N assimilation and transformation into organic forms. In this way, the results suggested that the insertion of the aquaporin *VgPIP1;2* in rice is a promising strategy to produce new rice cultivars that may present lower costs of production and a more sustainable cultivation.

**Key-words:** Ammonium; Aquaporin; Bromeliad; Rice; RNA-seq

## Acknowledgement

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# SEQUENCIAMENTO PARCIAL DO GENOMA DE *DYCKIA IBIRAMENSIS* REITZ (BROMELIACEAE).

**Joana Oliveira Zeist [1]; Liana Bittencourt Petrarca [1]; Yohan Fritsche [1]; Tiago Montagna [2]; Valdir Marcos Stefenon [2]**

[1]Bolsista. Rodovia Admar Gonzaga, 1346 - Florianópolis, SC. Universidade federal de Santa Catarina; [2]Docente. Rodovia Admar Gonzaga, 1346 - Florianópolis, SC. Universidade federal de Santa Catarina

**Abstract:**
*Dyckia ibiramensis* is a bromeliad endemic to Ibirama, Santa Catarina. Due to the hostile environment in which it grows, this species has a high adaptive capacity to environmental fluctuations, such as floods, droughts, and high luminosity, making it an interesting model for genomic studies related to resistance to these environmental stressors. The species is classified as "Endangered" on the MMA and CNCFlora lists of threatened species. With the advances in next-generation sequencing platforms, whole genome sequencing allows structural and functional inferences in uncultivated species, revealing genes and metabolic pathways of biotechnological and agronomic interest. This work aimed at sequencing and characterizing the genome of *D. ibiramensis* to obtain genomic resources applied to the species' conservation and biotechnological exploitation. A leaf sample of one single individual of the species was collected for DNA extraction, which was sequenced on an Oxford Nanopore Technologies MinION platform. The genome was assembled with the CLC Genomics software and the fastQ and fasta files were deposited in GenBank/NCBI (BioProject PRJNA872198). Genome annotation was performed using an *ab initio* approach in the MARKER Web annotation Service. The prediction of complete and partial genes was performed using the Augustus algorithm. The completeness of the orthologous genes sequencing was evaluated using the Benchmarking Universal Single-Copy Orthologs (BUSCO). A total of 1.42 billion bases were obtained, distributed in 903,133 reads with depth coverage of 12.7x. After trimming and removing adapters, 95% of the reads had a PHRED score > 20. The assembly generated 32,113 contigs with an N50 of 3,998 bases, totaling 78,578,411 bases (7.6% of the estimated genome). The Augustus algorithm predicted 5,519 proteins, while the BUSCO analysis recorded 456 complete or partial orthologous genes. BLAST analysis returned numerous CDSs > 200 base pairs and most of the positive alignments with similarity > 50%. Genes and metabolic pathways prospected and characterized via *ab initio* and BLAST strategies are being characterized and classified based on their agronomic and biotechnological importance with application to environmental adaptation. In addition to the conservation of this genetic resource endemic to the municipality of Ibirama/SC, there is a promising field of research intending to transfer this knowledge to cultivated species, towards their genetic improvement.
**Key-words:** NGS; Genomics; Conservation; ;

## Acknowledgement

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# UTILIZAÇÃO DE GENES MORFOGÊNICOS PARA POSSIBILITAR A EDIÇÃO GENÔMICA DE LINHAGENS TROPICAIS DE MILHO

**José Hernandes-lopes [1,2]; Maísa de Siqueira Pinto [1,2]; Letícia Rios Vieira [1,2]; Juliana Vieira Almeida Nonato [1,2]; Isabel Rodrigues Gerhardt [1,2,3]; Fernanda Rausch Fernandes [1,2,3]; Sophia Victorovna Gerasimova [4,5]; Laurens Pauwels [6,7]; Paulo Arruda [1,2,8]; Ricardo Augusto Dante [1,2,3]; Juliana Erika de Carvalho Teixeira Yassitepe [1,2,3]**

[1]. Campinas, Brazil. Genomics for Climate Change Research Center (GCCRC), Universidade Estadual de Campinas; [2]. Campinas, Brazil. Centro de Biologia Molecular e Engenharia Genética, Universidade Estadual de Campinas; [3]. Campinas, Brazil. Embrapa Agricultura Digital; [4]. Novosibirsk, Russia. Institute of Cytology and Genetics of the Siberian Branch of the Russian Academy of Sciences; [5]. Novosibirsk, Russia. Novosibirsk State University; [6]. Ghent, Belgium. Department of Plant Biotechnology and Bioinformatics, Ghent University; [7]. Ghent, Belgium. Center for Plant Systems Biology, VIB; [8]. Campinas, Brazil. Instituto de Biologia, Universidade Estadual de Campinas

**Abstract:**
Genome editing (GE) is a powerful tool to accelerate plant breeding, but its large-scale application still faces relevant obstacles. Prominently among these is the recalcitrance to traditional genetic transformation protocols exhibited by many genotypes of important crop species. Despite the widespread utilization of a few maize genotypes amenable to genetic transformation, these are unfit for high-confidence phenotyping in field trials or commercial ends. This hindrance is somewhat aggravated by the fact that most of the known transformable maize lines are adapted to temperate geographies, whereas a considerable proportion of maize production occurs in the tropics. Different strategies have been recently developed and are constantly being improved to overcome the low efficiency and genotype dependency of genetic transformation. Ectopic expression of morphogenic regulators (MRs) is among the most promising approaches to achieve "universal" GE capabilities in maize. Here, we report the successful GE of tropical maize lines using a MRs-based protocol. To this end, we used a CRISPR/Cas-based construct aiming at the knockout of the *VIRESCENT YELLOW-LIKE* (*VYL*) gene, which results in an easily recognizable phenotype. Three out of eight tropical lines were amenable to transformation, with efficiencies reaching up to 5%. Remarkably, most of the events showed the *vyl* loss-of-function phenotype, with 96.4% of the recovered events presenting indels at the target site. A zygosity analysis showed that approximately 40% of the $T_0$ regenerants are heterozygous for the mutations (i.e. only one allele edited). Further investigation revealed a SNP present in heterozygosis at the target site of some lines, explaining the high rate of heterozygous $T_0$ and highlighting the importance of investigating the target sequence in new genotypes for specific sgRNA design. Finally, these results demonstrate the efficient GE of relevant tropical maize lines, expanding the current availability of GE-amenable genotypes of this important crop.

**Key-words:** CRISPR/Cas; morphogenic regulators; maize transformation; ;

# NOVOS AGENTES DE BIOCONTROLE *PAENIBACILLUS* SP. A76 E *BACILLUS* SP. N72 MELHORA O ÍNDICE DE CLOROFILA NO CRESCIMENTO DE LIMOEIROS EM SOLOS CONTAMINADOS COM *PHYTOPHTHORA* SP.

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**Abstract:**
Brazil is one of the largest lemon producers in the world, producing about 20 million tonnes/year. Lemon plants can be affected by diseases such as *Phytophthora* gummosis, caused by the oomycetes *Phytophthora parasitica*, *P. citrophthora*, and *P. palmivora*, which can lead to the death of seedlings and adult plants. Biocontrol agents are an effective and safe alternative as they inhibit the development of phytopathogens and promote plant growth. Thus, this work aims to evaluate the chlorophyll index (CI) of lemon (*Citrus x limonia*) plants grown in soils contaminated and non-contaminated with the oomycete *Phytophthora* sp. and inoculated with three new biocontrol agents. For this purpose, lemon seeds were germinated, planted in Carolina soil® substrate and disposed in four treatments: control, inoculated with sterile water; T1, T2, and T3, inoculated with the new biocontrol agents *Priestia* sp. A50, *Paenibacillus* sp. A76, and *Bacillus* sp. N72, respectively. After three months in the greenhouse, plants were transferred from substrate to soil in two treatment blocks (soil contaminated with *Phytophthora* sp. and non-contaminated). At this time, the plants received an additional inoculation with the respective biocontrol agent. After two months, chlorophyll index (CI) was determined using clorofiLOG (CFL2060 Falker). The data showed that in contaminated soils, bacterial inoculation improved CI compared to the control treatment (from 17 to ~ 35). In non-contaminated soils, inoculation with the new isolates N72 and N76 showed the highest CI of all experiments (~ 46). Therefore, it is possible to conclude that bacterial isolates *Paenibacillus* sp. A76 and *Bacillus* sp. N72 can be an effective biocontrol agent against *Phytophthora* sp. and act as growth promoters in lemon plants.
**Key-words:** Biocontrol agent; Gummosis; Sustainable agriculture; ;

# PROTEÍNAS DO METABOLISMO ENERGÉTICO ESTÃO ENVOLVIDAS EM RESPOSTA AO ESTRESSE POR CD E DEFICIÊNCIA HÍDRICA DO SOLO EM *THEOBROMA CACAO*

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**Abstract:**
Cadmium can be incorporated into the food chain and irreversibly harm animals and humans. In plants, it promotes DNA mutations and RNA degradation, interferes with the function of proteins, altering their expression, stability and post-translational changes. On the other hand, Iron, an essential nutrient for the growth and development of all living organisms, participates in several important physiological processes in plants. The increased concentration of Cd in cocoa beans and, consequently, in their derivative products, has the potential to negatively impact the trade in cocoa beans produced in Latin America and exported to European Union countries. It is necessary to carry out research with Cd, aiming at reducing the absorption of this metal by cocoa trees grown in contaminated soils and, consequently, at mitigating its toxicity. The main objective of this work was to analyze, by means of Western blotting, the proteins involved in the metabolism: ATP synthase (ATP β synthase, 53 kDa) and heat shock protein (BiP, 80 kDa) in plants of the genotype of *Theobroma cacao* CCN 51, submitted soil water deficit and metal concentrations. The plants were subjected to water stress and different doses of Cd, Fe and Cd+Fe were added to the soil, along with the control treatment (no addition of metals and no water stress). Polyclonal primary antibodies were used in the ratio (1:2000) anti-ATP synthase and (1:2000) anti-BiP from *Arabidopsis thaliana*. The accumulation of ATP-β synthase and BiP were quantified from membrane images, using the software GelQuant.Net 1.8.0 (*www.biochemlabsolutions.com*). The ATP synthase protein showed significant expression in all treatments with metals and/or water stress and in the control treatment, with the treatment with isolated Cd, which showed a lower expression of the protein when compared with the control and with the other treatments. Regarding the BiP protein detection analysis, it was verified that the treatments with the different doses and combinations of metals (Cd and Fe) presented higher expressions when compared with the control treatment (Without addition of metals), and the treatments with Cd and Cd + Water deficiency those that showed lower expression of the protein. All treatments that were subjected to soil water deficit showed higher expressions of ATP synthase, which corroborates the action of the protein related to energy metabolism and regulatory mechanisms, acting on the plant's defense system against water stress. Bip (chaperones), responsible for maintaining the conformational integrity of proteins and protein folding, showed an increase in expression in treatments with metals and metals + soil water deficit, when comparing the results with the control treatment, which demonstrates a defense response of plants to stress promoted by metals and water stress. The analysis carried out showed results that prove plant defense responses to the stresses they were subjected to.

**Key-words:** ATP- *β synthase*; BiP; Cadmium; Cocoa; Western Blotting

# AVALIAÇÃO DO POTENCIAL DE GENÓTIPOS RECALCITRANTES DE CANA-DE-ENERGIA EM CULTURA DE TECIDOS PARA TRANSFORMAÇÃO GENÉTICA MEDIADA POR *AGROBACTERIUM*

**Paula Macedo Nobile [1]; Joice Oliveira [1]; Laísa Rocha [1,2]; Simone Ferreira Silva [1]; Greice Lubini [1,3]; Danyel Fernandes Contiliani [1,2]; José A. Bressiani [4]; Silvana Creste [1,2]**

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**Abstract:**
Genetic transformation of crop plants confronts barriers not encountered in model species, thus limiting the development of commercial transgenic varieties. Genetic transformation is even more challenging in organisms with highly complex genomes, such as *Saccharum* spp. The first limiting condition is the response of a given genotype to tissue culture. In addition, *Agrobacterium*-mediated transformation, despite its advantages, such as the insertion of a low copy number of a transgene and low cost, has the disadvantage of being a biological method that depends on the interaction of the bacteria with the plant. In order to evaluate the genetic transformation potential of 13 energy cane genotypes, immature leaf roll explants were inoculated *in vitro* for the production of embryogenic callus and genetic transformation via *Agrobacterium*. These genotypes were provided from the breeding program of the Nuseed Brasil S.A. company. The marker gene neomycin phosphotransferase (*nptII*) was used to select transformed events by the selection agent (geneticin antibiotic). Callus production (C) - in grams per explant unit (C/Ex) - varied between genotypes: seven genotypes produced 1-2 C/Ex, two genotypes produced up to 1 C/Ex, one genotype produced more than 2 C/Ex, and two genotypes did not produce enough to proceed the analysis. The efficiency of *Agrobacterium*-mediated transformation of ten genotypes was evaluated by the number of independent events (IEv) obtained per gram of transformed callus (IEv/C). Vx13-0136 and Vx16-3384 genotypes displayed transformation efficiencies around 0.3 IEv/C, and the Vx15-4288 genotype reached 0.2 IEv/C; conversely, the others showed efficiencies up to 0.1 IEv/C. Although genotype Vx15-1817 was recalcitrant to transformation, its callus production excelled among the others. Thus, not necessarily there is a relationship between callus production and transformation efficiency. Our findings indicate that most energy cane genotypes are recalcitrant to *Agrobacterium*-mediated transformation and callus production is highly variable among them. These genotypes have great commercial promise, and the optimizing tissue culture procedures and genetic transformation methods is of utmost relevance for developing genetically engineered seedlings by transgenesis and genome editing approaches
**Key-words:** embryogenic callus; biomass; bioenergy; energy cane; transgenic

## Acknowledgement

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# CONTROLE DA ATIVIDADE REPRESSORA DE LIMYB: SÍTIOS DE FOSFORILAÇÃO E REGULAÇÃO POR ESTRESSES BIÓTICOS E ABIÓTICOS

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**Abstract:**
LIMYB, a nuclear protein from the MYB family of transfactors (TF) that contains two MYB/SANT-like domains, is involved in the NIK1 (NUCLEAR SHUTTLE PROTEIN-INTERACTING KINASE 1)-mediated antiviral signaling, which defends plants from begomoviruses. Upon sensing biotic and abiotic elicitors, NIK1 is activated by phosphorylation at a key threonine residue at position 474. Ribosomal protein L10 (RPL10) is phosphorylated and then translocated to the nucleus due to NIK1 activation. L10 interacts with LIMYB inside the nucleus, and the complex binds to target promoters and suppresses the expression of translation machinery- and photosynthesis-relevant genes. Despite advances in understanding the processes underpinning NIK1-mediated antiviral immunity, it still needs to be determined how the activity of LIMYB, a downstream component, is regulated. In this study, we presented several lines of evidence suggesting that NIK1 or NIK2 activation mediates LIMYB phosphorylation at Ser157 to modify the transfactor repressive activity. First, we showed that the same elicitors that activate NIK1, including begomovirus-derived nucleic acids, the bacterial PAMP flg122, and heat, also induce LIMYB phosphorylation. The expression of genes associated with the translation machinery and photosynthesis was suppressed by phosphorylated LIMYB. Alanine was substituted for Ser-157 to reduce stress-induced phosphorylation, which in turn impacted LIMYB's ability to repress target genes in response to RNA isolated from begomovirus-infected plants. The fact that LIMYB was not phosphorylated in the double mutant nik1nik2 and that expression of the constitutively activated NIK1-T474D produced LIMYB phosphorylation in the absence of elicitors further proved that LIMYB phosphorylation is driven by NIK1 or NIK2 activation. These findings show that NIK1-mediated antiviral immunity controls LIMYB phosphorylation to modify the repressing activity of the LIMYB transfactor. Also, we showed that salicylic acid and ABA induce LIMYB phosphorylation and suppress the expression of the genes linked to NIK1 signaling. Although not yet confirmed, it is highly plausible that these hormones control NIK1 activation, enhancing the scope of the NIK1-mediated signaling hub.
**Key-words:** Fosforilação; Estresse Biótico; Estresse Abiótico; Sinalização Celular; LIMYB

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CNPq, Fapemig, CAPES, Finep

SOCIEDADE BRASILEIRA DE GENÉTICA

VIII Simpósio Brasileiro de Genética Molecular de PLANTAS

# Biodiversidade e Evolução de Plantas

# ANÁLISE TRANSCRIPTOMICA EM *EUGENIA UNIFLORA* REVELA O PAPEL DA VARIAÇÃO DA EXPRESSÃO GÊNICA NA ADAPTAÇÃO.

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**Abstract:**
*Eugenia uniflora L.*, commonly known as pitanga, Surinam cherry, or Brazilian cherry, naturally occurs in contrasting ecosystems within the Atlantic Forest (AF). It belongs to the Myrtaceae family, which includes other economically important fruit crops such as guava and pitanga. *Eugenia uniflora* presents high phenotypic plasticity, and its populations are genetically and spatially structured. It is a hardy plant that can grow in a wide range of environmental conditions, including drought, poor soil, and salt spray. This capacity allows that *E. uniflora* could be cultivated in many tropical and subtropical regions around the world. Although some previous studies have identified potential loci under positive selection suggesting local adaptation, the mechanisms driving the persistence of this species in such distinct environments are still poorly understood. In this study, we combined transcriptome analyses from samples collected in nature with greenhouse experiments to unveil the expression variation within and among adaptively divergent populations of *E. uniflora*. We found several differentially expressed genes related to stress response, metabolism, and growth regulation between the two natural populations, suggesting adaptation to their respective environments. Progeny plants grown in the greenhouse showed distinct gene expression patterns compared to their parental populations, indicating plasticity in gene expression. Our results demonstrated that plasticity in gene expression and evolutionary changes are important to local adaptation in this species. These findings shed light on the molecular mechanisms underlying environmental adaptation in *E. uniflora.*
**Key-words:** Neotropics; Myrteae; local adaptation; plasticity; RNAseq

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# 7TM-RGS: A CONSERVAÇÃO ESTRUTURAL EM PLANTAS TERRESTRES AO LONGO DA EVOLUÇÃO

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**Abstract:**
There is evolutionary pressure on three-dimensional structure even within proteins with low sequence similarity. Therefore, analysis of tertiary structure coupled with Molecular dynamics (MD) simulations may reveal the marks of evolutionary constraints on proteins, though few studies used crystal structures or structural models to infer evolutionary relationships and to discover essential functions. The structure and function of the heterotrimeric G-protein complex is structurally conserved in eukaryotes but have important functional differences among the phylogentic super groups. The paradigm established in animals involves an activation/inactivation mechanism that is based on a GTP/GDP binding cycle to the alpha subunit. However, plants and other organisms lack proteins functionally homologous to canonical GPCRs because their Gα subunits exchange GDP for GTP spontaneously in a GPCR-independent mechanism, suggesting distinct regulatory mechanisms. Regulator of G protein Signaling (RGS) accelerates the intrinsic GTPase activity of Gα, which modulates the strength, duration and specificity of G protein signaling. Plant RGS homologues, unlike cytosolic RGS proteins in animals, contain a 7TM domain at the N-terminal half, a flexible linker region, and a catalytic RGS. Post-translational modification on C-terminal and subsequent endocytosis of AtRGS1 rapidly decrease the activity of AtRGS1 on plasma membrane. Plant 7TM RGS proteins have many di-serine phosphorylation sites, showing a related landscape to the C-terminal phosphorylation of animal GPCRs, which has been showed lead to receptor endocytosis and desensitization. Thus, plant G signaling may be activated through a de-repression mechanism. The di-serine regions grant a type of phospho-bar code that controls downstream signaling, such as sugar and pathogen response pathways. It is noticeable that the 7TM-RGS proteins modulate the self-activating type of heterotrimeric G protein through the regulation of catalytic activity and subcellular localizations, even though the N-terminal 7TM and linker regions unique to plants have remained poorly understood. To shed light in this mechanism, our work focused on evolutionary pressure on protein structure to discover new functional motifs and residues to regulate 7TM-RGS and heterotrimeric G protein in plants. The tertiary structure of 7TM-RGS and G-alpha complex were modeled from multiple distant plants. MD simulations revealed a role of the linker region of 7TM-RGS1 in interacting and defining the corresponding position of RGS domain surface to Gα protein. Structural testing between the wildtype and phospho-mimetic/phosphor-null mutants of 7TM-RGS1 further revealed that a phosphorylation of Ser278 residue in the linker regulates RGS1 interface by interacting with positively charged residues in the RGSbox domain. We also demonstrated the phosphorylation of Ser278 is essential for its stability regulation and internalization. Thus, our study raises a novel concept, namely that evolutionary constraint of a flexible region is based on phosphosite-driven domain orientation and modulation. If validated with resolved structures, it may also introduce a pipeline to infer protein functional motifs with the conservation of structural motifs and residue-interacting patterns in a tertiary space.
**Key-words:** RGS1; G-proteins; Tertiary structure; phosphorylation; molecular dynamics

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# DUPLICAÇÃO GÊNICA E PSEUDOGENES LIGADOS À ADAPTAÇÃO À INUNDAÇÃO EM UMA ERVA AMAZÔNICA *ISCHINOSIPHON GRACILIS* (MARANTACEAE)

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**Abstract:**
Recent studies have demonstrated that duplication and pseudogenization can contribute to the adaptive process. The flood regime presents temporal and spatial variation and is a major environmental filter to the dispersal of the Amazonian biodiversity. Despite its importance, little is known about the mechanisms that drive the evolution of species to these wetland environments. To investigate the early stages of the adaptive processes in populations of *Ischnosiphon gracilis*, we analyzed structural genomics variants to detect duplicated genes varying in copy number within species as well as the onset of pseudogenization processes along the genome. We collected leaf tissue from 48 individuals along the riparian zone of the Falsino River (Amapá, Brazil). The maximum water column level above each population was measured during a complete hydrological cycle between 2018 and 2019. We extracted and sonicated DNA to build libraries to be further sequenced in the llumina HiSeq platform. We used Trimmomatic v0.39 for read quality control and assembled the genome in Spades v3.14.1. The BUSCO pipeline was used to obtain complete single copy genes in angiosperms that exhibited duplicate copies present in individuals along of the hydrographic gradient. Analysis of natural selection on coding sequences was carried out in the HyPhy v2.5.7 software with the aBSREL tool. We found 11 duplicated genes present in all individuals of *I. gracilis* along of the hydrographical gradient, three of which were inferred to have undergone positive selection in at least one of the copies (*VAB*, *FYPP1* and BUSCO ID 76489AT3193 non-annotated). The *VAB* gene was present in individuals of non-flooded forests and in flooded populations. *FYPP1* genes was observed only in flooded populations, and one of the individuals presented two copies under positive selection. Furthermore, six of the eleven genes showed premature stop codons along their sequences, which indicates pseudogenization process. Our results imply the presence of exclusive paralogous copies within this species, i.e., genes with recent duplication (in-paralogs). Additionally, the inference of positive selection may indicate that selection is exerting pressure for diversified molecular responses under flooding stress.
**Key-words:** Adaptation; Natural selection; Flooding; Duplication; Pseudogenes

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FAPERJ, CNPq, FAPESPA and CAPES

# USO DE MARCADORES SSR E MORFOMETRIA GEOMÉTRICA PARA INVESTIGAR A CLASSIFICAÇÃO TAXONÔMICA DE *PIMENTA PSEUDOCARYOPHYLLUS*

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**Abstract:**
In the Vale do Ribeira, SP, Brazil, *Pimenta pseudocaryophyllus* Landrum (Myrtaceae) is a well known and appreciated plant for its flavor and medicinal properties, being used by the communities in different preparations of popular medicine. The plant is obtained, predominantly, by predatory extraction, causing concern in relation to the maintenance of its genetic diversity. However, little is known about this species, including whether the name *P. pseudocaryophyllus* corresponds to a species or a species complex. This species is widely distributed in South America, but occurrence records are concentrated in the Atlantic Forest and Cerrado. There are three taxonomic varieties defined on the basis of vegetative morphology, especially the shape of the leaves. *P. pseudocaryophyllus* var. *hoehnei* (DC.) Landrum is in the coastal region of southern to southeastern Brazil, mainly in herbaceous, shrubby, and arboreal coastal sandy habitats; *P. pseudocaryophyllus* var. *pseudocaryophyllus* (Gomes) Landrum, is distributed in southeastern Brazil mainly in the highlands of ombrophilous dense forests, and *P. pseudocaryophyllus* var. *fulvescens* (Burret) Landrum that occurs in the Cerrado, with rare exceptions in the Bolivian Chaco. Thus, the objective of this work was to investigate whether there is correspondence between genetic and morphological properties across taxonomic varieties. To access genetic diversity and describe population genetic structure, we genotyped 153 *P. pseudocaryophyllus* individuals across four sites with a set of 11 polymorphic microsatellites. To investigate the morphology, we used a geometric morphometry of leaves from a subset of the total samples. We considered the leaf closest to the site's average and normalized them using General Procrustes Analysis and performed a Principal Component Analysis (PCA) to describe how taxonomic varieties are organized considering the leaves' shapes and sizes. The PCA of the morphology showed that individuals comprised groups according to sampling site and variety. On the other hand, the genetic structure of the population did not show similar pattern. Although we observed clearly separated groups, var. *pseudocaryophyllus* and var. *fulvescens* were grouped in the uppermost hierarchical level of structure (2 groups), whereas var. *hoehnei* was clearly differentiated. Our preliminary findings indicate that due to the divergence between leaf morphology and genetics, leaf shape is likely not a reliable trait to the properly identify independently evolving lineages. Also, our results highlight that the taxonomy of *P. pseudocaryophyllus* likely needs to be revised.

**Key-words:** genetic diversity; cataia; polymorphism; varieties; population genetics

# CARACTERIZAÇÃO MOLECULAR E MORFOLÓGICA DE VARIEDADES DO GÊNERO CITRUS

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**Abstract:**
Citriculture is a market of global importance, in which it presents citrus species with variability in their phenotypic and molecular composition. Thus, it is important to carry out studies that characterize and explore the intraspecific diversity among citrus varieties seeking advances in breeding programs. The objective of this work was to evaluate the genetic variability among genotypes of the genus Citrus from the germplasm collection of the Instituto Federal do Espírito Santo (IFES) Campus de Alegre, through morphological and molecular analyzes. The study was carried out with accessions of the species Citrus sinensis (L.) Osbeck, Citrus latifolia, Citrus aurantifolia (C.) Swingle, and Citrus reticulata Blanco. The genotypes were characterized during the fruiting period for the characters, leaf length, leaf width, length-width ratio, leaf thickness, fruit weight, fruit diameter, fruit length, mesocarp thickness, epicarp thickness. Genomic DNAs were extracted from three leaves with young tissues, using the Doyle and Doyle protocol with modifications and subsequently submitted to polymerase chain reaction testing 13 Simple Sequence Repeats markers (SSR). The genetic divergence between the varieties was calculated, generating a dissimilarity matrix used to perform the grouping analysis by the unweighted pair group method with arithmetic mean (UPGMA). All statistical analyzes were performed using the Genes program. The resulting dendrogram separated the varieties into two morphologically distinct groups. The characters fruit weight and leaf length were the ones that presented the greatest contribution (93.14%) to the genetic divergence of the genotypes. However, the molecular characterization obtained three groups. The analyzed varieties presented observed (Ho) average heterozygosity of 0.61 and expected (He) of 0.47, indicating a higher level of heterozygotes in the population. The mean content of polymorphic information (PIC) was 0.39, showing a moderately informative value. Of the SSRs used, a total of 36 alleles were amplified, with the most informative markers being CMS-16 and CCSMEc8 and the least informative CCSMEc1. Thus, the characterizations performed were efficient to estimate the genetic dissimilarity between Citrus varieties, showing a significant effect on phenotypic and molecular divergence.
**Key-words:** SSR; Genetic variability; Molecular marker; Polymorphism; Phenotyping

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Universidade Federal de Lavras - UFLAInstituto Federal de Ciência e Tecnologia do Espírito Santo - IFES Campus de Alegre Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - CAPES Fundação de Amparo à Pesquisa e Inovação do Espírito Santo - FAPES

# ESTUDO PRELIMINAR DO BACKGROUND GENÉTICO DE ALGUMAS VARIEDADES DE *CITRUS*

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**Abstract:**
The genus Citrus originates from the Asian continent, and has great economic interest due to high adaptability for different climatic conditions, and due the nutritional characteristics of its fruits. The genus Citrus is part of Rutaceae family, with species such as tangerines (Citrus reticulata Blanco), oranges (Citrus sinensis (L.) Osbeck), lemons (Citrus limon(L.) Osbeck), grapefruits (Citrus × aurantium L.) and others. The breeding programs focused on obtaining elite line with high production, productive longevity and tolerance to some biotic and abiotic stresses. However, this selection, linked to extensive monocultures, resulted in certain standardization of cultivars, with great risk for production break due to the restrictive genetic base of cultivars. Thus, in this work we carry out a preliminary study of intra and inter samples (cultivar and commercial varieties) variability of Citrus collected in different years and locations in Brazil and maintained in the Germplasm Bank for the genus at IFES Campus de Alegre. The study was carried out with ten Citrus samples and six plants per sample. Genomic DNA was extracted from young leaves using the Doyle and Doyle (1990) protocol modified by Abdelnoor et al. (1995). To acquire the number of haplotypes, the intra and inter sample haplotypic and nucleotide diversity values, we amplified thirteen SSRs genomics markers. The results were obtained by DNAsp and Arlequin software. The number of haplotypes, and intra and inter sample haplotypic diversity values were large for most samples. However, the nucleotide diversity values were low for these samples, suggesting great diversity with low fixed alleles. For the Limão Taiti and Limão Branco samples, both values were low for mentioned indices above, suggesting a narrow diversity (bottleneck). Thus, we suggest that new loci be analyzed to confirm this result of genetic narrowing, and/or that there are greater introgressions of materials for Limão Taiti and Limão Branco samples to initial increase of haplotypic indices and their genomic diversification. We also suggest that future studies carry out samplings not only in the Brazil's citrus belt, in state of São Paulo and Southwest region of Minas Gerais, but also in the states of Paraná, Bahia, Sergipe and Rio Grande do Sul, where citriculture has a significant importance for national production.
**Key-words:** SSR; Genetic erosion; Brazil; ;

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# ANÁLISE DA DIVERSIDADE GENÉTICA DE UM BANCO DE GERMOPLASMA DE BATATA-DOCE

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**Abstract:**
Sweet potato, *Ipomoea batatas* L. (Convolvulaceae), is native to Central and South America. It has wide use, ranging from human and animal consumption, to the production of flour and ethanol. In Vale do Ribeira, SP, Brazil, there is great morphological variation among the studied materials, with the varieties kept by small farmers, home gardens, indigenous communities and quilombolas, ethnic minorities in Brazil. However, the modernization of agriculture and the rural exodus have caused the loss of genetic diversity, and the latter factor also being responsible for replacing a large part of the consumption of sweet potatoes by products that are easier to prepare and more attractive. This scenario has led to the loss of genotypes that were kept by farmers, consequently requiring the acquisition and maintenance of these materials in collections, allowing the conservation of genetic variability and breeding programs for the species. Therefore, we established at Unesp (Registro, SP, Brazil) a germplasm bank of sweet potato clones collected in Vale do Ribeira and the present work aimed to carry out the genetic characterization of some accesses using SSR markers previously described in literature. We selected 49 accesses for individual DNA extraction and PCR reactions with eight polymorphic microsatellite loci (SSR). Each access was genotyped performing polyacrylamide gel electrophoresis of the obtained PCR products. These data were used to calculate Diversity Indices based on Simpson and Shannon, Roger's Distance and to perform a PCA (Principal Component Analysis). It was not possible to detect genetic structure in the analyzed pool and the overall diversity was low (Shannon: 3.89182; Simpson: 0). The PCA revealed the existence of two divergent groups, one of them containing nine individuals, but the divergence between them is discreet as confirmed by the heatmap generated from the Roger's Distance analysis. Our results suggest that evaluated accessions are genetically close, which makes it difficult to obtain divergent parents that may be necessary in future breeding programs. Future analyses considering morphological and agronomic characteristics will be fundamental, since there is a notorious diversity of these aspects in the established collection.
**Key-words:** Etnovarieties; Conservation; Plant breeding; ;

## Acknowledgement

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# USANDO SEQUENCIAMENTO RAD PARA RESOLVER INCONGRUÊNCIAS FILOGENÉTICAS DE *PSEUDOTRIMEZIA*(IRIDACEAE).

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**Abstract:**
Most studies on plant molecular systematics done so far have used informative Sanger sequencing markers to infer phylogenies in different taxonomic magnitudes. However, the selection of genetic markers to answer a scientific question, often including the design of custom markers, can be an expensive and time consuming stage of a scientific research project. Moreover, recent and current evolutionary events, such as incipient speciation and genetic flow, may result in different sets of genetic markers telling different evolutionary stories. The Trimezia tribe (Iridaceae family) is an example of a group in which systematics benefited from the use of Sanger sequencing markers to test hypotheses of morphological homology and phylogenetic classification. The species of this tribe concentrate in the Campos Rupestres, which is a very diverse Brazilian habitat, where many evolutionary radiations occurred in the last 10 million years. The great diversity and recent diversification hinder phylogenetic inference because, in many cases, the regions flanked by the usual Sanger genetic markers haven't accumulated sufficient mutations in each lineage after their evolutionary divergence. In the last revision of the tribe, 5 chloroplast markers and 1 nuclear marker were used. The inferences retrieved a good resolution for the oldest relationship nodes, and the genera were organized accordingly. However, it wasn't possible to unveil the topology of the internal nodes of each genus. *Pseudotrimezia* is a specially difficult genus, displaying incongruences between the chloroplast and nuclear matrices. In this work, I explored the history of *Pseudotrimezia* using a type of next generation sequencing named RAD (Restriction-site associated DNA) based on the Pst1 enzyme. I included 30 terminals (encompassing 16 of the 24 existing species in the genus) and 1 terminal outgroup. The assembly and alignment retrieved an average of 1472 base pairs per terminal (min 401/ max 2477). Thus the final alignment contained 33389 locus, of which 9114 were parsimony informative, including more than one allele. The phylogenetic inferences generated through Maximum Likelihood and Bayesian analyses showed high support for both bootstrap and posterior probability. Out of the 29 nodes, 26 had posterior probability over 0.999. I also tested the alternative hypothesis of genetic flow against lineage sorting using Petterson's D statistics, in order to probe the incongruence found among the different Sanger genetic marker sets from different genomes. My results pointed at significant gene flow events among 10 of the 16 species. Even though RAD sequencing is more often used in population studies, it proved to be an efficient and cost effective option to investigate relationship among species that diverged recently and are subject to genetic flow. Further analyses will be carried out to test the uneven direction of seed/pollen gene flow among the species.
**Key-words:** Pseudotrimezia; velloziaceae; Rad-sequencing; systematics; Campos Rupestres

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# DESVENDANDO A RELAÇÃO EVOLUTIVA DA FAMÍLIA GÊNICA ASCORBATO OXIDASE EM FABACEAE

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**Abstract:**
Ascorbate oxidase (AAO) is an enzyme found in the apoplast of plant cells that converts ascorbate into dehydroascorbate through a reduction reaction. AAO contains three functional domains of Multicopper Oxidase (Cu-oxidase 1, 2, and 3). Despite its importance in redox metabolism, AAO has primarily been studied using biochemical methods. However, there have been limited investigations into the evolution of the AAO gene family, and no studies have explored the evolutionary history of AAO in the Fabaceae plant family, which includes economically and nutritionally significant crops such as soybean, peanut, and bean. To better understand the evolutionary relationship of AAO in the Fabaceae plant family, we conducted a comprehensive analysis using a range of bioinformatics tools. A BLASTp search using the AAO sequence from *Glycine max* (Glyma.13G076900) as a query was performed against 21 Fabaceae genomes. *Arabidopsis thaliana*, *Oryza sativa*, and *Selaginella moellendorffii* were included as outgroups. The alignment was performed using MAFFT software and manually inspected to maintain only sequences with the three Cu-oxidase domains. A total of 70 sequences were maintained for the posterior analysis. The Maximum-likelihood phylogeny, performed in IQTree v2.1, revealed three main clusters (labeled Cluster I, II, and III) with most bootstrap values above 90%. *S. moellendorffii* AAO sequences were grouped in Cluster I. Monocots and dicots species were grouped in Clusters II and III, indicating that AAO originated before the divergence of these angiosperms. We conducted a *de novo* motif analysis, identifying ten conserved motifs with 20 to 50 amino acids each, all from the Cu-oxidase domains. This analysis helped to confirm that all the sequences were members of the AAO family. Also, a gene structure analysis revealed that the sequences varied in size from one to eight kb, with nearly all genes having five exons and four introns. These findings show that AAO sequences' gene organization and protein structure are highly conserved. In addition, the upstream promoter region from soybean *AAO* was analyzed to identify selected *cis*-regulatory elements. The analysis presented 25 transcription factor (TF) binding motifs, like BBR-BPC, HD-Zip, MYB, MIKC MADS, TALE, and bZIP. These TFs were involved in many important processes in plant metabolism, such as development, flowering, hormone regulation, and stress response. Taken together, our findings suggest that AAO proteins are highly conserved among the Fabaceae family and play a vital role in many processes, demonstrating their importance to this plant family.
**Key-words:** AAO; Fabacea; oxidative stress; molecular evolution;

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# Biologiade RNAs (miRNA, siRNAs, ncRNAs, circRNAs)

# CONTROLE DE QUALIDADE ASSOCIADO AO RIBOSSOMO EM PLANTAS: COMO AS PLANTAS LIDAM COM PROTEÍNAS INACABADAS NO RIBOSSOMO?

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**Abstract:**
Plants express numerous heat shock proteins and chaperones that help prevent "proteinopathies" caused by stress-damaged proteins. Nevertheless, recent studies in yeast and animals revealed that ribosomes stalled in uncompleted translation events are a relevant source of misfolded proteins. In these organisms, the molecular complex responsible for recycling the stalled ribosomes and targeting the uncompleted polypeptides to degradation is the ribosome-associated quality control (RQC), formed by LTN1, NEMF, and TCF25. LTN1 is an E3 ligase that recognizes defective 60S ribosomes and ubiquitinates the unfinished peptide, targeting it to degradation by the proteasome. RQC is activated when the translation cannot be terminated by canonical termination factors. This situation is induced by defective mRNAs that lack a stop codon or contain structural features that prevent the ribosome from reaching the stop codon during elongation. The neurodegeneration and proteotoxic stress caused by RQC failure in vertebrates and yeast, respectively, points to how important this pathway could be for plants. Protein sequence comparison analysis indicated that all the essential RQC genes are conserved throughout the major groups of plants. However, no experimental data exist on how plants cope with the unfinished protein products derived from unproductive translation events. To probe the RQC function in plants, we silenced the *NbLTN1* on *N. benthamiana* plants through virus induced gene silencing (VIGS). The silencing of *NbLTN1* mRNAs was verified by RT-PCR. LTN1 silenced plants were agroinfiltrated with a vector coding for a GFP transcript lacking a stop codon (GFP-NS). While the expression of the GFP-NS construct is completely suppressed in control plants, as tested with fluorescence detection under UV light and western blotting against GFP, *NbLTN1* silencing allowed the accumulation of GFP protein, which could be detected by both techniques. This result showed that plants rely on LTN1 for disposing of proteins generated from defective mRNA. Due to the relevance of RQC pathway for other organisms, we aim to understand the importance of LTN1 for plant development and resistance to abiotic or biotic stresses. For this, we tested Arabidopsis harboring a homozygous T-DNA insertion that blocks *AtLTN1* expression. These plants were subject to low light stress and the rosette area was evaluated for 40 days. The *Atltn1* plants had rosettes 50% smaller than the WT-col, indicating that LTN1 contributed to stress resilience. Future work will demonstrate how Ltn1 mutants are affected by other conditions requiring RQC activity, such as drought, heat stress, and viral infections.

**Key-words:** translation; RQC plants; ribosome quality control; proteostasis; mRNA decay

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FAPERJ, CNPq

# ALGODÃO GM PARA O CONTROLE DO BICUDO DO ALGODOEIRO MEDIANTE SILENCIAMENTO MULTIPLO DE GENES

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**Abstract:**
Cotton is the world's most important fiber production, with Brazil as the fourth largest producer country. However, cotton producers in tropical countries face a number of challenges related to insect pests. The cotton boll weevil (CBW), for example, causes significant economic losses in infested areas. Its pesticide-based management is burdensome and environmentally risky. Biotechnological approaches such as RNA interference have been successfully used to produce GM plants resistant to coleopteran insect pests. Here, we generated transgenic cotton lines expressing double-stranded RNA (dsRNA) molecules to trigger RNA interference-mediated gene silencing in CBW. We used a multi-target system against three essential genes: chitin synthase 2 (*chs2*), vitellogenin (*vg*), and ecdysis-triggering hormone receptor (*ethr*). In addition, the stability of expressed dsRNAs was improved by designing a structured RNA based on the viroid genome architecture and ribozyme self-cleavage mechanism. We transformed cotton embryos by inserting a promoter-driven expression cassette that overexpressed dsRNA in flower buds. Following the molecular characterization of the GM plants to confirm the presence of the transgene, the dsRNAs' expression was measured by RT-qPCR. Plants from the T1 cotton plant generation were then challenged with fertilized CBW females. We found significant expression levels of our multi-target dsRNA molecule in leaves and flower buds of GM cotton lines, while no trace of the dsRNA was detected in WT plants. In the bioassay, we observed high mortality rates (around 70%) in oviposited yolks from GM cotton, compared to WT cotton (< 10%) after 30 days. In addition, independent bioassays using the same plants showed that two of the target genes, *chs2* and *vg*, had reduced expression (~80-99%) in individuals growing within the GM lines. Furthermore, lethal phenotypes associated with the disruption of the peritrophic membrane were observed in early larval stages of insects fed on GM cotton buds. This effect was enhanced by the *vg* knockdown, which could affect the proliferation of insects continuously fed on the transgenic plants. Overall, our study provided a proof of concept for the use of stabilized hairpin dsRNA to generate sustainable GM cotton plants resistant to CBW through the plant-mediated RNAi approach.
**Key-words:** RNA interference; *Anthonomus grandis*; Viroid; ;

# O PERFIL DE EXPRESSÃO DE CU-MICRORNAS FORNECE INFORMAÇÕES SOBRE A REGULAÇÃO PÓS-TRANSCRICIONAL NA INTERAÇÃO ENTRE CANA-DE-AÇÚCAR E *HERBASPIRILLUM SOROPEDICAE* SOB ESTRESSE SALINO

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**Abstract:**

Plants have developed sophisticated mechanisms to tolerate abiotic and biotic stresses by regulating the expression of a large set of genes. The great potential of miRNAs to act as an important regulatory factor involved in plant development and stress response has led to a paradigm shift in the understanding of post-transcriptional gene regulation. Recent advances have demonstrated that miRNAs participation in regulating host immunity can be crucial in improving symbiosis with plant growth promoting bacteria (PGPB). In addition to contributing to the Biological Nitrogen Fixation, many studies have shown that the association with PGPB can provide tolerance to abiotic stresses. In the present study, we aimed to investigate the regulatory mechanisms triggered by copper (Cu) miRNAs with a functional role in sugarcane responses to salt stress, during association with a PGPB. The characterization of the sugarcane genotype RB867515 colonization by *Herbaspirillum seropedicae* HRC54 under salt stress (300 mM NaCl) demonstrated that inoculation appeared to promote plant growth due to the plant-bacteria interaction. Our results showed an increase in the shoot length, fresh and dry weight after 10 days of salt stress in comparison to non-inoculated (mock) plants. Moreover, the inoculation treatment resulted in an increase in shoot total chlorophyll and carotenoids amount, when compared to mock plants. To determine whether Cu-miRNAs display any role in the salt stress response in sugarcane associated with *H. seropedicae*, the expression profiles of possible stress-related Cu-miRNAs, miR408 and miR397, were analyzed by qRT-PCR after 0 and 7 days of stress. Both miRNAs were induced in mock plants under salt stress conditions. In contrast, miR408 and miR397 were differentially regulated in response to salinity stress during PGPR-plant interaction, showing an expression pattern very similar to non-stressed control plants. Both miRNAs regulate laccase genes, key enzymes for lignin biosynthesis. Increased lignification has been described as one of the reactions included in a general plant adaptation strategy in response to biotic and abiotic stress. Aiming to perform a functional validation study of miR408 expression regulation, we carried out similar experiments of stress and inoculation in mutant Arabidopsis plants with miR408 knockout. Inoculation promoted a better stress response in Columbia plants due to an increase in shoot growth, in addition to a greater number of promotion of thicker roots and lateral roots, in comparison to mutant plants. Inverse expression of target genes and target cleavage sites are being validated. Taken together, these results suggest that not only the inoculation of *H. seropedicae* may be promoting some type of tolerance to salinity stress, but also that the inoculation was able to differentially regulate key Cu-miRNAs that modulate the response to abiotic stresses in sugarcane.

**Key-words:** miRNA; diazotrophic bacteria; abiotic stress; ;

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# REVELANDO NOVOS MIRNAS E SEUS GENES ALVOS EM FEIJÃO (*PHASEOLUS VULGARIS*): UMA ABORDAGEM PREDITIVA

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**Abstract:**

Common bean (*Phaseolus vulgaris*) is an important crop with significant economic and nutritional value worldwide. However, its production is often impacted by various biotic stresses. To address this issue, it has been explored the use of microRNAs (miRNAs), a class of small non-coding RNAs, that regulate various cellular processes, including plant responses to stress. MiRNAs are known regulators of plant-pathogen interactions, especially during pathogen establishment and plant defense responses. Given the significance of *P. vulgaris*, this study aimed to apply an *in silico* approach to identify new miRNAs and their respective targets associated with biotic stress. To select miRNAs involved in the plant-pathogen response, an extensive search was performed in the Web of Science, Scielo, Pubmed, Wiley, ScienceDirect, and Google Scholar, using the keywords "microRNA" and "pathogen" within Fabaceae species. To avoid redundancy, we excluded *P. vulgaris* species miRNAs that had already been validated and were available in miRNA databases such as miRBase and PmiREN. After filtering out the previously validated *P. vulgaris* miRNAs, the remaining sequences underwent a blastN analysis with one allowed mismatch, aimed at selecting highly similar sequences for further assessment of miRNA precursors. The resulting sequences, with approximately 200 nucleotides, were subjected to RNAfold tests using ViennaRNA Web Services. Based on the folding analysis, we identified seven novel miRNAs in common bean: miR028, miR156g, miR828, miR5037a, miR5037b, miR9560, and miR10405. To further understand the biological functions of predicted miRNAs, putative target genes were identified in the psRNATarget web server. This analysis was performed to explore the prospective pathways of novel predicted miRNAs. Targets were investigated about their involvement in plant cellular processes and homology in other plant species. The miRNA miR156g targets an ARF4-related protein and might be involved in the regulation of auxin responsive genes, which are well characterized during plant-pathogen interaction. miR028 and miR10405 were predicted to target serine/threonine phosphatases. Protein phosphatase is described as a mediator of plant response to pathogen interaction, by regulating the duration of immune reaction. Serine/threonine phosphatase might be regulated by abscisic acid, jasmonate and salicylic acid during immune response. miR10405 also targets lipoxygenase (LOX), involved in oxidoreductase activity and lipid oxidation. Furthermore, LOX is related to defense responses during biotic stress by mediation of defense genes expression and cell death. This study shows that an *in silico* approach can effectively identify novel miRNAs and their targets that could be potentially involved in the *P. vulgaris* defense response to pathogens. Given the significant importance of this crop, the identification of these miRNAs can expedite future research that utilizes them as tools to improve crop resilience and productivity during biotic stresses.

**Key-words:** microRNAs; plant-pathogen interaction; biotic stress; ;

**Acknowledgement**

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# PERFIL GENÔMICO DE RNA LONGO NÃO-CODIFICANTE NO PARASITISMO VEGETAL

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**Abstract:**
Long non-coding RNAs (lncRNA) have a wide range of biological roles, such as interacting with macromolecules to influence chromatin remodeling, protein functional activity, and RNA metabolism at multiple levels (transcriptional and post-transcriptional). In plants, lncRNAs have essential regulatory roles in several processes, such as lateral root formation, plant response to phosphate deprivation, and fruit ripening. However, despite its relevance, little research has focused on how lncRNAs are involved in plant-plant interaction. The parasitic plants are angiosperms that evolved to heterotrophic lifestyle. Among them, some species become severe agricultural pests by being able to infect species of economic importance. *Phtheirospermum japonicum* is considered a model to understand the molecular basis of plant parasitism. In this work, we identified and analyzed through an in silico approach the putative lncRNAs in the genome of *P. japonicum*. Using the plant-exclusive GreeNC database, we selected 5,375 potential lncRNAs longer than 200 nt and with an open reading frame (ORF) smaller than 360 nt (120 amino acids). By using the CPC2 software to find potential coding and non-coding transcripts, we reduced the initial list by 24-28%. In *P. japonicum*, the analysis narrowed to 4,036 potential lncRNAs encoded by 3,976 genes (~13% of the species' gene apparatus). To rule out others RNAs types from lncRNAs, we used the CMSCAN and BLASTn software against the miRBase and Rfam databases to classify rRNA and tRNA. We also applied analyses with MIReNA software to identify miRNA and other kinds of small RNAs. The lncRNAs not ranked in either of these analyses were considered high confidence lncRNAs. *P. japonicum* has 1,015 high confidence lncRNAs. In comparison with non-parasite species (*Oryza sativa*, *Solanum lycopersicum*, and *Arabidopsis thaliana*), *P. japonicum* showed 22 shared lncRNAs and parasitic-specific 1,390 lncRNA transcripts. The findings of these lncRNAs in *P. japonicum* may open doors for further research addressing the specific functions of these molecules in parasite-host interaction.

**Key-words:** parasitic plant; lncRNA; *Phtheirospermum japonicum*; Orobanchaceae;

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# Desenvolvimento Vegetal

# DESVENDANDO A REDE DE INTERAÇÕES PROTEICAS DE APC7

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**Abstract:**
The genetic improvement of crops in Brazil using biotechnological tools aims to develop more productive and adapted cultivars, in order to reduce the use of chemical pesticides, contributing to a more sustainable agriculture. In the current panorama, it should be taken into account that the world climate is undergoing an accelerated change, which directly affects the productivity of crops, both due to the lack of adaptability of genotypes and due to the change in the plant-pathogen interaction dynamics. Throughout its evolution, plants have developed countless mechanisms to better adapt and an important way is through the accurate control of cell divisions. Our laboratory has been dedicated, since its creation until nowadays, to understanding how the modulation of cell cycle genes can impact plant development, under ideal and adverse conditions. Over the past few years, the LBMP/IBqM has generated a significant amount of promising data regarding the biotechnological applicability of modulating the expression of Anaphase-Promoting Complex (APC/C) genes, mainly in relation to the subunit seven of the complex (APC7), which has shown great potential to be an alternative to improve adaptability in crops. In this work, we seek to understand the physiological role of *APC7* and its truncated form *APC7-CT* in plant tissues when exposed to different environmental conditions. To this end, we exploit the network of APC7 protein interactions using *Tandem Affinity Purification* (TAP) approach, which was done in collaboration with the laboratory of Dr. Geert de Jaeger of VIB, Belgium. The proteins purified in TAP together with *APC7* and *APC7-CT* are being analyzed *in silico* to decipher a larger network of interactions, relying on what has already been described in the literature. In addition, we have used the loss of function mutants to assess the metabolic pathways involved in the stress response promoted by *APC7*. With these results, we aim to contribute to the understanding of the adaptability of plants and guide the science of plant breeding in the field of multiple stresses.
**Key-words:** Cell cycle Regulation; Anaphase-Promoting Complex; TAP-tag; ;

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# INDUÇÃO DE CALOS EMBRIOGÊNICOS DE GENÓTIPOS ELITES DE ARROZ DE TERRAS ALTAS

**Alisson Wilians Teixeira Silva [1]; Jocilene dos Santos Pereira [1]; Yasmin Vasques Brechembrock [1]; Ana Júlia da Silva [1]; Flávia Barbosa Silva Botelho [2]; Karen Eduarda do Lago [1]**
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**Abstract:**
Rice (*Oryza sativa*) is one of the most important cereals in human nutrition. As a source of carbohydrate, protein and vitamins, rice is fundamental in the food security, mainly for emergent countries. The crop productivity is extremely influenced by environmental factors. High throughput in plant breeding technologies such as CRISPR/Cas has allowed researchers to improve plant physiologic efficiency against environmental stress. However, one of the fundamental steps for the rice plant genetics transformation is to obtain embryogenic callus. Unlike model plants, the callogenesis protocols are not so efficient on elite crops available at the market. Therefore, the purpose of the present work was to evaluate the performance of four upland rice lines (Caçula, Douradão, Soberana and CMG1590 - being the last one from Upland Rice Breeding Program Melhor Arroz from the University of Lavras), to the embryogenic callus induction MS medium. It was used 50 seeds of each genotype. The palea was removed and healthy seeds were selected. Then, the seeds were submitted to a sanitizing protocol by immersion for two minutes in ethanol 70%, and immerged for 25 minutes in sodium hypochlorite 2%. After this, the seeds were washed with distillated and autoclaved water for five times. The seeds were inoculated in 90 x 15 mm petri dishes containing MS medium culture with 3% sucrose, 6 g $L^{-1}$ of agar, 1 mg $L^{-1}$ of 2,4D and 0,5 mg $L^{-1}$ of BAP. Afterwards, the seeds were transferred to a dark room and maintained at 27±0,2 °C degree. It was done six biological repetitions. The evaluation took place every three days along 21 days. The data were submitted to ANOVA analysis ($p < 0,0001$) and the means were compared by Tukey's test. Douradão line has showed the major amount of callus formation (48 ± 1,06), followed by Soberana (46 ± 1,23), CMG1590 (44,16 ± 1,01) and Caçula (38 ± 1,31). Soberana presented the major amount of friable callus formation with 65%, followed by Douradão with 60%, CMG1590 with 58% and Caçula with 53%. The MS medium to embryogenic callus induction (MS salts and vitamins + 1 mg $L^{-1}$ of 2,4D and 0,5 mg $L^{-1}$ de BAP) was efficient to induce callus formation in elite upland rice crop, although, MS medium and growth regulators modifications could be useful to improve the physiologic callus formation quality.
**Key-words:** *Oryza sativa*; Tissue culture; MS medium; Growth regulators; Plant transformation

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# UM BOOM DE CRESCIMENTO: O IMPORTANTE PAPEL DE APC7

**Bruna Gino de Araújo [1]; Flávia Thiebaut [1]; Patrícia da Fonseca Montessoro [1]; Aline Kohn Carneiro [1]; Janice de Almeida Engler [2]; Adriana Silva Hemerly [1]**

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**Abstract:**
The anaphase-promoting complex (APC) is a multi-subunit E3 ligase, widely conserved among eukaryotes, that regulates cyclin-dependent kinases (CDKs) activity by Cyclin (Cyc) degradation, at key cell cycle progression checkpoints. During plant development, this complex has an important role on controlling cell division. Many APC subunits have already been described, being important for the correct development and for increased productivity. Improving biomass production and seed yield to generate food, fuel and bioenergy for humanity are among the traits most desired among the breeders. Since plant development is modulated by the environment, through regulation of cell division rates, members of the APC complex could also play important roles on plant adaptation to climate changes. APC7 is an APC subunit identified by our research group, whose overexpression leads to increased biomass and accelerated growth in *Arabidopsis thaliana*. Therefore, the first part of this study aimed to analyze the role of APC7 on plant development, and its potential for increasing plant productivity, seeking to understand the mechanisms that lead to promotion of plant growth. In a second research line, we investigated APC7 potential to increase tolerance to heat stress, as the raise in global temperature is one of the most prominent impacts of climate changes. For these studies, we performed phenotypic, molecular, physiological and cell biology analyses of plants overexpressing APC7 ($APC7^{OE}$). Besides the increase of vegetative biomass, our analyses showed that, at reproductive stage, the length of the main inflorescence, lateral inflorescence and silique number is greater in $APC7^{OE}$. Kinematic analysis showed that overexpression of APC7 leads to an increase in leaf area due to the increased cell number and cell expansion, higher cell division rates on the proliferation phase and increased stomatal index, which indicate a balance among various growth processes. Higher ploidy levels were observed in $APC7^{OE}$, possibly being related to the increased cell expansion observed. Photosynthetic parameters were also higher in $APC7^{OE}$ plants. Altogether, our results revealed cellular and physiological mechanisms that were modulated by overexpressing APC7, that support the increased plant biomass and yield in $APC7^{OE}$ plants. In a second approach, we are investigating the potential of APC7 to increase tolerance to heat stress. For that, $APC7^{OE}$ plants were cultivated under prolonged heat stress (4 or 7 days of stress). Preliminary results suggested that $APC7^{OE}$ plants tolerate better heat stress, since photosynthetic measurements were higher in $APC7^{OE}$ plants growing in high temperatures. Also, all other improved growth parameters observed in $APC7^{OE}$ plants growing under normal temperature were maintained under heat stress. This work revealed important roles of an APC subunit in promoting plant growth, that can be of great importance in a world scenario of rising global temperature, and of increasing demand for food and bioenergy.
**Key-words:** anaphase-promoting complex; plant development; heat stress; APC7; photosyntesis

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# DIAGNÓSTICO DE DOENÇAS UTILIZANDO QPCR EM MUDAS CÍTRICAS REGENERADAS APÓS LIMPEZA CLONAL

**Candida Elisa Manfio [1]; Luana Aparecida Castilho Maro [1]; João Frederico Mangrich dos Passos [1]; Murilo Dalla Costa [1]**
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**Abstract:**
The citrus seedlings sector in Santa Catarina has experienced considerable expansion in recent years with an increase in the number of seedlings produced and also in the number of families involved in the activity. The lack of important diseases that commonly occur in other states of the federation could put the orchards in Santa Catarina, the seedling producing region and also the experimental collections kept in the open by Epagri at risk. In order for the activity to be profitable, whether as a fruit producer or seedling producer, it is essential that the propagation materials used for the formation of seedlings are free of any pathogens. Therefore, the diagnosis of diseases is extremely important, and clonal cleaning by micrografting is a primordial tool to ensure the absence of viruses that are highly harmful to the exploitation of this crop. In view of the above, the objective of this work was to certify the health of citrus seedlings regenerated after clonal cleaning. To obtain the seedlings, buds from parent plants of the SCS454 Catarina, SCS455 Reinaldo, SCS 456 Sigmar, SCS 457 Souza and SCS458 Osvino cultivars were used. The qPCR technique was used to verify the micrografting efficiency. Twenty-one seedlings were regenerated in vitro and had their RNA extracted using the Trizol protocol. The qPCR reactions for analysis of CEVd (Citrus exocortis viroid) and HSV (Hop stunt viroid) contained 1 µL of cDNA, 10 µL of master Mix (Sybr Green), 0.35 µM of primers, and water to make up the final volume of 20 µL, and for the qPCR reactions for psorosis, the protocol by Francesco et al., 2014 was used. Of the twenty-one seedlings, seventeen showed neither the presence of viroids nor Psorosis. The four seedlings that were positive did not show both diseases together. Although the four seedlings were not completely cleaned, they showed only one disease. Important results, since they confirm the efficiency of the clonal cleaning technique through micrografting in obtaining basic material with phytosanitary quality. The phytosanitary quality of the citrus seedling produced is a basic requirement for the frank development of the activity and to place the seedling in Santa Catarina on a differentiated level, precisely because it does not occur important diseases that are already diagnosed in other states of the federation. In this way, it will be possible to transfer the benefits of biotechnology to the productive sector for the use of healthy propagation material by nurserymen in the formation of seedlings and, consequently, contributing to the formation of long-lived orchards. In this sense, the clonal cleaning technique by means of micrografting was efficient for obtaining material free of viroids and psorisis.
**Key-words:** Citrus spp.; micrografting; seedling production; real-time;

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# A PERDA DE FUNÇÃO MEDIADA POR CRISPR/CAS9 DA *SLSBP13* AUMENTA O TAMANHO DO MERISTEMA APICAL DO CAULE EM TOMATE *SOLANUM LYCOPERSICUM*

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**Abstract:**
Plasticity allows plants to regenerate organs. Such plasticity depends on the activity of meristems and enables them to adapt their development in response to the environment. While the root apical meristem will form the root system, the shoot apical meristem (SAM) will form the shoot architecture. SAM is maintained by pluripotent stem cells that are controlled by the *CLAVATA3* (*CLV3*)-*WUSCHEL* (*WUS*) feedback signaling. In arabidopsis, WUS is regulated by feedback from *SPL* (or *SBP*) genes. *SBP* genes are a plant specific family of transcription factors that regulate plant growth and development at different levels, most of them regulated by the highly conserved microRNA156; however, the effect of the *SBP* genes on SAM of tomato is unknown. Understanding the molecular mechanisms underlying the *SBP* genes-regulated SAM size could help to generate plants with improved architecture. By reverse genetics approach we are exploring the effect of the *SBP* genes on SAM of tomato. By CRISPR/Cas9-mediated gene editing, we have obtained a loss-of-function mutant of *SlSBP13* (*sbp13_1*) from tomato Cv Moneymaker. *sbp13_1* plants display shorter height, more branching and bigger SAM compared to wildtype plants suggesting that *SlSBP13* is involved in different processes known so far; in addition, our data suggest that *SBP* genes also participate in meristem size regulation in tomato, and are possible targets to obtained plants with enhanced yield.
**Key-words:** Tomato; Shoot apical meristem; miR156; SlSBP13; Transcription factors

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# DECIFRANDO A BIOSSÍNTESE DO ÁCIDO ENT-CAURENÓICO NO CAFEEIRO ARÁBICA.

**Douglas Silva Domingues [1,4]; Suzana Tiemi Ivamoto-suzuki [2]; Samara Mireza Correia de Lemos [3]; Gabrielle Wyatt [4]; Philipp Zerbe [4]**

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**Abstract:**
Coffee is a popular beverage consumed worldwide, and it is and important crop for several developing countries. The allotetraploid Coffea arabica is the most planted species, where Brazil is the biggest producer and exporter. One of the longest-known plant hormones is Gibberellin (GA), which plays a vital role in regulating different developmental processes such as stem elongation, germination, dormancy, flowering, flower development, and leaf and fruit senescence. In the first portion of the GA biosynthesis pathway, the precursor geranylgeranyl diphosphate (GGDP) is converted to ent-kaurene in a two-step process catalyzed by ent-copalyl diphosphate synthase (CPS) and ent-kaurene synthase (KS). A subsequent reaction is catalyzed by the cytochrome P450 enzyme ent-kaurene oxidase (KO) to produce ent-kaurenoic acid. In this study, we aimed to functionally characterize the genes responsible for kaurenoic acid synthesis in Arabica coffee, using transient expression in Nicotiana benthamiana, including one copalyl diphosphate synthase, two ent-kaurene synthase genes, and two ent-kaurene oxidase genes. Through our characterization of these genes, we identified that each of these genes were able to produce the initial steps of GA biosynthesis. Our findings provide valuable insights into the genetic mechanisms underlying the biosynthesis of kaurenoic acid and its role as a precursor of gibberellins and other diterpenes in Arabica coffee. Further research is needed to fully understand the functional diversity of terpene synthases in Arabica coffee. The knowledge gained from this study may have practical applications for improving the quality and health benefits of coffee crops.
**Key-words:** ent-kaurenoic acid; diterpenes; plant plathways; heterologous expression;

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# OSBHLH035: UM FATOR DE TRANSCRIÇÃO ENVOLVIDO NO DESENVOLVIMENTO DA ANTERA DO ARROZ E NA SENESCÊNCIA DA FOLHA BANDEIRA

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**Abstract:**
The bHLH-type transcription factors form the second largest family of transcription factors in plants, involved in response to various stresses, coordinating plant growth and development. We performed a functional analysis of the OsbHLH035 transcription factor (TF), a poorly characterized member of the bHLH TF family. To provide further inside into OsbHLH035 function, we obtained plants overexpressing the *OsbHLH035* encoding gene (*OsbHLH035*OE). Phenotypic, physiological, and molecular parameters were evaluated and revealed that *OsbHLH035*OE plants are smaller, produce fewer seeds, have lower photosynthetic and gas exchange capacities, and their spikelets have deformed anthers compared to the non-transformed (NT) plants. In addition, the *OsbHLH035*OE plants showed early senescence of flag leaves when cultivated in soil, probably due to nitrogen deficiency, however, new experiments need to be performed to confirm this hypothesis. Furthermore, to identify differentially expressed genes and potential regulatory targets of OsbHLH035, transcriptome analysis of transgenic plants and non-transformed (NT) plants were performed. Through these analyses, differentially expressed genes related to the nitrogen assimilation pathway, antioxidant metabolism, and biosynthesis of secondary compounds were identified. In conclusion, the increase in *OsbHLH035* expression causes damage to the normal development of rice plants, affecting anther formation and photosynthesis and, consequently, leading to a reduction in seed production and plant height. Therefore, OsbHLH035 is related to different metabolic pathways, plant development, anther formation, and senescence.
**Key-words:** Functional analysis; bHLH; overexpression; transcriptome;

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# CARACTERIZAÇÃO DA AUXIN REPRESS PROTEIN (ARP) EM *SOLANUM LYCOPERSICUM* CV. MICRO-TOM

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**Abstract:**
The floral transition is a complex and dynamic mechanism and its involves different proteins in a cellular signaling cascade. Using subtractive libraries, a sequence with homology to *AUXIN REPRESSED PROTEIN* (*ARP*s) was identified in *S. lycopersicum* shoot apical meristem. The aim of this work was to understand the role of this sequence and a possible signal cascade involved. By two hybrids system and bioinformatics tools as STRING 10.0 and Cytoscape 3.7.2 software. Eight clones were identified using the two hybrid approach. From these, two sequences had homology to putative proteases and the third sequence had homology to CYTOCHROME C-OXIDASE protein. These three proteins can be associated to cell energy metabolism. The other five sequences identified codify the PHYTOEN SYNTHASE protein. Then, to understand the possible role of ARP1 protein in plants, it was used the Cytoscape 3.7.2 software to build an interactome network. It was observed the presence of five clusters, which were associated to plant energy metabolism, growth mechanism and cell differentiation. Moreover, transgenic lines having overexpression cassettes in sense and antisense orientation showed that the antisense lines had an early-flowering phenotype compared to that of the wild-type. The qPCR results showed repression for ARP expression and auxin levels measured by HPLC also showed low levels for these antisense lines. These results suggest that ARP1 might be a new target for the auxin control of reproductive plant development.
**Key-words:** florescimento; dois hibridos; plantas transgenicas; super-expressão; auxina

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# EFEITOS DA MODULAÇÃO NEGATIVA NA EXPRESSÃO DO GENE APC5 EM ARABIDOPSIS THALIANA

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**Abstract:**
The development process of organisms is directly associated with the cell cycle, which is divided into four phases: G1, S, G2 and Mitosis. Precise progression of the cell cycle is crucial for the correct cell division, and its regulation is carried out by cyclin-dependent kinase complex associated with its regulatory subunit, Cyclins. The Anaphase Promoting Complex/Cyclosome (APC/C) is an E3 ubiquitin ligase, which is one of the main components of a multienzyme cascade of protein degradation, the ubiquitin proteasome system (UPS). APC/C plays an essential role in regulating the cell cycle machine through the recognition of target proteins for degradation, such as cyclins, allowing the proper functioning of the cycle. Studies in rice, tomato and *Arabidopsis* have shown that APC/C is involved in several processes, such as embryogenesis, gametogenesis, growth regulation, hormone signaling and symbiotic interactions. Additionally, APC/C subunits are essential in all species, since the null allele of their individual components is lethal. In the last years, our group have demonstrated that changes in the expression levels of the APC/C subunits can affect plant growth, leading to an increase or decrease in plant biomass. The present work aims to functionally characterize the effects of APC5 down regulation in *Arabidopsis thaliana* through analysis of T-DNA insertion and artificial interference micro-RNA (amiRNA) lines. For this, we obtained the SALK_024997 line, hereinafter referred to as *apc5.1*, harboring a T-DNA insertion located in the 11th intron. The PCR analyzes showed that despite the 120 seedlings analyzed, we could not find homozygous plants, confirming the heterozygous insertion, thus proving the essentiality of APC5 for plant viability. The *APC5* mRNA levels were measured by RT-qPCR in the *apc5.1* mutant line, and the construct pAPC5:APC5 was used for the complementation experiments. The *apc5-amiRNA* lines were generated by floral dip, and the plants are being segregated for further characterization.
**Key-words:** Cell cycle; Plant development; Anaphase Promoting Complex (APC); ;

# DESENVOLVENDO UM TRANSCRIPTOMA DE REFERÊNCIA PARA O FRUTO DO ABACATE COMERCIAL (*PERSEA AMERICANA* VAR. HASS)

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**Abstract:**
During the development of genetics, some model organisms were adopted for the study of certain groups. In plants, the main one is the Brassicaceae Arabidopsis thaliana, which has a dry fruit. For fleshy fruits, the tomato (Solanum lycopersicum, Solanaceae) is often used. However, even this organism has some limitations regarding what it can show us about some other phylogenetically distant fleshy fruits, such as avocados (Persea americana, Lauraceae). This fruit is well known for its economic, cultural, gastronomical, and health benefits worldwide. It was shown that the lipid content in avocados is very important for the promotion of healthy levels and constitution of lipids in humans' bloodstream, as well as the availability of lipid-soluble proteins. However, until the present moment we just have de novo transcriptomes for this fruit, which are not anchored in a reference genome, hence holding probably a lower resemblance to the real fruit's transcripts. That way, the present study aims to contribute to our knowledge about the avocado fruit through the development of a reference transcriptome. For that, we gathered all the available RNAseq data for the avocado fruit available in the NCBI database until December 2021, along with its reference genome and annotation files. The reads were then trimmed, mapped to the reference genome and assembled into the aimed transcriptome. Subsequently, we compared this transcriptome to the proteome of 94 angiosperms. The transcripts that weren't matched to any protein were characterized as potentially non-coding. A transcript quantification and a differential expression analysis were also made. The differentially expressed genes were annotated through a BLASTX similarity analysis against the A. thaliana's proteome. As a result, the assembled transcriptome contained 54,434 expressed loci, resulting in 76,440 transcripts, while the reference genome has 24,616 protein-coding genes, being unknown the number of non-coding. We also got 25,211 transcripts differentially expressed along the development of the fruit. From the annotation we found 2,608 proteins related to 18,576 differentially expressed transcripts, some of them being found in uncommon developmental stages. Lastly, we found 72,219 coding transcripts and 4,221 potentially non-coding, being some of them close to 100,000 base pairs of length. Thus, we were able to assemble for the first time a reference transcriptome to the avocado fruit, anchored in a reference genome and considering data from various studies and growth conditions. We also obtained a landscape of some of the most important transcripts for this fruit's development through the gene expression analysis and compared the size and composition of coding and potentially non-coding transcripts in this transcriptome.
**Key-words:** Avocado; *Persea americana*; Hass; Transcriptome;

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# *SCI1* É TRANSCRICIONALMENTE REGULADO POR *NICOTIANA TABACUM* AINTEGUMENTA (NTANT) E ESTIMULADO POR AUXINA

**Joelma de Oliveira Cruz [1,2]; Greice Lubini [1]; Fernanda Maiara Nogueira [1]; Sara Coelho [3]; Vitor Favareto Pinoti [1]; Pedro Bosacariol Ferreira [1]; Vanessa Thomé [1,2]; Edward Strini [1]; Andréa Carla Quiapim [1]; Maria Manuela Ribeiro Costa [3]; Maria Helena Souza Goldman [1,2]**

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**Abstract:**
Understanding the molecular mechanisms that control flower development and successful reproduction is crucial for basic science and agricultural purposes. Reproduction in angiosperms involves a complex regulation of the floral meristem initiation and floral organ formation. *SCI1*, a regulator of cell proliferation, is expressed in all floral meristematic cells since *Nicotiana tabacum* floral meristem specification and affects the final size of the pistil. *In silico* analyses of the *SCI1* genomic sequence identified several putative *cis*-regulatory elements, among them for binding the transcription factor AINTEGUMENTA (ANT). ANT is a member of the AP2/ERF family and is involved in ovule initiation and development. This work aimed to determine if *SCI1* and *NtANT* are co-expressed in the pistil and to analyze if NtANT binds to *SCI1* genomic sequence. *In situ* hybridization experiments showed that *SCI1* is expressed in the pistil-specialized tissues, like the stigmatic secretory zone, stylar transmitting tissue, and the placenta inside the ovary. *SCI1* is also detected in the ovule integument and funiculus. Similarly, *in situ* hybridization showed that *NtAN*T is expressed in the same regions of *SCI1* in the placenta and functional regions of ovules. Through yeast one-hybrid (Y1H), we showed that the transcription factor NtANT binds to the *SCI1* promoter. NtANT interaction with the *SCI1* promoter was confirmed by electrophoretic mobility shift assay (EMSA). The luciferase assay showed that NtANT activates the expression of *SCI1*, and its expression is enhanced in the presence of auxin (5µM NAA or 5µM IAA). Our results indicate that *SCI1* is important during ovule development and that its expression is induced by NtANT and is stimulated by auxin. These results support our proposal that *SCI1* participates in the auxin signaling pathway during pistil development.
**Key-words:** ovule initiation; ovule development; pistil expression; ;

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# CARACTERIZAÇÃO FUNCIONAL DO GENE *APC5* EM *ARABIDOPSIS THALIANA*

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**Abstract:**
As a multicellular organism, plants have their growth and development directly dependent on the cell division. This process brings together dozens of molecular reactions, which ensures cell proliferation in the most varied plant tissues. The four sequential phases that make up the cell cycle (S, G1, M and G2) have the presence and absence of different molecular components of the cell division program, such as cyclins, CDKs and securins, acting together in order to guarantee the unidirectional progression of the process, in an irreversible way . The ubiquitin-proteasome system (UPS) is a multi-enzymatic cascade that target specific substrates for degradation through 26S proteasome. The Anaphase Promoting Complex/Cyclosome (APC/C) is one of the enzymatic machines responsible for recognizing the substrate to be ubiquitinated by the UPS. In Arabidopsis thaliana, the APC/C has 14 subunits. Several studies have reported that perturbations in the expression levels of some subunits can lead to changes in the plant phenotype, and compromise process such as gametogenesis. The cell division process occurs in synchrony with the other metabolic processes of the cell, as well as of the entire plant. Changes in nutrients or even in intracellular demands can lead to changes in metabolites concentration and their conversion rates, altering the biological processes of the organism. Metabolic changes occur at a rate fast enough to escape transcriptional regulation, forcing the cell to use other ways to regulate its reactions to deal with these changes, such as the action of metabolites as regulators. In this way, our project aims to characterize functionally the APC5 subunit from Arabidopsis thaliana, as well as to investigate the changes in the plant metabolism due to alterations in the expression levels of the APC5, possibly correlating the changes with already known process that drive plant growth. For this, we generated overexpression lines driven by the CaMV 35S promoter . The APC5 mRNA levels were investigated by RT-qPCR in the homozygous APC5OE lines. The APC5 promoter-reporter construct were analyzed and staining for GUS activity showed strong expression at root meristem and in developing lateral root primordia. Phenotypic analyses and GC/MS metabolites measurements has been carried out.
**Key-words:** APC/C; Cell Cycle; Metabolomic; GC/MS;

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# POTENCIAL ATIVIDADE ANTIOOMICETO DOS ÓLEOS ESSENCIAIS DE LIPPIA ALBA CONTRA O AGENTE CAUSATIVO DA GOMOSE DOS CITROS (PHYTOPHTHORA PARASITICA)

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**Abstract:**
The use of essential oils (EO) represents a promising role in the control of phytopathogenic fungi, due to their capacity of direct fungitoxic action by inhibiting spore germination and mycelial growth, or indirectly action by inducing the plant's defense system. *Lippia alba* belongs to a group of most popular medicinal and aromatic plants in Brazil and it is one that has antifungal activity against Aspergillus and Penicillium genera as well as against human pathogenic microorganisms. However, there are no records on its effect against oomycetes such as citrus gummosis causative agent *Phytophthora parasitia*. Belonging to the taxon stremanopyle group, oomycetes are eucaryotic microorganisms similar to filamentous fungi. *Phytophthora ssp* are hemibiotrophic pathogens that have been described as disease-causing agents in economically important crops in the world. The species *P. parasitica* Dastur and *P. citrophthora* cause great damage in citrus culture. The objective of this study was to examine the potential antioomycete activity of pre-selected essential oils of *L. alba* for inhibition of *P. parasitica*. Pure EOs were extracted from dried plant material by hydrodistillation in apparatus type Clevenger, in a closed circuit, for a period of two hours for each genotype. Subsequently the EOs were separated from the aqueous phase and placed in glass vials (5ml), kept away from light and refrigerated at 20°C. They were impregnated in filter paper discs (30 µL) and deposited in the center of Petri dishes with Juice V8-agar cultural medium (containing 200 ml of V8, 4,5g of $CaCO_3$, 17g of agar and 0,4g of pentantibiotic) together with the inoculation of mycelium discs (5mm) of the *P. parasitica*. The plates were maintained in the dark at 27°C. The inhibition potential was satisfactorily observed in two of the Lippia genotypes tested ("A and B"), which in 96 hours showed an inhibition of the growth and development of the mycelium. On the other hand, genotype "C" did not inhibited *P. parasitica* development. We believe, that this research contributes for the potential of essential oils as an agroecological alternative to alleviate the phytosanitary problems in crops of economic importance, by reducing the use of agro-toxins, consequently, contributing for a lower environmental impact.
**Key-words:** Lippia alba; Phythophthora parasitica; Essential Oils; Biotic Stress; Phytochemical Control

# DESENVOLVIMENTO DE TESTE COM BIOMARCADORES DE EXPRESSÃO GÊNICA PARA OTIMIZAR A SELEÇÃO DE GENÓTIPOS DE MILHO (*ZEA MAYS*) RESPONSÍVOS AO USO DE BIOINOCULANTES

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**Abstract:**
Bioinoculants (biodefensives and biofertilizers) are composed of microorganisms, such as bacteria and fungi, and can promote several benefits for plant development, as well as a positive environmental impact and a better cost-benefit when compared to synthetic chemical fertilizer. However, the efficiency of the bioinoculant depends on the genotype of the plant and the environment where the plant-bacteria association is established. An important question is: how to select the best plant-bioinoculant combinations so that the inoculation is positive for the plant? For that, the goal of this project is the development and application of an innovative tool based on gene expression that allows the identification of plant varieties that respond positively to bioinoculants. Transcriptomic databases were previously generated by the Laboratory of Plant Molecular Biology (LBMP/UFRJ) in experiments performed with sugarcane and maize plants inoculated with diazotrophic bacteria, growing in different soil conditions. In this study, possible biomarker genes were selected from the databases, by searching for expression profiles common in the beneficial plant-bioinoculant associations. Next, these gene expression profiles are currently being evaluated in different maize genotypes and soil types, to validate them or not as good biomarkers. Initially, two maize genotypes (G1 and G2) and three different formulations of bioinoclants (Az, Bf and Bt) were selected to be tested. Plant growth promotion, bacterial colonization and biomarker expression profiles were analyzed in response to treatment with bioinoculant formulations and different concentrations of nitrogen fertilizers. The experimental results showed that the three formulations of bioinoculants tested promoted some gain in the maize genotypes analyzed, however, the Bf bioinoculant was better associated with both genotypes, which showed seed priming and better development for the evaluated phenotypic parameters (number of leaves, fresh and dry mass of shoots, root length and chlorophyll content) in relation to control plants. Furthermore, the G1 genotype was more responsive to inoculation with the Az bioinoculant. The results showed that: i) inoculated plants presented an optimization in relation to chemical fertilization, since inoculated and unfertilized plants grew similarly to control plants that were fertilized; ii) inoculated plants and fertilized with a dose of 25% nitrogen grew similarly to those inoculated and fertilized with 50%. The selected biomarker genes were tested on RNA samples extracted from the different treatments using RT-qPCR. Several of them corroborated with the observed phenotypic responses, showing to be good biomarker candidates. Finally, the validated biomarkers are being used to develop a test kit to identify plant varieties with higher yield and productivity due to better biofertilizer use. The technology will allow more accurate plant nutrient management, contributing to sustainable agriculture practices.
**Key-words:** climate changes; sustainable agriculture; food crops; plant development;

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# UMA REDE REGULATÓRIA DE *ARABIDOPSIS THALIANA* QUE FUNCIONA NA INTEGRAÇÃO DOS CONTROLES DO CICLO CELULAR E METABOLISMO VEGETAL COM ESTÍMULOS AMBIENTAIS

**Patricia da Fonseca Montessoro [1]; Joaquin Roca [1]; Laura Ducatti [1]; Adriana Flores Fusaro [1]; Leticia Tessaro [2]; Jelmir Craveiro de Andrade [2]; Carlos Adam Conte-junior [2]; Adriana Silva Hemerly [1]**

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**Abstract:**
Plants, being sessile organisms, have developed a sophisticated regulatory network capable of coordinating gene expression in meristematic zones as a means of survival in response to environmental conditions. Thus, understanding the molecular mechanisms that connect the regulation of plant development to stress situations is essential for obtaining plant varieties that can contribute to agricultural sustainability. Previous work by our group has identified ABAP1 (Armadillo BTB Arabidopsis protein 1) as a negative regulator of the cell cycle in the G1/S phase in plants. Several ABAP1 interactors were identified, and are being characterized by analyses of overexpressing and knockout/knockdown mutant lines. Modulation of expression of some of the member of this network leads to higher biomass, seed yields and increased tolerance to stresses in *A. thaliana*. In this work, we aimed to characterize the molecular and biochemical mechanisms by which this gene network regulate plant performance in a changing environment, by characterizing mutant plants of a member of this network (with unknown function) using different approaches: Illumina RNA-Seq transcriptomic analysis, physiological analyses of photosynthesis and generation of metabolic profiles through ATR-FTIR (attenuated total reflection Fourier-transform infrared). The expression profile of knockout mutant showed a large number of differentially expressed genes related to photosynthesis and carbon metabolism that were induced in young and mature leaves, indicating a possible relationship between mass gain and better photosynthetic performance. This hypothesis was supported by physiological analyses that showed a significant increase in chlorophyll and carotenoids contents, in addition to improved photosynthesis rates in mutant plants. In addition, the metabolic profile in young and mature leaves and seeds clearly discriminated the knockout mutant from Col-0 plants, due to the distinct metabolic differences between the two genotypes, with greater abundance of proteins, lipids and carbohydrates in the mutant, which was maintained throughout development until the seed stage. In this study, we demonstrate that the silencing of this gene coordinates the increase in biomass and the gain in productivity through better photosynthetic performance and carbon assimilation to metabolically supply its energy demand. Altogether, the results suggest that the ABAP1 gene regulatory network modulates transcriptional reprogramming of different genes and pathways involved in efficient carbon uptake and fixation in conjunction with fine-tuning of cell cycle rates, to increase plant survival in response to extreme environmental conditions.

**Key-words:** *Arabidopsis thaliana*; cell cycle; ABAP1; biomass; metabolism

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# ANÁLISE FUNCIONAL DO GENE *SAMBA* DESTACA SEU PAPEL NO CRESCIMENTO E DESENVOLVIMENTO DE PLANTAS DE TOMATE (CV. MICRO-TOM)

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**Abstract:**
The Anaphase-Promoting Complex/Cyclosome (APC/C) is an E3 ubiquitin ligase involved in ubiquitin-dependent proteolysis of key cell cycle regulators by the 26S proteasome. The spatio-temporal degradation of mitotic cyclins and securins ensures the correct onset of the cell cycle phases and exit from the cell division program, respectively. The identification of genes encoding APC/C subunits in *Arabidopsis thaliana* suggests that the complex has other specific functions during plant development, such as embryogenesis, gametogenesis, growth regulation, hormone signaling and symbiotic interactions. Despite extensive research on APC/C, only a few components have been functionally characterized in plants. One of these proteins, SAMBA, proved to be a highly promising candidate in Arabidopsis regarding to increased productivity, through production of larger leaves, roots and seeds. However, mutation in the maize SAMBA ortholog displayed dwarfism, erect upper leaves, reduced organ and tissue growth. Therefore, to better understand SAMBA's role in plant development, we used Clustered Regularly Interspaced Palindromic Repeats/CRISPR-associated protein 9 (CRISPR/Cas9) to generate sequence-specific mutations at *Solanum lycopersicum* (tomato) cv. Micro-tom in the *SAMBA* gene. We produced CRISPR-Cas9 *samba* mutants, which was confirmed by PCR and sequencing analysis. Surprisingly, we found phenotypes that contrasted dramatically from those of Arabidopsis; the mutant lines are delayed in growth and development compared to the wild-type plants (WT). Additionally, disruption of *SAMBA* function decreased pollen viability, reducing the average fruit size and weight, showing severe defects in their shape, seed number and embryo development. To investigate whether the observed modifications in fruit growth kinetics are associated with changes in fruit composition, fruit samples were harvested at three developmental stages (3, 5 and 8 dpa) from homozygous *samba* plants free Cas9, dissected and subjected to metabolomic analysis. We investigated the major pathways of primary plant metabolism by using an established GC-MS method. Our results suggest that SAMBA plays an important role in tomato development, as seen in Arabidopsis and maize, and provides important insights for further discoveries of fundamental biological processes that will improve the performance of this commercial crop.
**Key-words:** Plant development; Cell cycle; Ubiquitin-protein ligases; APC;

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# EFEITO BIOESTIMULADOR DO EXTRATO DE MICROALGAS NO DESENVOLVIMENTO DE PLANTAS DE ARROZ

**Thainá Inês Lamb [1,3]; Emilio Berghahn [1,4]; Fernanda Miyagi Pita [1,6]; Leonardo de Oliveira Neves [1,6]; Édina Aparecida dos Reis Blasi [1,5]; Jamili Seibel Hofstetter [1,3]; Mariana Dammann [1,2]; Luiz Carlos Oliveira da Silva [1,6]; Giseli Buffon [1]; Anja Dullius [1]; Camille Eichelberger Granada [1,7]; Raul Antonio Sperotto [1,7]**

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**Abstract:**
Population growth requires increased food production and improvement of the production process to maximize crop performance without enhancing the arable areas. Classic chemical fertilizers, which are widely used in agriculture, can cause a number of environmental problems. Therefore, environmentally healthy solutions capable of increasing the production and quality of food are needed. Microalgae are photosynthetic organisms capable of producing a range of compounds with great potential to be applied in agriculture. Several of these compounds have already been described as biofertilizers/biostimulants. This study evaluated the production of indolic compounds, exopolysaccharides, siderophores, and the development of microalgae strains (Chlamydomonas sp., Chlorella sp. and Desmodesmus sp.) isolated from lakes and freshwater deposits in four Brazilian states. The microalgae that produced a larger range of compounds and developed faster were selected for aqueous extracts production (0.1 and 0.5 g/L) to evaluate their potential as biostimulants of rice (Oryza sativa L.) plants. Rice seeds were inoculated with 10 mL of the extracts. Also, leaf application was performed on 20-day-old plants, being evaluated 10 days later (30-day-old). The seed inoculation and leaf application of the extracts provided beneficial changes in the physiological characteristics of the plants. Shoot length increased 6.4 and 10.3% after the application of SYN 16 aqueous extract at 0.1 and 0.5 g/L concentrations, respectively, and 4.3 and 8.7% when SYN 90 was applied. The roots were also larger with SYN 16 treatment (7.7 and 9.3% at 0.1 and 0.5 g/L concentrations, respectively). Regarding the shoot dry weight, increases of 22.3 and 36.1% were detected with the application of SYN 16 strain at 0.1 and 0.5 g/L, respectively. The strain SYN 90 at 0.5 g/L increased 27.2% the shoot dry weight when compared with control plants. Still, the root dry weight was 37.9 and 43.4% higher after the application of SYN 16 at 0.1 and 0.5 g/L, respectively. The strain SYN 90 at 0.5 g/L increased 35.5% the root dry weight when compared with control plants. Altogether, our results clearly show the biostimulatory effect of microalgae extracts in rice plants development, especially the genera Chlamydomonas sp. and Desmodesmus sp.. Our efforts are now aimed at identifying the bioactive compounds of these microalgae that can stimulate plant growth.
**Key-words:** agriculture; algae; biofertilizer; Chlamydomonas; Desmodesmus

# ALGODÃO TRANSGÊNICO EXPRESSANDO DUAS NOVAS TÓXINAS CRY CONFERE ALTA RESISTÊNCIA AO BICUDO DO ALGODOEIRO

**Thuanne Pires Ribeiro [1]; Gustavo Casecá Ruffo [2]; Leonardo Lima Pepino Macedo [3,4]; Isabela Tristan Lourenço Tessutti [3,4]; João Pedro Abreu Souza [2]; Osmundo Brilhante Oliveira Neto [1,5]; Maria Cristina Mattar da Silva [3,4]; Maria Fátima Grossi de Sá [3,4,6]**

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**Abstract:**
One of the main challenges in growing cotton (*Gossypium hirsutum*) is to control the damage caused by insect pests. In particular, the cotton boll weevil (CBW), *Anthonomus grandis*, is considered one of the most important pests of the Brazilian cotton crop. Although genetically modified (GM) cotton plants expressing Cry toxins are used worldwide applied for insect resistance, no commercial GM cotton event has been successfully developed for CBW control. This study addresses the development of GM cotton plants with high resistance to CBW. A plant vector was designed to express the described Cry proteins (Cry23Aa and Cry37Aa) encoded by *Bt* genes. The genes in question were regulated by two constitutive cotton promoters; the *cry23Aa* gene by the *FS4* promoter and the *cry37Aa* gene by the *FS1* promoter. Both promoters drive expression towards the flower buds. The expression cassette was introduced into the embryonic axes of a Brazilian cotton cultivar using the agrolistic technique. Cotton embryonic axes were bombarded and seedlings were selected for imazapyr resistance conferred by the *ahas* gene. The transgenic cotton plants pre-selected for tolerance to imazapyr showed a transformation efficiency of 5.75% and the PCR analysis confirmed the insertion of the expression cassette into the cotton genome. Relative expression analysis by qPCR indicated one copies of the transgene in the genome of $T_0$ plants. ELISA immunodetection tests were also performed on 45 positive PCR events. From these, seven events were selected that expressed high levels of Cry27Aa (32.41-40.91 µg/g of fresh tissue) and Cry37Aa toxin (30.59-48.77 µg/g of fresh tissue) in flower buds. Western blot analysis confirmed the presence of both proteins: Cry27Aa, with about 32 kDa and Cry37Aa, with about 32 kDa. Mortality rates of the target insect fed on $T_0$ generation plants ranged from 83 to 100%. As a result, the CBW resistant cotton plants generated in this study represent a major advance in the pest management and can significantly improve and increase cotton production worldwide.
**Key-words:** *Anthonomus grandis*; Cry23Aa toxin; Cry37Aa toxin; *Gossypium hirsutum*; *Bacillus thuringiensi*

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# CARACTERIZAÇÃO DA FUNÇÃO DO GENE *FLC-LIKE* DURANTE A TRANSIÇÃO DA ENDO- PARA ECODORMENCIA NA MACIEIRA

**Tiago Sartor [1,2]; Vítor da Silveira Falavigna [1,2,3]; Amanda Malvessi Cattani [1,2]; Carolina Pereira Silveira [2]; Jaiana Malabarba [1,2]; Diogo Denardi Porto [4]; Priscila Grynberg [6]; Roberto Coiti Togawa [6]; Marcos Mota do Carmo Costa [6]; Henrique Pessoa dos Santos [2]; Giancarlo Pasquali [1]; Luis Fernando Revers [1,2]**

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**Abstract:**
The MADS-box *MdFLC-like* gene was previously described within the major QTL for time of bud break in apple chromosome Chr09, and its expression gradually increases towards dormancy release. The molecular mechanisms in which MdFLC-like modulates the transition from endo- to ecodormancy and from ecodormancy to bud break are unknown. Here, the *MdFLC-like* gene was characterised to better understand its role during dormancy progression. The increase in *MdFLC-like* expression coincides with a decrease in *MdDAM1* transcript levels during ecodormancy establishment. In agreement, a transactivation assay using Arabidopsis protoplasts revealed that MdFLC-like represses the *GUS* reporter gene controlled by the *MdDAM1* promoter. Moreover, apple calli overexpressing *MdFLC-like-3xFLAG* showed decreased *MdDAM1* expression and delayed growth, suggesting that MdFLC-like may also repress growth-related genes. A deeper characterization of the mRNA levels of *MdFLC-like* during bud break demonstrated that ambient temperature performs as an environmental trigger modulating its expression, which rapidly switched when temperatures changed between cold and warm. Notably, *MdFLC-like* and *MdFT2* expression dynamically oscillated in a contrasting pattern of expression under these conditions. Our results suggest that MdFLC-like is a repressor of *MdDAM1* and *MdFT2* during the transition from endo- to ecodormancy and from ecodormancy to bud break, respectively, in apple trees.
**Key-words:** *Malus* x *domestica*; dormancy; *FLOWERING LOCUS C*; *DAM1*; *FT2*

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# *SOLANUM LYCOPERSICUM SCI1* (*SLSCI1*) AFETA O CRESCIMENTO DOS ÓRGÃOS REPRODUTIVOS, ANTESE, E É ALTAMENTE EXPRESSO EM SEMENTES

**Vanessa Thomé [1,2]; Joelma Oliveira Cruz [1,2]; Vitor Favaretto Pinoti [3]; Greice Lubini [2]; Pedro Boscariol Ferreira [4]; Andrea Carla Quiapim [2]; Karla Gasparini dos Santos [4]; Mateus Henrique Vicente [4]; Cassia Regina Fernandes Figueiredo [4]; Fábio Tebaldi Silveira Nogueira [4]; Lázaro Eustáquio Pereira Peres [4]; Maria Helena S. Goldman [1,2]**

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**Abstract:**
The development of floral organs is regulated by complex genetic pathways that ensure fine control of cell division and differentiation. Our previous work showed that *SCI1* (Stigma/Style Cell-cycle Inhibitor 1) regulates the final size of stigma/style in *Arabidopsis thaliana* and *Nicotiana tabacum*. Although some mechanisms of *SCI1* have been elucidated in these species, the involvement of *SCI1* in post-fertilization events was not previously investigated. Large-scale gene expression studies in tomato plants suggest that the *Solanum lycopersicum SCI1* homolog (*SlSCI1*) is expressed in flowers and fruits. Thus, the present work aims to expand the study of *SCI1* function from the early stage of floral development to fruit development in tomato. For this purpose, gene expression analysis was performed by RT-qPCR in several organs and tissues of *S. lycopersicum* cv Micro-Tom (root, stem, leaf, vegetative apex, floral meristem, sepal, petal, anther, ovary, stigma/style, immature green fruit, ripe green fruit, and seed). In addition, *SlSCI1* silencing transgenic tomato lines have been developed, via *Agrobacterium* transformation, to characterize traits associated with flowering and fruit formation. The results of the RT-qPCR demonstrated that *SlSCI1* is equally expressed in the floral meristem, petal, stigma/style, ovary, and immature green fruit and is highly expressed in seeds. The highest expressions were found in the most proliferative tissues/organs. This indicates that *SlSCI1* may have a role in regulating cell proliferation in all these organs derived from the floral meristem. *SlSCI1* silencing plants showed a reduction in anther and style length, and also a reduction in fruit size. Moreover, the silencing of *SlSCI1* resulted in early anthesis. Taken together, these results support the hypothesis that *SlSCI1* affects tomato floral development, floral organ growth, and acts in post-fertilization events.
**Key-words:** cell proliferation; flower development; fruit size; ;

## Acknowledgement

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# CARACTERIZAÇÃO DE PLANTAS TRANSGÊNICAS DE TOMATE COM SILENCIAMENTO DO GENE SWI2/SNF2 ATPASE MINUSCULE REMODELADOR DE CROMATINA E SEU IMPACTO NO DESENVOLVIMENTO VEGETATIVO E REPRODUTIVO

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**Abstract:**
SWI2/SNF2 ATPases, a protein family of chromatin remodelers (CHR), cause epigenetic alterations at nucleosome level; regulating the accessibility of transcriptional machinery. Arabidopsis thaliana has four complexes of SWI2/SNF2 ATPases: BRAHMA, SPLAYED, MINUSCULE 1, and MINUSCULE 2. The single mutant of MINU1 and MINU2 do not differ from the wild type, but the double mutant minu1;2 shows strong alterations at the embryonic and post-embryonic levels. Tomato (Solanum lycopersicum) has a single highly conserved gene for ATPase MINU (SlMINU). Our research group has generated SlMINU-RNAi transgenic tomato plants using cv.M82 as background, with the purpose of characterizing and understanding how the chromatin remodeling MINUSCULE epigenetically regulates the vegetative and reproductive stages of tomato. Tomato development until the first inflorescence was evaluated to know the internode distance, leaf number, plant height, and number of days to 1º inflorescence. Statistical analysis using Graph Pad Prisma 7.0 showed that transgenic plants are smaller than M82 ($p < 0.05$), but with a similar number of leaves to the control. To compensate for this, SlMINU-RNAi plants have closer internodes. Morphometric analyses during the early vegetative development showed that shoot apical meristem of 4DAG and hypocotyls with 6DAG of SlMINU-RNAi plants are smaller than those of cv.M82. During the reproductive development, flowers and reproductive structures like anthers and style showed the same trend. All these measurements were made with the Image J software and analyzed with the Graph Pad Prisma 7.0, indicating that all these differences are statistically significant ($p < 0.05$). The morphometric characterization of mature tomato fruit was performed using the Tomato Analyzer 3.0 to evaluate parameters as height, width, area, and perimeter; which showed that the fruits of iRNA minu plants have smaller dimensions than M82. Moreover, SlMINU-RNAi fruits presented fewer seeds per fruit. All these differences are statistically significant ($p < 0.05$). Among all the characteristics described, only the ovary size and the number of locules did not present statistically significant differences. On the other hand, preliminary genome-wide gene expression analyses comparing apices from M82 and transgenic plants with 10DAG at long-day conditions showed genes upregulated and downregulated by the knockdown of MINU. Using p-value<0.05 and Foldchangue 1.5, the DEGs are mainly involved in developmental processes, photosynthesis, and the circadian clock. One candidate gene is CONSTANS, which is up-regulated by MINU knockdown. To better understand the role of MINU, an MNase assay was performed for tomato apices. The results revealed that the expression of CONSTANS gene could be affected by an alteration in its nucleosome occupancy. This fact shows that this regulation at chromatin level could be possible in others MINU target genes, altering their genetic expression and impacting tomato development.
**Key-words:** SWI2/SNF2 ; ATPases; MINUSCULE; tomato; MNase

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SOCIEDADE BRASILEIRA DE GENÉTICA

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# PLANTAS

# Estresse Abiótico

# ANÁLISES FUNCIONAIS DE GLUTATIONA PEROXIDASE-LIKE 8 (GPXL8) DE ARABIDOPSIS EM RESPOSTA A ESTRESSES ABIÓTICOS

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**Abstract:**

Cell metabolism produces toxic by-products, such as reactive oxygen species (ROS), which excess can compromise cell membranes, protein function and also DNA integrity. Glutathione peroxidase-like (GPXL) enzymes are peroxidases responsible for detoxifying cells, since they convert peroxides into non-toxic products, using thioredoxin as a reductant. Yeast GPXL3 (*syn*. Orp1p) can also act as a redox sensor, by reacting with hydrogen peroxide and oxidizing the transcription factor Yap1, which eventually leads to the expression of defense genes. *Arabidopsis thaliana* GPXL8, like Orp1, is found in the cytosol and nucleus, thus highlighting its possible role in redox signalization, besides its peroxidase function. In this study, we aim to analyze how the GPXL8 is involved in different abiotic stresses, evaluating the phenotypic responses of different lines that overexpress, or are knockout mutants for this gene. Moreover, the dual function of GPXL8 also raises the question of whether these two activities could be differentially triggered by abiotic stresses, leading to different plant adaptations. The presence of heavy metals is one of the major conditions that limit plant development and can, consequently, affect food productivity. Cadmium and cesium are both considered toxic heavy metals for plants, even in low concentrations, disturbing plant development and physiology through the generation of ROS. To evaluate how GPXL8 affects plant development in response to heavy metal stresses, transgenic plants overexpressing FLAG-GPXL8, as well as knockout *gpxl8* mutants complemented with 35S::FLAG-GPXL8 construction were generated by floral dip method, and, alongside to knockout *gpxl8* mutants, were submitted to heavy metal stress conditions containing cadmium or cesium. To assess the phenotypic differences among the different lines, the primary root length, lateral root growth, rosette diameter and chlorophyll content will be measured. This study will provide new insights into how GPXL8 is involved in plant adaptation and development in response to abiotic stresses.

**Key-words:** GLUTATHIONE PEROXIDASE; ABIOTIC STRESS; HEAVY METALS; ARABIDOPSIS;

## Acknowledgement

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# A FAMÍLIA DE FITOCIANINAS DE CANA-DE-AÇÚCAR (*SACCHARUM* SPP.) E A SUA RESPONSIVIDADE AO ESTRESSE DE SECA

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**Abstract:**
Drought significantly threatens sugarcane crops - the main feedstock source for sugar and biofuel production. Phytocyanins (PCs) comprise a family of plant-specific copper-binding proteins, which are characterized as plantacyanins (PLCs), uclacyanins (UCs), stellacyanins (SCs), and early nodulin-like proteins (ENODLs) according to their structural features. PCs are involved in various plant processes and may also function in abiotic stress responses, including drought. However, the sugarcane PC family has yet to be characterized, and their involvement in drought scenarios remains to be explored. Here we decided to characterize the sugarcane (*Saccharum* spp.) PC family, investigate its responsiveness to water deficit and perform a functional study in transformed plants. Sugarcane PCs were retrieved using *Arabidopsis thaliana*, *Oryza sativa*, and *Sorghum bicolor* PC sequences as queries in TBLASTN searches against the SUCEST database and Sugarcane Genome Hub, and confirmed as plastocyanin-like domain-containing sequences by InterPro. Classification of sugarcane PCs was based on the prediction of signal peptides (SignalP 6.0), glycosylphosphatidylinositol (GPI)-anchor signal (Big-PI Plant Predictor), N-glycosylation sites (NetNGlyc 1.0), and copper-binding sites (multiple sequence alignment via MUSCLE 3.52). A phylogenetic tree was constructed in MEGA X software using the neighbor-joining method with bootstrap analyses (1,000 replicates). Relative expressions of eight PCs were evaluated by RT-qPCR in drought-tolerant ('IACSP94-2094') and -sensitive ('IACSP97-7065') sugarcane genotypes at 21 days without irrigation. Functional analyses of a candidate PC gene were conducted using heterologous expression and knockout (CRISPR-Cas9) approaches in rice as a model plant. For heterologous expression, a sugarcane PC coding sequence was cloned into pYPQ203 vector. For gene knockout, two guide RNAs were designed (CRISPR-P v2.0), assembled into a polycistronic cassette, and cloned into pDIRECT-25H vector. For both strategies, we performed *Agrobacterium*-mediated transformation of rice embryogenic *calli*. Gene-edited rice events were identified by PCR and Sanger sequencing decoding (TIDE). Considering only the monoploid reference genome (R570 cultivar), we uncovered 70 sugarcane PCs, including 2 SCs, 17 UCs, 19 PLCs, and 32 ENODLs. Among differentially expressed genes in drought, we found a transcriptionally contrasting *UC* gene between the sensitive (up-regulated) and tolerant (down-regulated) genotypes. Interestingly, this negative drought responsiveness in the tolerant genotype is also reported in the closest rice homolog, *OsUCL23*, revealed by the phylogenetic tree. Therefore, we decided to overexpress this sugarcane *UC* and knockout *OsUCL23* in rice plants. To date, we generated 11 gene-edited plants, whereas the UC-overexpressing seedlings are under the regeneration process. Transformed rice T2 plants will be functionally studied to elucidate the role of these PCs in drought tolerance. Finally, here we report firsthand the sugarcane PC family and its transcriptional responsiveness to water scarcity, which may be useful for novel strategies in sugarcane molecular breeding.
**Key-words:** CRISPR-Cas9; drought; phytocyanin; sugarcane; rice

## Acknowledgement

This study was supported by the São Paulo Research Foundation (FAPESP - Process Numbers: 2021/13478-2, 2020/07045-3), the Coordination for the Improvement of Higher Education Personnel (CAPES - Finance Code 001), and the Foundation for Agricultural Research Support (FUNDAG).

# ASCORBATO DESIDROGENASE ESTROMAL (*OSAPX7*) REGULA A TOLERÂNCIA AO ESTRESSE DE SECA EM ARROZ (*ORYZA SATIVA*)

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**Abstract:**
Chloroplast ascorbate peroxidases exert an important role in the maintenance of hydrogen peroxide levels in chloroplasts by using ascorbate as the specific electron donor. In this work, we performed a functional study of the stromal APX in rice (*OsAPX7*) and demonstrated that silencing of *OsAPX7* did not impact plant growth, redox state, or photosynthesis parameters. Nevertheless, when subjected to drought stress, silenced plants (APX7i) show a higher capacity to maintain stomata aperture and photosynthesis performance, resulting in a higher tolerance when compared to non-transformed plants. RNA-seq analyses indicate that the silencing of *OsAPX7* did not lead to changes in the global expression of genes related to reactive oxygen species metabolism. In addition, the drought-mediated induction of several genes related to the proteasome pathway and the down-regulation of genes related to nitrogen and carotenoid metabolism was impaired in APX7i plants. During drought stress, APX7i showed an up-regulation of genes encoding flavonoid and tyrosine metabolism enzymes and a down-regulation of genes related to phytohormones signal transduction and nicotinate and nicotinamide metabolism. Our results demonstrate that *OsAPX7* might be involved in signaling transduction pathways related to drought stress response, contributing to the understanding of the physiological role of chloroplast APX isoforms in rice.
**Key-words:** Drought stress; Ascorbate Peroxidase; Reactive Oxygen Species; Photosynthesis; Stomata conductance

## Acknowledgement

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# ANÁLISE DO PERFIL DE EXPRESSÃO GÊNICA ASSOCIADO A AÇÃO DA METILAÇÃO DO DNA EM TECIDOS FOLIARES E RADICULARES DE GENÓTIPOS DE SOJA SUSCETÍVEIS E TOLERANTES AO DÉFICIT HÍDRICO

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**Abstract:**

Of Asian origin, soybean (*Glycine max*) belongs to the family Fabaceae and is one of the most economically important legume crops, with a great relevance for global food. However, approximately 50% of the soybean crop is impacted by abiotic stresses, which tend to increase with climate change. Allied to this, epigenetic mechanisms can assist in plant responses to different stresses and understanding how these mechanisms are associated with tolerance to various conditions is of great importance, since modulation of specific gene expression patterns can be the target of biotechnological tools. In order to reveal new perspectives on understanding the soybean transcriptome under water deficit conditions, this research compared the gene expression profiles of contrasting soybean genotypes (Embrapa 48 and BR 16) by RNA-seq, enabling an analysis of the main molecular features that differentiate these cultivars in leaf and root tissues under drought. In addition, it examined the action of DNA methylation under 5-azacytidine in the same genotypes. The transcriptomic data of soybean grown under water deficit conditions were obtained in the NCBI Sequence Read Archive (SRA) database (Accession: PRJNA615913). The reads were then filtered by FASTX toolkit and mapped to the soybean genome (*Glycine max* Wm82.a2.v1) by Hisat2. Then, the transcripts were separated by library by Stringtie tool and Stringtie merge and Gffcompare were used for comparison and union of the gene annotations from the GFF files. Finally, the data were normalized by TPM, thus the program Feature Counts was used to identify the reads mapped by genomic features and Heatmapper (http://www.heatmapper.ca/expression) was generated for gene expression analysis of tissues under water deficit conditions. To evaluate the action of global DNA methylation under 5-azac in soybean cultivars, seeds were germinated in test tubes containing 20 mL of MS 1/2 strength medium with sucrose (30 g/L) and with 25mM of 5-azac methylation inhibitor. The difference found in the response of Embrapa 48 and BR 16 in leaf and root samples is remarkable and explains the better performance of Embrapa 48 cultivar under drought conditions. Indeed, leaves generated a higher number of "up-regulated" genes, and the data showed that Embrapa 48 responds to water deficit faster than BR 16, showing more differentially expressed genes from moderate levels. Furthermore, analyses of DNA methylation action under 5-azac in soybean showed physiological changes in roots in both cultivars. However, the performance of the Embrapa 48 cultivar treated with methylation inhibitor stood out when comparing the plant development variables, such as length, number of lateral roots, root volume, and fresh and dry mass.Thus, a possible response of this cultivar to different stresses is suggested.

**Key-words:** Osmotic stress; DNA methylation; Gene regulation; Glycine max;

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# ANÁLISES MOLECULARES DA RELAÇÃO ENTRE A RESPOSTA ADAPTATIVA À DEFICIÊNCIA DE FOSFATO E OS NÍVEIS DE BRASSINOSTERÓIDES EM RAÍZES DE ARROZ (*ORYZA SATIVA* L.)

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**Abstract:**

Phosphate is an essential nutrient for plant growth and development, but its availability is often limited in soil, resulting in reduced crop yields and poor-quality grains. The natural phosphate sources are limited and non-renewable and present a rising concern for global food security. The use of mutants has been a promising strategy for understanding molecular traits and for the improvement of crop yields, particularly in conditions of environmental stress. Considering this, d2-1 rice plants possess a mutation in the D2 gene, which compromises the biosynthesis of brassinosteroid phytohormones. This mutant exhibits insensitivity to certain phosphate-deprivation morphological alterations, such as leaf inclination. Furthermore, it has been shown, in Arabidopsis, that brassinosteroid signaling is antagonistic to the stress response. Thus, understanding the relationship between this phytohormone and the molecular mechanisms by which d2-1 plants are phosphate-starvation tolerant presents a potential for rice improvement programs. To further investigate it, 10-day-old T65 wild-type and d2-1 plants were submitted to optimal and phosphate-stress conditions in a hydroponics system. The total RNA was extracted from the roots of 25-day-old plants, and specific primers will be used to measure the expression levels of genes related to phosphate deficiency and brassinosteroid signaling. Changes in the root architecture of the mutant under phosphate deprivation were previously noted by our group. In light of these observations, we expect to perceive a differential expression of genes related to phosphate transport and signaling pathway receptors between the control/treatment conditions and wild-type/mutant groups. These results will provide better insight into how the expression levels of brassinosteroids in rice roots affect the plant's molecular response to phosphate-starvation conditions.

**Key-words:** Abiotic Stress; Brassinosteroids; Differential Gene Expression; Phosphate Starvation; Plant Response.

## Acknowledgement

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# REDE DE SPLICING DE TRANSCRITOS DE ARROZ EM RESPOSTA AO ESTRESSE TÉRMICO: UM INDÍCIO DE COMO FATORES DE SPLICING AFETAM A TERMOTOLERÂNCIA BASAL

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**Abstract:**
To identify novel solutions to improve rice yield under rising temperatures, molecular components of thermotolerance must be better understood. Alternative splicing (AS) is a major post-transcriptional mechanism impacting plant tolerance against stresses, including heat stress (HS). AS is largely regulated by splicing factors (SFs) and recent studies have shown their involvement in temperature response. However, little is known of the splicing networks between SFs and AS transcripts in the HS response. In order to expand this knowledge, we constructed a splicing network based on a publicly available RNA-seq dataset that explored rice heat response over a time-course. Our analyses suggest that the HS-dependent control of the abundance of specific transcripts coding for SFs might explain the widespread, coordinated, complex, and fine AS regulation of critical genes during plant exposure to extreme temperatures. AS changes in these critical genes might affect many aspects of plant biology, from organellar functions to cell death, providing novel regulator candidates for future functional studies.
**Key-words:** Systems Biology; Oryza sativa L.; Co-expression Networks; ;

# ETAPAS INICIAIS VISANDO O NOCAUTE DE DOIS FATORES DE SPLICING LIGADOS AO ESTREESE AO CALOR EM ARROZ.

**João Henrique Servilha [1]; Bruno Luka de Souza Bambirra Silveira [2]; Abdellah Barakate [3]; Cristiane Paula Gomes Calixto [4]**

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**Abstract:**
Rice is one of the most consumed foods in the world, but heat stress affects its yield and grain quality. To identify mechanistic solutions to improve this crop under climate change threats, the molecular responses of thermotolerance must be identified. Alternative pre-mRNA processing is one of the mechanisms involved in plant response against stresses, but little is known about how the variation of transcribed isoforms is regulated by temperature changes. The rice genes LOC_Os02g40900 and LOC_Os05g30140 are candidates for heat-sensitive splicing regulators. To investigate this hypothesis, we generated plasmids based on the CRISPR-Cas9 methodology to knock out these genes in rice plants. Using the dual-targeting strategy, we selected two target sequences in the coding region of each gene. These target sequences were inserted in plant expression vectors via PCR, sub-cloning, and Golden Gate Assembly. The correct plasmids were confirmed by Sanger sequencing and they were then inserted into *Agrobacterium* and confirmed by colony PCR. For plant transformation, rice calluses were obtained by inducing undifferentiated embryo division in rice seeds. After induction, subculture was performed, which will allow for transformation via *Agrobacterium*. Our next steps are to obtain biallelic T0 plants, with significant deletions in the target genes. Finally, we intend to understand the effects of heat stress on non-transgenic homozygous T2 knockout plants through heat stress assays. In this way, part of the poorly understood question of the regulation and function of the alternative splicing mediated by heat stress in rice can be elucidated, providing an important data to unravel the mechanisms of heat stress tolerance in cereals and other grasses.
**Key-words:** Alternative splicing; Heat stress; Oryza sativa; Thermal stress; Climate change

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# CARACTERIZAÇÃO DE FAMÍLIAS GÊNICAS DE HISTONA ACETILTRANSFERASES EM *SETARIA VIRIDIS*

**João Marcos Fernandes Esteves [1]; João Travassos Lins [1]; Marcio Alves Ferreira [2]**

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**Abstract:**

Prolonged droughts and extreme heat waves are becoming more frequent because of rapid climate change and thus pose a challenge for plant science and agriculture. In this sense, C4 photosynthesis stands out for its high efficiency in water and nitrogen use. Moreover, C4 plants are typically more drought-tolerant, which highlights their potential for a more sustainable agriculture. Since plants are sessile organisms, they adapted to undertake transient abiotic stresses, such as molecular mechanisms that can keep information on how to respond in the future, known as genetic memory. Epigenetic modifications are a major part of genetic memory since they regulate gene expression and may be modulated by external stimuli. However, studies on the epigenetic response to abiotic stresses and their ties to adaptive memory are still scarce. In this work we aim to characterize histone acetyltransferase gene families that might be involved with epigenetic modifications in the model plant *Setaria viridis* under three abiotic stress conditions, such as water deficit, salinity and heat, through bioinformatics and gene expression analysis. Using HMMER, we identified ten genes involved with histone acetylation. Maximum likelihood phylogenetic trees were inferred using IQ-TREE to identify each homologue. Four genes belong to the CBP/300-like subfamily, two genes in TAFII250, only one gene in the MYST subfamily and three genes under the GNAT group. GNAT is composed by three subfamilies: ELP3, HAT1-like and GCN5, each having one gene identified. Additionally, we were able to successfully induce heat shock stress in 4-week-old plants using a heat chamber at 40 °C. The stress condition was assessed by electrolyte leakage measurements and a significant increase in leakage was observed in both leaves and roots in relation to control samples. Leaf samples have shown an increase of 120% in leakage, while root samples had an increase of 27%. These results were expected since the leaves are majorly impacted by the surrounding air temperature. In the following months we will perform a larger scale stress experiment, including salt and drought stress, and additional physiological measurements will be taken, alongside qPCR and RNA sequencing analysis to assess differential gene expression. We expect the results from the work to provide new and insightful information on how epigenetic modifiers respond to abiotic stresses, which may prove useful in future studies regarding genetic memory.

**Key-words:** *Setaria viridis*; Epigenetics; Memory genes; Abiotic stress; Phylogenetic analysis

# ANÁLISE FISIOLÓGICA E MOLECULAR DA RESPOSTA DE MEMÓRIA DE *SETARIA VIRIDIS* AO DÉFICIT HÍDRICO

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**Abstract:**
The main causes of agricultural losses are droughts and the stress caused by water deficit. This scenario has been aggravated by climate changes and, among the affected crops, C4 plants (such as maize and sugarcane) stand out due to their major water use efficiency and economic relevance. *Setaria viridis* has been widely used as a model organism for C4 plants in physiological and molecular studies. Although its response to abiotic stresses has been the focus of manifold papers, our knowledge of the memory response of C4 plants is still lacking. The memory response is defined as the ability to store the information from a previous stress event to achieve a faster or more effective response to a subsequent stress event and the main mechanism used by plants to store this information is through epigenetic changes. Therefore, our goal was to evaluate the memory response of *S. viridis* to water deficit cycles in a physiological, gene expression and epigenetic level. By integrating them, we hoped to better elucidate the memory response in *S. viridis* and the epigenetic mechanisms involved in memory formation. This knowledge might be useful for C4 crops breeding. To induce the memory response, we cultivated *S. viridis* plants in a hydroponic system and applied three seven hour cycles of water deficit with PEG-8000 intercalated with rehydration periods. Throughout the cycles, we performed physiological measurements, such as analysis of relative water content (RWC), electrolyte leakage (EL) and chlorophyll fluorescence. During the water deficit cycles, we observed significant reductions in the RWC and the photochemical efficiency (Fv/Fm), as well as an increase in the EL and non-photochemical quenching of the plants. These results were confirmed by multivariate analysis. To study the epigenetic machinery in *S. viridis*, a necessary step was the identification of its coding genes, hereafter referred to as epigenetic modifiers. Our first strategy was based on a review of epigenetic modifiers in arabidopsis, which was used to identify their orthologs in *S. viridis* through the use of orthology searching tools. Then, we recovered their expression data from an RNA-seq of roots exposed to drought cycles and established a fold-change and FPKM cut-off to identify which genes were involved in drought response. In the second strategy, we searched the literature for epigenetic modifiers associated with drought response in monocots and identified their orthologs in *S. viridis*. The genes identified by the two strategies had their expression evaluated by RT-qPCR, which allowed us to identify genes with memory profile. This work allowed us to establish the framework of memory response to water deficit in *S. viridis*, which will be further studied by knocking out epigenetic modifiers by CRISPR-Cas9 and evaluating the position of epigenetic modifications by chromatin immunoprecipitation.
**Key-words:** *Setaria viridis*; Memory; Water deficit; Epigenetic modifiers;

# FENOTIPAGEM PARA ANÁLISE DE TOLERÂNCIA AO DÉFICIT HÍDRICO EM LINHAGENS DE ARROZ DE TERRAS ALTAS SUBMETIDAS AO ESTRESSE POR POLIETILENOGLICOL 6000

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**Abstract:**
Water deficit is a limiting factor for rice (*Oryza sativa*) production in upland conditions compromising productivity and maintenance of the crop. Thus, plant breeding program aim to select lines and develop cultivars tolerant to water deficit, as an alternative to guarantee rice productivity in regions affected by water irregularities. Seed and seedling phenotyping has been shown to be a tool that allows early, quick selection with low space demand. Thus, the present work aimed to analyze seeds and seedlings of upland rice subjected to water deficit by Polyethylene glycol 6000. The experiment was carried out in the laboratory under two conditions, water deficit simulation and environmental control. Thus, germitest paper moistened with a PEG 6000 solution at -0.9 Mpa concentration (environment with water deficit) and with water (control environment) was used. Germination tests were monitored with counts at the 7th and 14th days and phenotyping by image analysis to verify shoot length and main root. The images were taken on the 3rd, 5th, 7th, 9th and 11th days after sowing and the measurements were taken using the Image J software. The data were transmitted to analysis of variance and Skott-Knott test. Based on the statistical data, significant differences were observed at 5% probability between the evaluated strains. The Skott-Knott test revealed that the lines CNA-70, CNA-73 and CNA-98, presented better performance for germination in the stress condition. Some strains delay germination but manage to develop over the course of days, as was the case with the control, Douradão. The image analyzes allowed observing the development of the root area, with better performance of the lines CNA-38, CNA-70, CNA-139 and CNA-144. For shoots, a better performance was observed for the following lines CNA-70, CNA-73, CNA-139, CNA-144. The CNA-70 line stood out in all the analyzes carried out, which suggests a high potential for germination and development in conditions of water scarcity. The data indicate the presence of promising upland rice lines for tolerance to water deficit, among those analyzed.
**Key-words:** Plant breeding; Seedling; Germination; ;

## Acknowledgement

CAPES, CNPq and FAPEMIG.

# ALUMÍNIO ALIVIA CLOROSE INDUZIDA POR DEFICIÊNCIA DE FERRO EM ARROZ CULTIVADO (*ORYZA SATIVA*) E EM SEU ANCESTAL SELGAVEM (*ORYZA RUFIPOGON*)

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**Abstract:**
Rice is one of the most cultivated cereals in the world and is considered a staple food for more than half of the world's population. Cultivated rice (*Oryza sativa*) was domesticated from *Oryza rufipogon*. Because it is cultivated in all continents, rice farming areas have a wide range of environmental characteristics, climatic and agronomic conditions and, consequently, are exposed to several abiotic stress, such as low levels of essential nutrients in the soil solution and the presence of toxic elements. Among the stresses to which rice is exposed are iron (Fe) deficiency and aluminum (Al) toxicity, two major problems in agriculture. In this study we evaluated the responses of *Oryza rufipogon* and *Oryza sativa* (cv. IRGA 429) to Fe deficiency, Al excess and both stresses combined to understand if Al stress impacts the Fe deficiency response. Rice plants were cultivated in a controlled condition in a green room, using hydroponics. Treatments used were control nutrient solution, Al subtoxic treatment (300 µM AlCl3 added to nutrient solution), Fe deficiency (no Fe added) and combined -Fe + Al. Shoots of plants treated with -Fe +Al showed no clear difference compared to controls, while plants under -Fe had leaf chlorosis. Interestingly, shoots of plants treated with -Fe + Al showed little to no chlorosis, which indicates that the addition of Al to the nutrient solution can result in alleviation of the typical Fe deficiency symptoms in leaves. Photosynthetic pigments quantification supports this observation: -Fe treatment decreased chlorophyll a, chlorophyll b, total chlorophyll and carotenoid concentration compared to controls, while +Al and -Fe+Al treatments alone showed no difference. We confirmed these results using SPAD. Results were consistent for *O. sativa* and *O. rufipogon*, indicating that the mechanism for such phenomenon is conserved in both species. We tested expression of Fe responsive genes OsIRO2, OsIRT1 and OsYSL15 in roots. In both species, OsIRO2 is up regulated by Fe deficiency, and a similar expression level is observed in -Fe+Al?treated plants. The three genes were down regulated in the +Al treatment, suggesting that Al is able to down regulate OsIRO2, OsIRT1 and OsYSL15. Interestingly, OsIRT1 and OsYSL15 in the combined treatment were expressed at levels similar to control. Our data shows that -Fe+Al treated plants decreased the root expression of Fe deficiency up?regulated genes, OsIRT1 and OsYSL15, which encode two key Fe transporters, but not the expression of transcriptional regulator OsIRO2. Therefore, our data suggest that Al might be impacting Fe utilization in rice plants and could account for the hormetic effect of Al in rice.
**Key-words:** Aluminum; Iron deficiency; *Oryza rufipogon*; *Oryza sativa*;

**Acknowledgement**

CNPq, CAPES, FAPERGS.

# OTIMIZAÇÃO DA TRANSFORMAÇÃO MEDIADA POR AGROBACTERIUM PARA O ACESSO A10.1 DE *SETARIA VIRIDIS* E O ESTABELECIMENTO DO PROTOCOLO DE TRANSFORMAÇÃO PARA ACESSO AST.1

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**Abstract:**

Several characteristics make *Setaria viridis* an ideal plant model for monocots with C4 metabolism. Among them, *S. viridis* has a well-established *Agrobacterium tumefaciens* mediated transformation protocol, which is crucial for a model plant. However, *S. viridis* has several accessions and multiple factors can influence their transformation efficiency. Previous studies showed that the ME034V accession has a higher transformation efficiency when compared to A10.1. However, the majority of published genomic studies focus on the A10.1 accession, which makes it the best target for forward genetic studies. In addition to A10.1, the Ast-1 accession may also be a good target for forward genetic studies, due to its drought sensitive phenotype. Therefore, the objectives of this work were to optimize the transformation protocol for the A10.1 accession and the establishment of a transformation protocol for the Ast-1 accession.For the transformation experiments, the accessions A10.1, Ast-1 and ME034V (used as control) of *S. viridis* were used. For the selection and identification of transgenic plants, we used hygromycin as a selective agent on medium and PCR, respectively. CRISPR/Cas9 constructs and RD29 promoter fused to the GUS/GFP constructs were used for transformation. Induction of embryogenic callus of Ast-1 resulted in an average of 1 callus per 4.5 seeds introduced in tissue culture. In our experiments, the accessions ME034V and A10.1 showed an average of 1 callus per 4.7 seeds and 1 callus per 6.4 seeds, respectively. Studies conducted with the accession A10.1 have shown an embryogenic callus rate of 1 callus per 1.8 seeds. There is no data on embryogenic callus induction of the ME034V and Ast-1 accessions. In out experiment, the A10.1 accession showed an average transformation efficiency of 6.3%. Previous studies with *S. viridis* showed that the transformation rate is usually low. In the present study, the transformation efficiency among the regenerated plants from the ME034V accession was approximately 13,3%. However, we observed a high number of escapes. Furthermore, our future goal is the improvement of the transformation method by optimizing the concentrations of selection agent with a focus on the reduction of escape plants to obtain more transgenic lines for the accessions ME034V, A10.1 and Ast-1 for both CRISPR/Cas9 and pRD29::GUS-GFP constructs.

**Key-words:** Setaria; Agrobacterium-mediated; transformation; Ast.1; A10.1

# EXPRESSÃO DO GENE MDDHN11 DA MACIEIRA (MALUS DOMESTICA) EM SOJA VISANDO MAIOR TOLERÂNCIA AO ESTRESSE ABIÓTICO

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**Abstract:**
Soybean (Glycine max (L.) Merril) is one of the most important legumes in the world as it is an excellent source of oil and protein. Soybean production is highly dependent on rainfall or abundant irrigation. Under dry conditions soybean yield can reduced by more than 50%, causing substantial financial losses. Drought is a significant climate risk for soybeans and requires effective mitigation strategies. Genetic engineering techniques can be used to develop plants with tolerance to water stress. Dehydrins (DHNs) are protective proteins related to several developmental responses in apple (Malus × domestica Borkh) that involve dehydration, such as seed desiccation and abiotic stresses. Transgenic *Arabidopsis* plants expressing MdDHN11 under severe water stress confirmed the protective relevance of DHNs during long-term water deficit. In view of the importance of the soybean crop, and the risks that changes in precipitation patterns represent for the crop, the objective of this work was to genetically transform soybeans to express the *MdDHN11* gene in order to increase tolerance to water stress. It was generated a vector to express the *MdDHN11* in soybean, under the control of the *actin* 2 promoter from *Arabidopsis thaliana.* In addition, it was added the *Atahas* gene that confers tolerance to herbicides of imazapyr, which was used to select transgenic events. The vector was used to transform soybean embryos by the biolistic method. Presence of the transgenes in regenerated plants was confirmed by PCR amplifications. Plantlets were allowed to set seeds and the transgenes segregated in a Medelian fashion. Effect of *MdDHN11* transgene expression on physiology of transgenic soybean plants will be presented.
**Key-words:** Drought tolerance; Glycine max; Biotechnology; ;

**Acknowledgement**

CNPq

# EFEITOS PROTETORES E CICATRIZANTES DA APLICAÇÃO DE EXTRATOS DE MICROALGAS EM PLANTAS DE ARROZ SUBMETIDAS A ESTRESSE DE BAIXA TEMPERATURA

**Leonardo de Oliveira Neves [1]; Thainá Inês Lamb [3]; Emilio Berghahn [3]; Fernanda Miyagi Pita [1]; Édina Aparecida dos Reis Blasi [5]; Jamili Seibel Hofstetter [1]; Mariana Dammann [1]; Luiz Carlos Oliveira da Silva [1]; Giseli Buffon [2]; Anja Dullius [2]; Camille Eichelberger Granada [2,4]; Raul Antonio Sperotto [2,4]**

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**Abstract:**
Rice (Oryza sativa L.) is one of the most produced grains in the world, being consumed by more than half of the world's population. However, productivity losses are caused by abiotic stresses such as salinity, cold and drought. Rice plants have high sensitivity when exposed to low temperatures, severely decreasing crop productivity. One plausible strategy to overcome this issue is the application of microalgae, which are capable of protecting stressful plants by producing various compounds with biological activity. Therefore, the objective of this work was to evaluate the capacity of microalgae extracts to stimulate a protective/healing effect in rice plants subjected to low temperature stress. Rice seeds were inoculated with 10 mL of microalgae strains of the genera Chlorella sp., Chlamydomonas sp. and Desmodesmus sp., along with the consortium of these strains, at 0.1 and 0.5 g/L concentrations, and then subjected to 7 cold nights (10ºC). In addition, 20-day-old plants (vegetative stage) were subjected to a second cold exposure (10ºC) for 4 days, with two forms of microalgae extract application: before cold exposure (to evaluate the protective effect), and after cold exposure (to evaluate the healing effect). The shoot and root length of the seedlings was analyzed after 7 days of germination. Total chlorophyll concentration and shoot/root dry weight was analyzed in 30-day-old plants. It was seen that the seed inoculation increased the shoot length of the seedlings. The highest increase (23%) was seen with the inoculation of Chlorella sp. extract at 0.1 g/L concentration. With the application of the consortium of the three strains at a 0.5 g/L concentration, a 35% increase was seen in the root length. Regarding tests performed in the vegetative phase, the total chlorophyll concentration was not changed in any treatment. However, leaf application of the extracts after exposure to cold resulted in higher shoot and root lengths. Regarding the shoot dry weight, only the application of the strain Desmodesmus sp. at 0.1 g/L concentration before cold exposure resulted in an increase of 62.5% compared to control plants. Plants treated with microalgae extracts showed higher root dry weight in all forms of application and for all strains tested, including the strains consortium. Our results suggest that inoculation in seeds and leaf application of microalgae extracts promote a protective and healing effect to low temperature stress during germination and vegetative stages of rice plants.
**Key-words:** low temperature; microalgae; rice; stress;

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# ENGENHARIA GENÉTICA DE PRECISÃO PARA TOLERÂNCIA À SECA EM SOJA E SEU EFEITO NA VIA DE MORTE CELULAR PROGRAMADA DO RETÍCULO ENDOPLASMÁTICO

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**Abstract:**
Understanding plant molecular pathways associated with drought tolerance has become essential for crop genetic improvement in a climate change scenario. Considering the different drought tolerance mechanisms, the endoplasmic reticulum (ER), responsible for protein synthesis and processing, is one of the main targets of severe stresses in plants. The efficiency of ER performance is linked to the activity of molecular chaperones such as *GmBiP* (binding protein). In severe and prolonged stress, *GmBiP* plays a crucial role as a negative regulator of the NRP/DCD-mediated cell death response, attenuating the modulation of expression and activity of the signaling components of this circuit. This integrated ER stress response pathway converges on N-rich proteins (NRPs) containing the cell death and development domain (DCD) to induce activation of the vacuolar processing enzyme promoter and the programmed cell death. Thus, in this study, we applied two strategies to suppress senescence induced by the DCD-NRP circuit to increase drought tolerance. Firstly, we investigated the potential of CRISPR/dCas9 system fused with transcription activators (VP64) in positively modulating the expression of *GmBiP*. For this, the efficiency of three single guide was tested in transient tobacco transformation assays. We found that the sgRNA position affects the transcription modulation, being the best sgRNA, the closest to the transcription start site. These results were replicated in a soybean transient transformation system confirming that overexpression of endogenous *GmBiP* leads to the repression DCD-NRP circuit genes. After validation tests, we obtained stable transformant soybean plants overexpressing *GmBiP*. As expected, DCD-NRP circuit genes were repressed in these plants and *GmBiP* modulation remained stable over generations. Drought stress trials are ongoing. To develop a non-transgenic strategy for DCD-NRP regulation, we identified a potential downstream gene (*GmNAC030*) that positively regulates this circuit to be knocked out via CRISPR/Cas9 technology. We designed and validated a sgRNA in a soybean hairy root system. The selection of CRISPR-edited plants is ongoing. Our results confirm the potential of DCD-NRP circuit regulation and CRISPR technology for developing drought tolerance crops.
**Key-words:** CRISPR/Cas9; CRISPR/dCas9; *Glycine max*; *GmBiP*; *GmNAC30*

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# ANÁLISE *IN SILICO* DO GENE RESPONSIVO A ESTRESSE TÉRMICO *TELOMERE REPEAT-BINDING FACTOR 1* EM ARROZ (*ORYZA SATIVA* L.)

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**Abstract:**

Rice is particularly susceptible to the effects of heat stress (HS), especially at reproductive stages. Thus, as this crop is a major food staple worldwide, the effects of climate change threaten food security. The characterisation of heat-responsive genetic elements could therefore aid crop improvement efforts. In this sense, a study recently carried out by our group has identified a set of key genes found to undergo differential expression (DE) and/or alternative splicing (DAS) during HS, such as the *TELOMERE REPEAT-BINDING FACTOR 1* (*OsTRBF1*, LOC_Os01g40670). This gene codes for a potential transcription factor (TF) that binds to telomeric dsDNA, but little is known of its biological role. To assess the putative function of *OsTRBF1*, diverse biological databases have been explored. A search through Monocots PLAZA 5.0 indicated that GO terms such as 'nucleosome assembly' and 'transcription cis-regulatory region binding' were associated with this gene. Analysis of *OsTRBF1* promoter region using PlantPAN 3.0 identified binding sites for multiple TF families responsive to stress, development and hormone signalling. We explored the *OsTRBF1* interactome with these TFs through transcriptional co-expression networks under 'stress', 'developmental' and 'hormone treatment' conditions. Half of the TFs found to be co-expressed with *OsTRBF1* are ethylene-responsive, some of which implicated in abiotic stresses, while others are involved in flower development. Analysis of *OsTRBF1* expression in BAR ePlant indicated that this gene has an ubiquitous expression encompassing different plant developmental stages, including reproductive tissues. We also observed a putative response to drought and salt stresses. A brief investigation into *OsTRBF1* post-transcriptional control confirmed AS occurring at the C-terminal region - suggesting different protein isoforms and changes in mRNA stability. Lastly, we have carried out a phylogenetic analysis of *OsTRBF1* by extracting and reanalysing data from Phytozome and Monocots PLAZA 5.0. *OsTRBF1* homologues were identified in all land plants analysed, hinting at multiple duplication events. In *Arabidopsis*, found *TRBF1* homologues were also associated with nucleosome assembly, one of them interacting with H3K27me3 target genes, and seem to be ubiquitously expressed. In summary, *TRBF1* could pose a novel epigenetic interface with stress and developmental responses in plants and will be further investigated in functional genomics studies in rice.

**Key-words:** Heat Stress; Alternative Splicing; Epigenetics; Bioinformatics; Systems Biology

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# A SUPEREXPRESSÃO DE UMA OSMOTINA DE *SOLANUM NIGRUM* (SNOLP) POTENCIALIZA AS VIAS DE RESPOSTA À SECA EM SOJA

**Maria Helena Bodanese Zanettini [1]; Luisa Abruzzi de Oliveira Busatto [2]; Lariane Frâncio [3]; Fernanda Lazzarotto [4]; Giulia Ramos Faillace [5]; Frank Guzman [6]; Débora Favero [7]; Ricardo Luís Mayer Weber [8]; Christian Bredemeier [9]**

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**Abstract:**
Environmental stresses are responsible for limiting soybean yield. In order to mitigate the impacts generated by water deficit, molecular biology tools are being used to develop genetically modified plants. Previous studies showed that two independent events (B1 and B3) of soybean transgenic plants expressing a *Solanum nigrum* osmotin (SnOLP) had an increment in drought tolerance. The present study aims to investigate the drought tolerance promoted by osmotin overexpression in soybean. Transgenic and non-transgenic (NT) plants in vegetative stage were submitted to water deficit by the irrigation suppression during seven days. Control plants were kept irrigated. Physiological variables were monitored and confirmed that the transgenic plants present better performance when compared to the NT plants. The total RNA extracted from leaves was sequenced and data was normalized by DESeq2. A total of 2044 and 1505 differentially expressed genes (DEGs) were identified in B1 and B3 events, respectively. Regarding B1 event, 769 genes were upregulated and 1275 downregulated. For B3, 541 genes were upregulated and 964 genes were downregulated. The exclusion of DEGs in common between transgenic and NT plants resulted in 395 genes upregulated and 234 downregulated, shared by B1 and B3 events. The metabolic pathways and gene ontology categories identified are known to be involved in plant responses to drought. Hormonal, photosynthetic, carbohydrate and amino acid metabolism, reactive oxygen species, and post-translational modifications pathways were significantly modulated in transgenic plants. Altogether, the results suggest that osmotin promotes tolerance through an increment in the plant responses elicited by drought.
**Key-words:** RNA-Seq; Transgenic plants; Gene Ontology; Metabolic pathways;

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# A SUPEREXPRESSÃO DO GENE SCTPX2-LIKE AUMENTA A TOLERÂNCIA AO DÉFICIT HÍDRICO EM CANA-DE-AÇÚCAR TRANSGÊNICA.

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**Abstract:**
Sugarcane is a vital component of the Brazilian economy, and its productivity is often limited by environmental factors such as drought. The *ScTpx2-like* gene from sugarcane is induced by drought, and its overexpression in Arabidopsis has been shown to enhance tolerance to water deficit. Here we investigated the effects of increased levels of *ScTpx2-like* expression in transgenic sugarcane plants exposed to water deficit. Transgenic sugarcane overexpressing *ScTpx2-like* under the control of the maize pUbi-1 promoter was generated. Both transgenic and wild sugarcane plants were subjected to water deficit for ten days at a pot capacity of 30%. Transgenic plants displayed 12-23% higher efficiency in net photosynthetic rate than wild-type plants. Furthermore, the proline content in both wild-type and transgenic plants increased under stress. However, while wild-type plants had a 48% increase, transgenic plants showed 50-72% higher proline levels. Malondialdehyde increased by 129% in wild-type plants, and in transgenic plants, the increase was lower, ranging from 20-107% in different events. Experiments aiming at the determination of the subcellular localization of ScTpx2-like::GFP fusions are underway and will help gain insights into the mode of action of this protein. Overall, the study suggests that overexpression of the *ScTpx2-like* gene can help mitigate the adverse effects of water deficit in sugarcane, thereby offering the potential for biotechnological applications in developing new drought-tolerant commercial cultivars.
**Key-words:** TPX2; sugarcane; drought; water defict; transgenic

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# ANÁLISE DO TRANSCRIPTOMA DE RAÍZES DE ARROZ REVELA INSIGHTS MOLECULARES SOBRE O PAPEL DAS PROTEÍNAS ASR NA TOLERÂNCIA À DEFICIÊNCIA DE FOSFATO

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**Abstract:**
The high demand of crops for soluble inorganic phosphate is worrisome, not only because phosphorus (P) is a macronutrient that is insufficiently bioavailable in agricultural soils but also due to the low efficiency of fertilizer applications. The latter comes from phosphate rocks poorly distributed globally that are a slow restoration resource. Besides, Brazil is the third largest importer of phosphorus in the world and, in the previous year, invested more than 300 million dollars in fertilizers. Considering this, the cultivation of rice (*Oryza sativa* L.) exemplifies the strong dependence on this nutrient since its deficiency leads to productivity losses. Therefore, molecular tools may represent ecofriendly alternatives capable of, in the future, favoring crops' nutrient uptake to mitigate the adverse economic effects that this abiotic stress establishes. ASR (Abscisic acid, Stress, and Ripening) proteins are unique to plants and are involved in development processes and responses to biotic and abiotic stress via, for example, transcriptional regulation. The genome of cultivated rice has six copies of ASR genes, whose silencing by RNAi compromised the modification of the root system architecture in response to phosphate deficiency, making mutant plants more sensitive to stress compared to the wild type. Also, our research group concluded that OsASR5 expression is induced by P deficiency in lateral roots, suggesting that this gene is crucial for these abiotic stress responses. However, the molecular mechanisms through which it performs this role have not yet been elucidated. To shed light on this, *O. sativa* cv. Nipponbare wild-type and ASR-RNAi plants were grown in Yoshida's hydroponic solution with and without $NaH_2PO_4$. After five days of treatment, the total RNA of the roots was extracted with the Direct-zol RNA MiniPrep Plus kit, according to the manufacturer's instructions. The samples were grouped in triplicates, sequenced by the Illumina HiSeq 2500 platform, and analyzed with the 3D RNA-seq tool. Phosphate deficiency promoted differential expression of transcripts such as the Phosphate transporter family (PHT), Inorganic pyrophosphatase 2 (PPA2), and Purple acid phosphatase (PAP10a) in ASR-RNAi rice plants. The protein encoded from these transcripts are involved in uptake, root-to-shoot translocation, metabolism, signaling, and phosphorus usage. The Gene Ontology (GO) annotation indicated the enrichment of differentially expressed genes in cellular and metabolic processes such as catalytic activity in response to this abiotic stress. Thus, these data could broaden the knowledge about the role that ASR proteins play in the transcriptional reprogramming of rice roots during phosphate deficiency. It could also contribute to understanding the molecular basis of previously observed phenotypic responses and favoring functional characterization of potential new stress response components.

**Key-words:** Abiotic stress; Nutritional deficiency tolerance; Molecular responses; Rice transcriptome;

# GERENCIAMENTO DOS RECURSOS ENERGÉTICOS EM ARABIDOPSIS: ENVOLVIMENTO DA VIA SNRK1-BZIP1/53 E 63

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**Abstract:**

Light is the source of energy for photosynthesis, fixation of $CO_2$ and production of carbohydrates, which constitute the primary energy source of the plant cell. During the night, when there is no photosynthesis, plants rely on the carbohydrate they stored during the day in the form of starch, a polymer of glucose. Starch is degraded in a controlled manner, providing a continuous supply of carbohydrates (energy) throughout the night to sustain metabolic activities and growth until dawn. Under energy deprivation (i.e. conditions that limit photosynthesis and/or respiration), the available energy reserves must be efficiently managed in order to ensure survival and growth. The conserved *SUCROSE NON-FERMENTING RELATED KINASE 1* (*SnRK1*) plays a central role in this process. This kinase is activated under energy starvation, triggering a metabolic reprogramming to slow down energy-consuming processes and to activate pathways for energy production from the reserves. In *Arabidopsis thaliana*, the response triggered by *SnRK1* recruits the bZIP-type Transcription Factor (TF) *bZIP63* and its dimerization partners *bZIP1* and *bZIP53*, which will work on the reestablishment of energy balance in the cell through regulation of genes related to starch degradation and energy deficit responses. Further understanding of how plants optimize the usage of their energy resources to ensure vigorous growth is needed to develop new approaches aiming yield improvement , The objective of the Ph.D. intends to investigate the role of TFs *bZIP1*, *53* and *63* in controlling the usage of energy resources. For this, analysis of changes in growth, developmental, metabolism and gene expression during the diel cycle in single, double and triple mutants for *bZIP1*, *53* and *63* are being developed. Additionally, we are analyzing two silenced lines (L9 and L5) for expression of *bZIP63(% WT)*, *1* (%WT) and *53(%WT)* by interference RNA (RNAi). Gene expression analysis by RNAseq and RT-qPCR between wild type (WT) and mutant lines which ones? Showed that genes related to starch, sugar and amino acids metabolism, short day growth control among others were specifically deregulated in these silenced lines. These data further support the role of bZIP1/53 and 63 partnership in defining the gene expression program related to energy homeostasis.

**Key-words:** Arabidopsis; Energia; bZIP TF; Gerenciamento;

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SOCIEDADE BRASILEIRA DE GENÉTICA

VIII Simpósio Brasileiro de Genética Molecular de PLANTAS

# Estresse Biótico - interação planta-micro-organismos

# MAPEAMENTO DE QTL E IDENTIFICAÇÃO DE SNP HAPLÓTIPOS QUE AFETAM A RESISTÊNCIA À PODRIDÃO-PARDA DE *THEOBROMA CACAO L.*

**Abstract:**
The cacao tree (*Theobroma cacao* L.) is globally an important crop due to its beans being used as raw material to produce chocolate. However, cacao production has been impacted by pest and diseases worldwide, amongst them the black pod disease caused by *Phytophthora spp*. Hence, to identify quantitative trait loci (QTL) associated with resistance to black pod disease, we performed a QTL mapping in a segregating mapping population comprising 459 trees of a cross between 'TSH 1188' and 'CCN 51'. A chip with 3,500 SNP markers and ten years of field data were also utilized. QTL peaks (LOD threshold of 3.1) were mapped from 3.13 to 3.28 Mb on chromosome IV, comprising a genomic region of 0.15 Mb. Based on the cacao Matina 1-6 genome, 292 transcript sequences of genes were reported within this genomic region. To identify candidate genes for resistance against black pod disease, we examined trees exhibiting recombination events for both parental haplotypes within the QTL region and between flanking SNP markers. Analysis of parental haplotypes revealed that the black pod disease resistance is inherited from the maternal haplotype ('TSH 1188'). We discovered recombination events for at least of the parental haplotypes occurring at five distinct genomic locations, but some recombination events occurred at the same spots. Near these recombinant events, we found important genes related to well-known defense mechanism processes during plant-pathogen interactions, such as recognition of pathogen effectors, hypersensitive response, programmed cell death, systemic resistance response, and phytohormone-dependent defense responses to pathogen infections. These genes are suggested to play a role disease resistance of cacao trees to the Black Pod Disease, and this knowledge can be used by breeding programs all over the world to facilitate marker-assisted selection.
**Key-words:** QTL ; SNP; *Theobroma cacao* L. ; *Phytophthora spp*.; haplotype

# DIVERSIDADE E MICROBIOMA FUNCIONAL EM FEIJOEIRO

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**Abstract:**
The plant microbiome is a complex ecology that serves vital purposes for its host. Recent extensive metagenomic research has shed light on its structure and potential functions. The impact of the microbial repertoire on plant growth and development begins to be unraveled. The relevant economic crop, the common bean (Phaseolus vulgaris), is the second largest legume crop worldwide. The lack of effective management against abiotic and biotic stresses seriously impacts its productivity, including the Fusarium wilt disease caused by Fusarium oxysporum f. sp. phaseolus (FOP). Here, we explored the high diversity of Amazon Dark Earth (ADE) soil to mine beneficial bean-interacting partners. With this goal, we evaluated the microbiota of ADE in two bean genotypes, IAC Millennium (resistant) and IAC Alvorada (susceptible), with different levels of FOP resistance. Our data pointed out that an ADE-enriched microbiome increases the biomass of both genotypes. In IAC Alvorada, ADE microbiota increased fresh weight by 28% in the aerial part and 44% in the root system. The dry mass increased by 40% (stem) and 54 % (roots). The length of the aerial part was 37%. For roots, the size and volume increased by 51 % and 54%, respectively. In comparison, the IAC Millennium genotype cultivated under ADE microbiota showed an increase of 20% (stem) and 41% (roots) in fresh. And 27% (stem) and 46% (roots) in dry mass. The length increased by 29 % (stem) and the root system by 23%. The optical emission spectrometry indicated that the two genotypes cultivated in the ADE microbiome assimilated more nutrients than the control, especially the micronutrients. In addition, the IAC Alvorada, when grown for 14 days in the presence of ADE microbiota, showed a slight increase in resistance to FOP. To further investigate the leading players of this community, we isolated 43 bacteria using three different cultivation media. The bacteria of the genera Pseudomonas, Stenotrophomonas, Paenibacillus, and Brevibacillus showed antagonistic activity to FOP in vitro. Currently, we are investigating the entire diverse picture of ADE microbiota using the 16S-sequencing approach. These findings will contribute to studies seeking a deeper understanding of plant-microbiome interactions.

**Key-words:** microbiome; Phaseolus vulgaris; Fusarium oxysporum f. sp. phaseolus; plant-microbiome interactions; 16S-sequencing

# GENE SERINA ENDOPEPTIDASE IDENTIFICADO EM ALGODÃO NO LOCUS DE RESISTÊNCIA À DOENÇA AZUL DO ALGODOEIRO É MODULADO DURANTE INFECÇÃO VIRAL.

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**Abstract:**

Cotton (*Gossypium sp*) is a widely cultivated plant, ranking as the most important fiber crop in the world. Cotton blue disease (CBD) is one of the most important diseases affecting cotton crops worldwide. The disease is transmitted by the aphid *Aphis gossypii* and caused by *cotton leafroll dwarf virus* (CLRDV). Belonging to the *Solemoviridae* family and *Polerovirus* genus, CLRDV presents non-enveloped icosahedral symmetry, with a single-stranded RNA positive genome and contains 7 open reading frames. We identified 2 ORFs among the molecular markers of CBD resistance (R) locus in the commercial cotton's genome *Gossypium hirsutum*. This work aims to functionally characterize one of these ORFs, named CBD1. CBD1 sequence showed that it is a serine endopeptidase. CBD1 protein sequence analysis shows a peculiar composition, rich in proline, serine and threonine, a fact that may explain its structural organization, showing an intrinsically disordered folding pattern. This set of constitutive characteristics would be able to demonstrate the protein ability to form liquid-liquid separation. Analyzing the amino acid sequence in a bioinformatics tool that predicts liquid-liquid separation capacity (LLPSDB v2.0), CBD1 is shown to be highly capable of forming such separation. In order to understand the role of GhCBD1 in CLRDV infection, quantitative real-time PCR reactions (qRT-PCR) were performed to evaluate the expression of these genes in CBD resistant (R) (Delta Opal and Cedro), moderately resistant (IMACD) and susceptible (S) (FM966 and CNPA ITA90) cotton varieties in leaves, stems and roots. In general, the IMACD cultivar showed lower levels of CBD1 expression in all the analyzed organs. Unexpectedly, FM966 and Delta Opal were the ones that showed the highest levels of expression of this gene in the three organs evaluated. In order to check if virus infection is modulating cbd1, its mRNA relative expression was evaluated in S and in R plants after CLRDV infection. Plant's samples were collected at four different points: 24 hours, 5 days, 15 days and 25 days after infection. Plants were also inoculated with aphid without virus. Cbd1 mRNA levels decreased strongly (100x) 24 hpi (hours post infection) after aphid contact and 1.000x after aphid+virus contact in the susceptible cultivar. After that, the expression starts to increase throughout the infection, peaking at 15 dpi (days post infection), where it is about 150 times more expressed than in the aviruliferous aphid. In R plants, a repression of 10x after inoculation of the aphid, and 100x after aphid+virus inoculation were observed. Curiously, in the resistant cultivar, there is a slight increase of cbd1 expression after 15 dpi, followed by a small repression at 25 dpi. Further studies are in progress to understand the role of this serine endopeptidase (CBD1) in CLRDV resistance.

**Key-words:** Gossypium; CLRDV; CBD1; Plant-pathogen interaction;

# LUTANDO CONTRA A HERBIVORIA: RESPOSTAS TRANSCRICIONAIS DE ALGODÃO (GOSSYPIUM HIRSUTUM) SOB INFESTAÇÃO PELO BICUDO DO ALGODOEIRO (ANTHONOMUS GRANDIS)

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**Abstract:**
Cotton (*Gossypium hirsutum*) plants have high economic importance, since these are the main source of fiber for the textile industry. However, pathogens and pests are a limiting factor of cotton development, causing severe losses in productivity of the plantation. The cotton boll weevil (CBW, *Anthonomus grandis*) is a chewing herbivore known for being one of the most harmful pests to attack cotton, due to its high reproductive capacity and difficulty of control. Several studies have been elucidating mechanisms of plant-insect interaction, but cotton responses when under herbivore attack still need more exploitation in order to elucidate the molecular mechanisms in which cotton responds to CBW attack, the global gene expression analysis of cotton floral buds infested by cotton boll weevil larvae during 2h and 12h was evaluated by mRNA-seq. Besides, a previous study of 48 h of cotton boll weevil infestation was also integrated, which allowed us to evaluate a time-course response of cotton floral buds to herbivore attack. The results obtained in our study will extend our knowledge on cotton-CBW interaction allowing to reveal the endogenous pathways of defense. For the transcriptomic analysis, floral buds of 6mm were drilled and had an egg of CBW inoculated. Control samples consisted of drilled cotton flower buds in which no egg was inoculated and both control and inoculated floral buds were collected after 2h and 12h for RNA extraction and sequencing. Over 631 million reads were generated using Hiseq 4000 Illumina sequencing of three biological replicates. The reads obtained were mapped to the *G. hirsutum* genome, allowing the accurate identification of differentially expressed genes (DEGs). CBW larvae feeding of floral buds triggered an intense transcriptome reprogramming, with 1656, 1698 and 4473 DEGs found at 2h, 12h and 48h post infestation, respectively. Enrichment analysis of genes ontologies of DEGs highlighted the modulation of processes related to plant defense and development after the recognition of CBW larvae, already in 2h of infestation and the response becomes more complex along the time-course of the infestation. Regarding transcription factors, a hyper-geometric test found the families ERF, NAC, GRAS and WORKY to be over-represented among DEGs. Our data also found the jasmonate pathway to be specifically modulated, with genes being up-regulated on the first time-point of analysis and down-regulated on later time-points. For defense compounds, the transcriptomic analysis revealed several DEGs related to secondary metabolites pathways, such as terpenoids, phenylpropanoids and polyamines, which contribute to the cotton plant defenses. In conclusion, we believe the greater comprehension of induced defenses in cotton plants will pave the way to the establishment of new biotechnological strategies, facilitating the achievement of new insect-resistant cultivars.
**Key-words:** cotton; biotechnology; biotic stress; RNA-seq;

# DEGS MARCADORES DO PROCESSO DE GERMINAÇÃO DE ESPORO DE *MONILIOPHTHORA RORERI*

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**Abstract:**
*Moniliophthora roreri*, is a phytopathogenic fungus that causes moniliasis in *Theobroma cacao* and severely threatens global almond production. In 2021 it was detected for the first time in Brazil, configuring a high risk for cocoa plantations. Since this pathosystem is important, the understanding of the genes involved in the development of the fungus is still incipient. Therefore, the objective of this study was to identify genes involved in *M. roreri* spore germination by comparing the transcriptional profile of four spore germination times. For this, the relative quantification of the number of transcripts available in *M. roreri* spore libraries produced in MiSeq at time 0, 08, 16 and 48h were analyzed. The TPM (*Transcript per million*) values were transformed into a Log2 scale and used in the PCA in the R 3.3.2 program. For the differential analysis of gene expression, the DESeq2 software package and edgeR in the R software were used, considering $p < 0.05$ and LogFC < 1 and -5 for Sp0x8, Sp0x16 and Sp0x48 comparisons. Heatmap was generated for the visualization of the profile of differentially expressed genes (DEGs) also in the R statistics software. The functional classification analysis was done based on the annotation of the DEGs from the gene ontology made in Revigo. In the PCA, components 1 and 2 explained 82.9% of the variation in the data, 57.1% and 25.8%, respectively, considering the total DEGs, which are capable of differentiating the germination times of the spores. Component 1 with greater variation allowed a high separation of the DEGs resulting in two clusters, one for spore 0H (in red) and one for spore 8, 16 and 48H (in blue). A total of 474 transcripts were considered differentially expressed throughout *M. roreri* spore germination. Of these 58% is down regulated and 42% up regulated, the differential expression profile was high in Sp0x16 compared to the initial (Sp0x8) and final (Sp0x48) times of germination. In the transcriptional profile revealed in the heatmap it is possible to identify marker genes that are crucial in germination times based on the expression pattern. In cluster 2 there are DEGs that are exclusively expressed in the spore only at the time of 8 hours (Sp0x8), in contrast at the time of 16 hours highly repressed DEGs were identified. The annotated DEGs were distributed into three functional classes: biological processes, cellular component and molecular function. All germination times showed DEGs in the three functional classes. With detach on the molecular function that grouped the largest number of transcripts regardless of germination time. Future analyzes will allow the characterization of the DEGs considered markers in the germination times of the *M. roreri* spore, which may confer greater efficiency in the contingency of the disease.
**Key-words:** Differential expression; *Theobroma cacao*; moniliasis; ;

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# CARACTERIZAÇÃO FUNCIONAL DE CANDIDATOS A EFETORES DE *MONILIOPHTHORA PERNICIOSA*

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**Abstract:**

Plants have a system to recognize microorganisms through plasma membrane receptors known as pattern-recognition receptors (PRRs). These receptors identify microbe-associated molecular patterns (MAMPs), which are conserved molecules among microorganisms. Recognition of MAMPs triggers a response called pattern-triggered immunity (PTI) that inhibits microbial colonization. However, most pathogens use effector molecules to suppress PTI and manipulate host physiology and immunity. Nucleotide-binding, leucine-rich-repeat (NLR) proteins recognize effectors, leading to effector-triggered immunity (ETI), often characterized by cell death. PTI and ETI are interconnected and share many components. While effectors have been studied in bacterial pathogens, their function in filamentous pathogens like *Moniliophthora perniciosa* is largely unknown. *M. perniciosa* is a major pathogen of cacao trees, causing significant losses in cocoa bean production. Through genome analysis, *M. perniciosa* was found to encode 17,008 proteins, including 247 candidate secreted effectors. Furthermore, 78 candidate effectors were identified and selected for functional characterization. Our goal is to identify the ability of these effectors to suppress the plant immune system. Initial experiments assessed the ability of candidates to suppress cell death, and none were able to do so when expressed intracellularly. Therefore, a plasmid for extracellular expression was constructed, and tools and protocols for quantifying species reactive oxygen during PTI activation are being established. Once characterized, the effectors will be subject to further studies.

**Key-words:** Plant immune system; Witche's Broom Disease; Cacao tree; *Moniliophthora perniciosa*; Effectors

# AVALIAÇÃO DE BACTÉRIAS ASSOCIADAS À SOJA QUANTO À CAPACIDADE DE INIBIR O CRESCIMENTO DE FUNGOS FITOPATOGÊNICOS

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**Abstract:**
Brazil is the largest producer and exporter of soybean (*Glycine max* (L.)) in the world. However, Brazilian crops are constantly threatened by multiple pathogens (mainly fungi), which limit production and cause billionaire losses to the country. Currently, disease control is achieved through chemical fungicides, which can be harmful to the environment and human health if used improperly. As an alternative, studies have been focusing on plant-pathogen-microbiome interactions in order to identify potential biocontrol agents in the microbiota naturally associated with plants. The objective of this work is to evaluate a collection of bacteria associated with soybean (*Soybiome*) in terms of their ability to inhibit, *in vitro*, the most important pathogenic fungi of this crop. Specifically, the collection is being evaluated for its ability to inhibit the germination of urediniospores of the fungus *Phakopsora pachyrhizi* and the mycelial growth of *Fusarium tucumaniae*, *Colletotrichum truncatum*, *Macrophomina phaseolina*, *Sclerotinia sclerotiorum* and *Corynespora cassiicola*. So far, 92 bacteria have been tested for their ability to inhibit the germination of *Phakopsora pachyrhizi* spores. Of these, 16 isolates showed inhibitory activity, causing, on average, a reduction of 71% in the rate of spore germination. In addition, we identified 14 other bacteria that, despite not preventing germination, delayed the development of the spore germ tube. In parallel, 195 bacteria were evaluated for their ability to inhibit the growth of five other phytopathogenic fungi in Petri dishes, including all 92 isolates tested against *P. pachyrhizi*. In these assays, 7 bacteria inhibited the growth of all 5 fungi. We are currently performing the taxonomic characterization of the *Soybiome* collection through the sequencing the 16S gene in order to identify the isolates. The results obtained so far show the importance of the *Soybiome* collection as a valuable reservoir to search for bacteria of agricultural interest. In addition to evaluating a greater number of isolates for their ability to antagonize pathogens *in vitro*, we will perform *in planta* experiments in the next stages of the project. We expect to identify a set of bacteria that can be used as biocontrol agents against diseases of one of the most important crops for the Brazilian agriculture.
**Key-words:** Biocontrol; Microbiome; Plant-pathogen-host; ;

# EDIÇÃO MEDIADA POR CRISPR/CAS DO GENE *PP2B12* VISANDO O DESENVOLVIMENTO DE NOVA VARIEDADE DE *CITRUS SINENSIS* VAR. HAMLIN TOLERANTE AO HLB

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**Abstract:**
Huanglongbing (HLB) is the main disease of citrus species worldwide and is caused by *Candidatus* Liberibacter asiaticus (*C*Las) bacteria. Since all sweet orange (*Citrus sinensis*) varieties are susceptible to HLB, the development of novel tolerant commercial varieties becomes imperative. Based on the feasibility of triggering mutations and silencing target genes by using CRISPR technology, it makes possible to modulate physiological response against HLB in citrus species by targeting susceptibility genes as a promising approach. Transcriptome analyses comparing HLB-susceptible, tolerant and resistant citrus genotypes were previously performed and identified genes that may not only help to explain correlations with occurrence of HLB symptoms, but also serve as promising candidates for gene editing. Among them, the *PP2* (*Phloem Protein 2*) gene family stands out and encode conserved phloem lectins in plants. *PP2* genes act as a defense mechanism against pathogens, leading to the cell wall strengthening and blocking of sieve elements in phloem. Hence, during *C*Las infection, PP2 proteins block transport of photoassimilates to different plant organs, leading to symptoms and possibly death of tissues and even whole trees. Thus, in this work we aimed to apply CRISPR technology to genome editing of sweet orange varieties, in order to regenerate HLB-tolerant plants. In this way, Hamlin variety was transformed with a plasmid-based CRISPR/Cas9 system by using *Agrobacterium tumefaciens* EHA105 harboring apDIRECT_22C vector containing a two-sgRNA cassette targeted to the *pp2B12* gene. Target host sites were first genotyped through Sanger sequencing, then the gene construct was delivered to epicotyls for genetic transformation. A total of 3641 explants was submitted to transformation and, in total, 20 PCR-positive shoots for the genes of interest were subsequently micrografted onto Carrizo rootstock. Our next steps will be to characterize indels in candidate edited plants, as well as cloning the edited ones and perform infection experiments with *C*Las. Therefore, this workpaves the way for expanding the knowledge of mechanisms involved in the response against HLB and has a great potential to establish strategies for tolerance acquisition in susceptible genotypes through modern biotechnological tools.
**Key-words:** CRISPR/Cas9; genome editing; Huanglongbing; phloem protein 2; sweet orange

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# INTERAÇÃO ENTRE ARGINIL T TRANSFERASE (ATE) DO ALGODÃO E PROTEÍNAS VIRAIS DO CLRDV PODEM SER A CHAVE DE UM NOVO MECANISMO DE RESISTÊNCIA DA PLANTA CONTRA O POLEROVIRUS

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**Abstract:**
ATE (Arginyl t-RNA transferase) is a component of the N-degron pathway that controls the half-life of proteins by targeting them to degradation via the Ubiquitin Proteasome System. In the Arg/degron branch, the N-terminal tertiary destabilizing residues (Cys, Asp, and Glu) can be converted into secondary destabilizing residues (oxidated-Cys, Asn, Gln) by the enzymes Plant Cysteine oxidase (PCO), Nt Asparagine amidase (NTAN), and Nt Glutamine amidase (NTAQ), respectively. The cascade continues with ATE, which adds an Arg to these secondary destabilizing residues. The arginylation is recognized by the E3 ligases Proteolysis 1 (PRT1) or Proteolysis 6 (PRT6). Previous results indicate that high levels of ATE mRNA impair cotton leafroll dwarf virus (CLRDV, *Polerovirus*, *Solemoviridae* family) replication and spread in cotton. So, we speculated that ATE might be involved in the degradation of some viral proteins. *Nicotiana benthamiana* leaves were co-infiltrated with agrobacteria vectors harboring the CLRDV P0 (silencing suppression protein), P3 (capsid protein), or P4 (short distance movement protein), and cotton ATE (*Gh*-ATE). Then, the protein levels were analyzed by western blot. This experiment showed that all three CLRDV proteins were drastically reduced in the presence of cotton ATE. To confirm that ATE-induced protein degradation was not a general process triggered by high levels of ATE, we evaluated ATE substrate specificity using agroinfiltration assays. Combinations of vectors expressing ATE, GFP, or GUS were agroinfiltrated in three true leaves of *N. benthamiana* plants. GFP and GUS proteins are known to be stable proteins in plants. Five days after co-agroinfiltration of *N. benthamiana* leaves with constructs ATE-HA + GFP + p19, ATE-HA + GUS + p19, or ATE-HA + p19 + empty vector, total protein was extracted and analyzed by western blot and luminescence assays. An anti-HA antibody (1: 5000) was used for the western blot to detect ATE, and an anti-GFP antibody (Invitrogen; dilution 1: 2000) to detect GFP. Li-Cor Odyssey 1: 10000 (IRDye 800CW Goat anti-Rabbit, green) was used as the secondary antibody. The results showed that ATE does not degrade GFP. Luminescence analysis of GUS also showed that the co-infiltration of GUS with ATE does not affect GUS levels. Analysis of co-immunoprecipitation and BiFC are in progress to confirm the direct interaction between ATE and the putative target viral proteins. Our results indicated that degradation of CLRDV P0, P3, and P4 protein induced by *Gh*-ATE might confer the resistance phenotype to CLRDV infection observed in certain cotton varieties.
**Key-words:** ATE; Arg/degron; *CLRDV*; *Gossypium hirsutum*;

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CAPES, FAPERJ

# TRANSFERÊNCIA DE SISTEMAS CRISPR/CAS BASEADOS EM PLASMÍDEOS PARA INTERRUPÇÃO DO GENE CALOSE SINTASE 7 (CSCALS7) VISANDO O DESENVOLVIMENTO DE *CITRUS SINENSIS* TOLERANTE AO HLB

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**Abstract:**
Huanglongbing (HLB) is the most devastating citrus disease worldwide, leading to significant economic losses in citrus industry. HLB is mainly caused by *Candidatus* Liberibacter asiaticus (*C*Las), gram-negative bacterium able to proliferate in the plant phloem. Previously, expression of some genes was reported to be related to callose deposition within phloem, which leads to HLB symptoms. Thus, performing CRISPR-based gene editing may be a promising alternative in the development of HLB-tolerant plants, by aiming to disrupt susceptibility genes. There are no HLB-resistant or tolerant varieties of sweet orange (*Citrus sinensis*), which makes urgent the development of innovative approach to control this disease. Therefore, our goal is to transform the commercial varieties of *C. sinensis* (Hamlin and Valencia), using Agrobacterium-mediated transfer of CRISPR plasmids in citrus cells. This transformation will allow us to silence the HLB susceptibility genes and develop robust screening protocols for both transformation and gene-edited shoots. In this way, transformation experiments were carried out using pDIRECT_22C vector harboring a multiplex cassette for 3 sgRNAs targeting callose synthase 7 gene (*CsCalS7*). EHA105 strain of *Agrobacterium tumefaciens* was used to transform epicotyls whose shoots were screened for transformation through duplex PCR for Cas9 and sgRNA cassettes. In a total of 5019 epicotyls, the regeneration rate was 3.21% and 6.97% for Hamlin and Valencia's explants, respectively. Regarding transformation efficiency, it was 0.082% and 2.68% for Hamlin and Valencia, respectively. Although all shoots are screened based on duplex PCR, it was found that some of them are partially recombinant, harboring an integrated cassette for the sgRNAs but absent of Cas9 cassette. Considering that both components of the CRISPR system are mandatory for functionality of gene editing, partially recombinant shoots are eliminated. Hence, we can conclude that a suitable and robust screening of transgenic shoots is essential and must be incorporated in protocols, making possible to better select gene-edited plants. Based on this, double-positive buds will be used for grafting onto Rangpur lime rootstocks and the events obtained will be challenged with *C*Las and morphologically evaluated for callose deposition and HLB symptoms.
**Key-words:** callose; CRISPR/Cas; genetic transformation; Huanglongbing; sweet orange

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# BACTÉRIAS METILOTRÓFICAS FACULTATIVAS DE PIGMENTAÇÃO RÓSEA COMO PROMOTORAS DE CRESCIMENTO DE PLANTAS E INDUTORAS DE CRESCIMENTO

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**Abstract:**
Biological products based on microorganisms have emerged as viable and sustainable alternative to agrochemicals. Pink-pigmented facultative methylotrophic bacteria (PPFMs) are largely associated with roots, leaves and seeds of most terrestrial plants and utilize volatile C(1) compounds such as methanol generated by growing plants during cell division as sole source of carbon and energy. PPFMs have been well-studied in agricultural systems due to their importance in crop seed germination, yield, pathogen resistance and environmental stress tolerance. These methylotrophic bacteria can benefit plants in different ways, for example by producing phytohormones or even acting as biofertilizers by providing nutrients such as nitrogen and phosphorus. In this work, we selected four isolates and two consortia of methylotrophic bacteria previously screened by multifunctional properties (nitrogen fixing, phosphate solubilization, auxin and siderophores production, and ACC deaminase activity). These selected bacteria strains were cultivated in liquid CHOI-3 medium with methanol as sole carbon and energy to treat seed of soybean (*Glycine max*, Zeus cultivar) aiming to promote plant growth and/or induce resistance against phytopathogens. The methylotrophic bacteria strains explored in this work were able to positively affect parameters associated with plant growth, including seed germination, increase in stem diameter and root and shoot elongation. The PPFM bacteria treatments also increased almost all the available macro and micronutrients in the soybean leaves 60 days after inoculation, suggesting a strong biofertilizer effect in the soybean plants at greenhouse conditions. Furthemore, some methylotrophic bacteria isolates reduced symptoms of sclerotinia stem rot (white mold) caused by *Sclerotinia sclerotiorum*, up-regulating salicylic and jasmonate/ethylene marker genes evaluated by qPCR analysis. Our results demonstrated the great potential of the PPFM bacteria to be explored in the development of biopesticide and biostimulant products to increase crop growth and yield in a sustainable manner.
**Key-words:** biopesticide; biostimulant; biofertilizer; biotechnology;

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# INOCULAÇÃO BACTERIANA DE PLANTAS DE ARROZ COM OBJETIVO DE AUMENTAR A PRODUTIVIDADE DE GRÃOS

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**Abstract:**
Rice (Oryza sativa L.) grains are an excellent source of carbohydrates, being the main food for approximately half of the world's population. Brazil is the ninth largest rice producer in the world, and Rio Grande do Sul state accounts for 70% of total production. To sustain world population growth, an increase in food production without extending agricultural boundaries is necessary. New strategies aiming to develop sustainable practices of agriculture have been developed, aiming to improve grain production. Bacterial inoculation techniques are a sustainable biotechnological approach that can improve grain quality, plant productivity, and resistance to biotic/abiotic stresses. Thus, this work aimed to verify whether inoculation of two new Plant Growth Promoting Rhizobacteria (PGPR) increases rice grain yield. With this purpose, Bacillus sp. S26, Burkholderia sp. CIR3, and a consortium (S26+CIR3) were inoculated in rice seeds during germination. The seedlings were grown in Carolina soil substrate and maintained in greenhouse conditions. New inoculations were performed at tillering and heading growth stages. After 150 days of plant emergence, the panicles were harvested and seed number/weight per plant, percentage of full seeds, weight of 1,000 seeds, plant height, and tiller number were evaluated. The weight of 1,000 seeds was higher in treatments inoculated with CIR3 and S26+CIR3 compared with non-inoculated control. Principal component analysis performed with agronomic data of inoculated and non-inoculated treatments explained 74.5% of the total variability. This analysis showed that plants inoculated with CIR3 and S26+CIR3 were related to the percentage of full seeds and weight of 1,000 seeds, while plants inoculated with S26 were related to seed number/weight per plant, plant length, and tiller number. These data show that rice inoculation with bacterial isolates CIR3 and CIR3+S26 can improve grain yield of rice plants, being an interesting biotechnological tool for the development of sustainable agriculture.
**Key-words:** Sustainable; Agriculture; Biotechnology; Plant Growth Promoting Rhizobacteria;

# SILENCIAMENTO DE GENES DE SUSCEPTIBILIDADE GERA PLANTAS RESISTENTES A BEGOMOVÍRUS

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**Abstract:**
Begomovirus is a genus of very well-adapted and emerging plant viruses. Vectored by whitefly, the begomoviruses cause enormous damage to various crops such as tomatoes, cassava, peppers, cotton, and beans. The virus' predisposition to recombine its genomes, mutation events and the broad coverage of its vectors result in an increase in its diversity and, therefore, its evolutionary success. Host gene mutations, focusing on recessive susceptibility genes, are an exciting tool to monitor and delay viral adaptation. Therefore, this work aimed to verify whether the silencing of two susceptibility genes can generate plants resistant to begomovirus. We used seeds from the *Arabidopsis thaliana* Columbia (Col-0) model plant and susceptibility gene knockouts generated by T-DNA from ABRC. The knockouts were confirmed by PCR and RT-qPCR. The plants were kept in a growth chamber at 22 ºC, in two photoperiods (long days - 16 h of light and short days - 10 h of light) and evaluated during 56 days after germination. Ten plants from each genotype (Col-0, susceptibility gene knockout 100) were infected by the cabbage leaf curl virus (CabLCV) through biolistics and 5 plants were used as controls. Every 7 days for 28 days, the infection development was monitored by a visual analysis of symptoms and PCR-based diagnosis. In parallel, the rosette diameter and the inflorescence length were measured. Also the number of leaves from 24 plants of each genotype (Col-0, susceptibility gene knockout 100, susceptibility gene knockout 200) was counted to assess plant development and growth. RT-qPCR analysis showed a higher viral load in control plants than in the knockouts. Analyzes of rosette diameter, number of leaves and inflorescence length showed no significant differences between knockouts and control, only differing between photoperiods. Under long days, it was possible to observe a more significant number of leaves and a greater rosette diameter, as well as the floral tassel up came 7 days earlier than in plants incubated under short days. As knock outing these genes can confer resistance to begomoviruses, they may be potential targets for engineering recessive resistance. Furthermore, silencing these susceptibility genes did not cause significant adverse effects on growth, development, and productivity in *Arabidopsis*, an intrinsic property of recessive resistant genes.
**Key-words:** Recessive resistance; Susceptibility genes; Begomovirus; Resistance to infection; CabLCV

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# MILHO E *HERBASPIRILLUM*: ANÁLISE DE TRANSCRIPTOMA REVELA COMO A DISPONIBILIDADE DE NITROGÊNIO PODE INFLUENCIAR ESSA INTERAÇÃO

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**Abstract:**
Plant growth-promoting bacteria, such as *Herbaspirillum seropedicae*, have been recommended as commercial inoculants for several crops, including maize. Studies show that inoculation, associated with a dose of nitrogen (N) with the *H. seropedicae*, increased maize productivity and in the absence of fertilizer this increase was lower. The main goal of our study is to understand how different doses of nitrogen can alter the metabolism of maize and diazotrophic bacteria at molecular levels. Here, we deployed RNA-seq to determine the global gene expression of *H. seropedicae* ZAE94 associated with maize roots grown with different doses of N (0.3 and 3.0 mM, denominated N- and N+, respectively). Our results provide an overview of differentially expressed genes (DEGs) in both maize and ZAE94 associated in response to different N availability, revealing new insights into pathways involved in grass-diazotrophic bacteria association. mRNA transcriptomes of maize seedlings and ZAE94 were characterized eighteen days after inoculation. The transcriptome profile revealed a total of 1061, 511, 1040 and 598 DEGs in maize in the following analyzed comparisons: Control N+ vs Control N-, Inoculated N+ vs Inoculated N-, Inoculated N+ vs Control N+ and Inoculated N- vs Control N-, respectively. While for the bacteria, 57, 482 and 111 DEGs were found for the following analyzed comparisons: Inoculated N+ vs Inoculated N-, Inoculated N+ vs Control N+, Inoculated N- vs Control N-, respectively. Our results support the modulation of maize nitrogen metabolism, phytohormones, cell wall and defense responses in plants inoculated with ZAE94 and grown at different N doses. Analysis of qRT-PCR revealed that genes of nitrogen metabolism are induced in Inoculated N- vs Control N-, validating the transcriptome result. These genes can be considered a marker for diazotrophic bacteria inoculation. In addition, a modulation in the metabolism of the bacteria can be observed by transcriptome analysis regulating genes involved in transport, secretion system, cell mobility, oxireductases, chemotaxis when associated with maize roots and cultivated at different doses of N. By qRT-PCR we can observe induction of bacterial genes involved in nitrogen metabolism in plants inoculated at both N+ and N-. Therefore, we next addressed if inoculation of diazotrophic bacteria combined with different doses of nitrogen fertilizers can improve nitrogen metabolism in maize. Then, we performed another experiment using three doses of N (0.3, 1.5 and 3 mM). Our result showed that at concentrations of 1.5 and 3.0 mM the inoculation promoted an increase in the root surface, which we can associate with a better uptake of the nutrients present in the nutrient solution. This study provides a valuable contribution to how different nitrogen doses can alter the metabolism of maize and diazotrophic bacteria. A better understanding of this could in turn contribute to more sustainable agriculture practices.
**Key-words:** diazotrophic bacteria ; gene regulation; nitrogen metabolism ; plant-bacteria interaction;

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# LEPROSE DOS CITROS: UM ESTUDO DOS FATORES DA PLANTA E DO VÍRUS ENVOLVIDOS NA INTERAÇÃO PATÓGENO-HOSPEDEIRO

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**Abstract:**
Citrus leprosis virus C (CiLV-C, genus *Cilevirus*, fam. *Kitaviridae*) causes citrus leprosis, a disease endemic in the Americas. Economically, citrus leprosis is the most important viral disease affecting citrus orchards in Brazil, costing millions of dollars per year for the chemical control of the viral vector, the mite *Brevipalpus yothersi*. Scientifically, the virus draws attention due to its atypical inability to spread systemically in any of its known plant hosts, remaining restricted to local lesions around the feeding sites of the vector. We have dissected molecular mechanisms involved in plant/CiLV-C interplay using transcriptomic and histochemical analyses. The host response to the viral infection is spearheaded by the activation of the plant immune system involving RNA silencing and salicylic acid (SA)-mediated pathways, reactive oxygen species (ROS) burst, and cell death, including the upregulation of genes involved in the hypersensitive response (HR). Moreover, the ectopic expression of CiLV-C proteins unveiled that P61, a putative glycoprotein of cileviruses, produces an HR-like and mimics the primary responses observed during plant/CiLV-C interaction, placing P61 in the epicenter of the processes leading to the HR-like symptoms associated with the virus infection. Studies on the subcellular localization of CiLV-C proteins showed that P61 accumulates in the ER lumen, likely causing its disruption. Disturbance of the ER by P61 raised the hypothesis that P61 induces an unmitigated ER stress which, in turn, triggers the HR-like. Upon ER stress, plant cells induce unfolded protein response (UPR) to restore the ER homeostasis; instead, the inability to revert the ER stress may lead to plant cell death, a process interconnected with the SA pathway. In this study, we have confirmed the occurrence of ER stress and the upregulation of UPR marker genes after the transient expression of P61. Accordingly, expression of a truncated version of P61, lacking its signal peptide, drastically reduced both the cell death phenotype and transcript levels of HR and ER marker genes, suggesting that P61 entry into the ER is essential for triggering the HR-like response. Conversely, the expression of P61 in *nahG* transgenic plants caused an enhanced cell death phenotype and stronger induction of HR and UPR marker genes. The spliced form of the bZIP60 mRNA, which characterizes the activation of the UPR pathway during plant virus infection, was also detected in P61-expressing plants. Altogether, our findings support the involvement of ER stress in the development of the HR-like in presence of P61 and, hypothetically, in the symptoms caused by the CiLV-C infection.
**Key-words:** plant-virus interaction; *Cilevirus*; hypersensitive response; endoplasmic reticulum stress; pathogen effectors

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# AVALIAÇÃO FUNCIONAL DO MICROBIOMA SOJA

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**Abstract:**

Brazil is the largest producer and exporter of soybeans, a grain that is widely consumed everywhere in the world. Conventional cultivation methods are largely dependent on chemical fertilizers and defensives that increase production costs and often harm the environment. In this regard, the large diversity of microorganisms that live in association with plant tissues (microbiomes) constitute promising alternatives for the identification and development of novel plant growth promotion strategies. These microorganisms can often synthesize plant hormones, promote nutrient absorption, and mitigate biotic and abiotic stresses. This study aims to evaluate a large collection of soybean-associated bacteria (denominated *Soybiome*) for their ability to promote plant growth through the production of indole compounds, solubilization of inorganic phosphate and calcite, and degradation of ACC (1-aminocyclopropane-1-carboxylate), the precursor of the plant stress hormone ethylene. So far, 282 bacterial isolates have been tested, resulting in the identification of 30 strains that solubilize phosphate, 30 that solubilize calcite, 33 that degrade ACC, and 26 that produce high amounts of indole compounds. Several strains have tested positively in more than one assay. The most promising strains will be selected for in planta assays. Our results suggest a significant potential for these bacteria to act as biofertilizers to promote soybean growth.

**Key-words:** Biofertilizantes; Microbioma; Soja; ;

# CARACTERIZAÇÃO DE PROMOTOR INDUTÍVEL E TECIDO-ESPECÍFICO PARA CONTROLE DE PRAGA EM ALGODÃO (*GOSSYPIUM HIRSUTUM*)

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**Abstract:**
The cotton plant (*Gossypium hirsutum*) is a plant species cultivated for the production of fibers and seed oil, amounting to more than 90% of the total fiber production, Brazil is the fourth largest cotton producer. However, there's still space for productivity growth following genetic engineering strategies aiming for greater pest resistance. Cotton crops are substantially affected by the cotton boll-weevil (*Anthonomus grandis*, CBW) confirming the importance of the development of new biotechnological tools as pesticides are less effective against CBW infestation and if not successfully dealt with, the infestation could result in up to 75% loss on fiber production. Therefore, our goal is to improve the Bt stategy into a more specific tool against CBW by combining the toxic gene to a promoter that's significantly more reactive to insect herbivory in the tissues that the beetle damages, making the molecular response inducible upon herbivory and tissue-specific, resulting in less energy consumption by the plant and decreasing the risk of effect in non-target organisms. In order to select candidate promoters with these characteristics, a transcriptomic analysis was performed in cotton plants at 2 and 12 hours post inoculation with CBW eggs. After normalization, Log2FC values were obtained and an ACO1 (aminocyclopropane-1-carboxylate oxidase) gene that showed high up-regulation on both time-points was chosen for further analysis.After selection, quantitative analysis was performed on the *ACO1* gene expression. qPCR analysis confirmed that *ACO1* is upregulated after 2h, 12h and 48h of CBW infestation. Stamens and carpels present the more elevated expression levels of *ACO1*, the organs that are the main target of boll weevil larvae. It was also observed a high expression of *ACO1* in cotton flower buds sizing 2mm and 10mm in diameter. The *ACO1 gene is also* expressed in fruits at 7 and 15 days post anthesis. After that, I*n silico* analysis of *ACO1* 2Kb long promoter was performed using the Jaspar database and FIMO algorithm on the platform MEME-Suite. On the promoter, were identified 5 cis elements associated with biotic stress, and 17 other cis elements that were specific to pollen. Fragments of 700bp, 1Kb and 2Kb were selected for isolation based on the in silico analysis. All three fragments were successfully amplified from cotton genomic DNA and the 700bp fragment was cloned. Promoter fragments will be fused to GUS/GFP and used for transformation in *A. thaliana* plants. The promoter activity will be observed through *uidA* (GUS) reporter gene assays . If their inducible activity using CBW elicitors are confirmed, constructs will be also used for stable transformation of cotton plants.

**Key-words:** *Gossypium hirsutum*; *Anthonomus grandis*; Promoters; Inducible promoters; Cotton Boll-Weevil

# MODULAÇÃO DA EXPRESSÃO DE GENES DE PLANTAS PODE MELHORAR A RESPOSTA A BIOINOCULANTES COM BACTÉRIAS BENÉFICAS

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**Abstract:**
World agriculture has been facing extreme climate changes, which have a major impact on plant productivity, especially food. Water scarcity, extreme changes in temperatures and soils deficient in nutrients are some of the main factors that reduce the productivity of plant species of economic interest. The use of bio-inoculants, composed of bacteria that promote plant growth, are becoming an alternative way to minimize the impacts generated by chemical fertilizers, but the benefits of plant-bacteria interactions may be limited, since they depend on several factors such as plant species and associative bacterial species. Plant breeding and the implementation of new precision gene editing technologies are important tools to produce cultivars with greater tolerance to environmental stimuli (biotic and abiotic). Our research group has identified several genetic networks involved in the establishment of a beneficial plant association with diazotrophic bacteria. We have shown that at early steps of the interaction, plants need to sense the bacteria as beneficial and the modulation of expression of several plant receptors is involved. At the end, the final outcome of plant growth promotion involves genes related to growth, such as the ones regulating plant cell division. In this context, the goal of this work is to study the function of two plant genes - DESC2 and LRR5.4, involved in the modulation of cell divisions in responses to the environment and in the signaling of the plant interaction with beneficial bacteria, respectively. Previous studies performed in these pathways have shown that the DESC2 protein, which acts as a negative regulator of cell division, and the LRR5.4 gene, a specific receptor for diazotrophic bacteria, must be repressed to obtain an increase in plant biomass and establish a better association with bioinoculants. Our results in *Arabidopsis thaliana* plants with repression of DESC2 and LRR5.4 genes showed a better association with bioinoculants and an increase in root and rosette biomass with a gain of 34% compared to wild type plants. Furthermore, the DESC2 mutant plants inoculated showed an increase in seed productivity. On the other hand, DESC2 and LRR5.4 mutant plants inoculated showed a greater number of associated bacteria, suggesting that the repression of these genes is important for a beneficial association with bioinoculants. These results can provide new tools for increasing the efficiency of the use of bioinoculants and consequent reduction in the use of chemical fertilizers. These new tools will be able to be applied in the country's agricultural crops, directing them towards the sustainability of world agricultural practices.
**Key-words:** Association; Beneficial bacteria; LRR receptor; Cell cycle; Plant growth

## Acknowledgement

INCT, CNPq, FAPERJ and CAPES

# AUMENTO DA PRODUTIVIDADE EM PLANTAS DE *PASSIFLORA EDULIS* INFECTADAS PELO COWPEA APHID-BORNE MOSAIC VIRUS (CABMV) EM CAMPO PELO TRATAMENTO COM UMA PEPTIDGALCTOMANANA DE FUNGO

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**Abstract:**

Viral diseases are a major threat to sustainable and productive agriculture around the world, leading to economic crop losses. Brazil is the main producer of passion fruit, and *Passiflora edulis* represents the most abundant species. The passion fruit crop is severely affected by woodiness disease caused by cowpea aphid-borne mosaic virus (CABMV). The virus is present in all Brazilian growing areas and causes deformation of the fruits and leaves associated with blister and foliar mosaic symptoms in passion fruit plants. Infected plants show a marked development reduction, and their productivity is drastically reduced (approximately 60%). In this study, we evaluated the damage caused by this pathogen at the molecular level and observed that the expression of different defense-related genes was repressed in infected plants. Genes such as *pathogenesis-related protein 3* (*PR-3*), *phenylalanine ammonia-lyase* (*PAL*) and *lipoxygenase 2* (*LOX2*) and some involved in phytohormone pathways, such as *auxin-responsive protein SAUR20* and *gibberellin 2-beta dioxygenase 2,* were downregulated by infection. The management of woodiness disease is faulty, as vectors are resistant to most of the available insecticides. As a sustainable alternative for the management of this disease, we evaluated the effect in the field of the treatment of passion fruit plants with the bioestimulant Hariman (peptidogalactomanann from *Cladosporium herbarum*) before virus infection. Aerial parts of young plants, just before field planting, were treated by Hariman pulverization at 100 µg.ml$^{-1}$. After pulverization, all the plants were infected mechanically with CABMV and transferred to the field. Treatment led to a significant increase in the expression levels of all the genes mentioned above in the infected plants. This was associated with a reduction in virus accumulation and an important mitigation of CABMV symptoms. Infected treated plants showed an increase in height, number of leaves, number of flowers and fruits. At harvest, eighteen months after planting, an increase of 80% of yields in ton/h was observed in treated plants compared to water-treated controls. Our data show that Hariman treatment allows plants to respond efficiently against CABMV infection, mitigating symptoms and blocking yield losses associated with the disease.

**Key-words:** CABMV; virus resistance; peptidogalactomannan; *Cladosporium herbarum*; phenylalanine ammonia-lyase

## Acknowledgement

CAPES, CNPq, UFRJ and FAPERJ.

# AVALIAÇÃO DO MICROBIOMA DA SOJA NA BUSCA POR MICRORGANISMOS SUPRESSORES DE DOENÇAS

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**Abstract:**
Plant-associated microorganisms can exert beneficial effects on the host, offering possibilities for the improvement of agronomical traits in crops through the manipulation of their microbiomes. Among these traits, resistance to pathogens is of major relevance for agriculture. In this work, we investigated how infection of soybean with Asian Soybean Rust (ASR) affects the leaf and root/rhizosphere microbiome. We hypothesize that leaves infected with ASR present an altered bacterial community that is enriched in strains with the ability to antagonize the fungus *Phakopsora pachyrhizi*, the causal agent of ASR. We also hypothesize that soybean plants exposed to aboveground infections can modulate the rhizosphere microbiota and recruit beneficial microbes via root exudates. To test these hypotheses, we evaluated the bacterial communities that colonize healthy and infected field-grown soybean leaves and roots via high-throughput sequencing of the 16S ribosomal gene. We found remarkable differences in the bacterial populations inhabiting these leaves and identified taxa that show either higher or lower relative abundance in infected leaves. These results confirm that ASR affects the soybean leaf microbiome. In the roots/rhizosphere, however, we did not detect significant differences between healthy and infected plants. We also isolated bacteria from soybean leaves and roots to assemble a culture collection of 'soybean-associated bacteria'. This collection (named *Soybiome*) currently consists of 3,038 strains and constitutes a valuable resource for the identification of microorganisms that can exert any kind of beneficial effects on plants. Indeed, initial screenings have revealed strains that can antagonize a set of 6 soybean pathogens, including *Phakopsora pachyrhizi*. The characterization of such strains will pave the way for the development of biofungicides effective against one of the most important plant diseases in soybeans plants.
**Key-words:** phyllosphere; rhizosphere; microbiota; 16S sequencing;

# CONTROLE DE *MELOIDOGYNE INCOGNITA* EM ALGODOEIRO MEDIADO POR UMA GLICOPROTEÍNA DE *CLADOSPORIUM HERBARUM*

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**Abstract:**
The root-knot nematode (RKN) *Meloidogyne incognita* has become a serious threat to cotton production. The use of biological control agents that elicit plant defense mechanism has been considered a promising durable biocontrol strategy. A glycoprotein (Hariman) isolated from *Cladosporium herbarium* mycelium has demonstrated antiviral activity against TMV and CABMV by inducing systemic acquired resistance in tobacco and passion fruit, respectively. We investigated the RKN reproduction after the treatment of cotton leaves with pGM under greenhouse conditions. Twenty-two days after germination (dag) 20 cotton young plants were sprayed with 100 ug.ml$^{-1}$ pGM and the treatment was repeated each 15 days until day 45. As control, 20 plants were sprayed with water at the same time points. All the plants were inoculated with 3.000 eggs of *M. incognita* at 25 dag. The evaluation of nematode reproduction (eggs$^{-1g}$ of roots) at 90 days after inoculation (dai) showed a reduction of 60% in eggs/ g of root. According to the gall index, 80% of the pGM treated plants showed highly resistance phenotypes (score 1), while all control water-treated plants remained highly susceptible (score 5). The yield parameters did not differ among treatments. RT qPCR assays showed that the transcription of pathogen-related 1 (PR-1) was 10 fold induced at 45 days after pGM treatment. Analysis of other defense related genes are in progress to try to understand which defense pathways maybe be associated the significant reduction in nematode reproduction induced by pGM treatment. This is the first report to describe the nematicidal activity of pGM from *C. herbarum* against *M. incognita* in cotton plants. The use of this biostimulant to the control of nematodes in cotton crop may represents an important alternative tool for sustainable cotton agrotech.
**Key-words:** Peptidogalactomannan; fungal glycoprotein; root-knot nematode; *Gossypium hirsutum*; defence-related genes

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INCT Planta PragaEmbrapa Recursos Genéticos e Biotecnologia - Cenargen Universidade Federal do Rio de Janeiro - UFRJ Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - CAPES

# ANÁLISES DE ESTRUTURA E INDUÇÃO DA FAMÍLIA DE GENES MLO DE SOJA SOB INFECÇÃO POR *PHAKOPSORA PACHYRHIZI*

**Matheus Mertz Ribeiro [1]; Adriana Brombini dos Santos [3]; Liliane Santana Oliveira [1]; Valeria Y. Abe [1]; Fernanda M. Castanho [1]; Ricardo V. Abdelnoor [2]; Francismar Corrêa Marcelino-guimarães [2]**

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**Abstract:**
INTRODUCTION: The MLO (*Mildew Locus* O) gene family act as susceptibility gene (S-gene) towards fungi causing the *powdery mildew* (PM) disease. Inactivation of these genes through loss-of function mutations or silencing results in resistance in several plant species. The members of this family are characterized by the presence of seven transmembrane domains integral to the plasma membrane with an extracellular N-terminus and an intracellular C-terminus. In addition, S genes are characterized as responsible for encoding proteins that contribute to a compatible interaction between the pathogen and the host, enabling infection and disease development. Our objective is to characterize the soybean MLO gene family and identify its members that are induced during *P. pachyrhizi* infection in soybean using transcriptomics.MATERIALS AND METHODS: *In silico* identification of the MLO protein members were conducted on the Phytozome.v13 database, using the BLAST tool and the W82.v1.a4 reference genome. Each gene was characterized based on total length, chromosome localization, strand, START and STOP positions, as well as number and size of exons and introns. The catalytic domains, alignment of sequences and phylogenetic tree were performed by Pfam, MUSCLE and IQ-TREE software, respectively. In the RNAseq experiment, we utilized two near-isogenic lines of *Glycine max*, namely BRS 184 and BRS 184 carrying the Rpp5 gene from the plant introduction Kinoshita were used. The experiment was installed in a completely randomized design with at least three biological replicates. The plants were divided into two groups: (I) control and (II) inoculated with *P. pachyrhizi*. Thus, the leaves of the groups, were collected in specific time points after infection (6, 12, 24, 48 and 72 hours). Initially, the RNA-Seq reads were aligned with the W82.v1.a4 soybean reference genome using Hisat2 software. Aligned reads were mapped from the reference genome and reads counts were performed using Hisat2 software. Normalized read counts and differential expression analysis were performed with DESeq2. Genes with a log2 fold change greater or equal to two and PADJ less than 0.05 were identified as differentially expressed genes. RESULTS: In this work 40 genes representing the MLO family were found, with protein sizes ranging from 130 to 598 amino acids. We identified the *Glyma.12G169332* as a new member of the family. A slight difference was observed in the distribution of gene models across the chromosomes, as previously reported. Results of the phylogenetic tree revealed the division of the family into eight clades, as described in the literature. Gene expression results were obtained through RNA-seq data, in which differentially expressed genes were observed in the first hours of infection. Most of the genes in this family were expressed in the presence of the pathogen, especially the representatives of clade V. CONCLUSION: Members of MLO in clade V were induced by *P. pachyrhizi* infection.
**Key-words:** Soybean; *Phakopsora pachyrhizi*; MLO; Plant-pathogen; Transcriptome

## Acknowledgement

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# RESISTÊNCIA À MOSCA-BRANCA (*BEMISIA TABACI*) EM PLANTAS DE TOMATE GENETICAMENTE ENGENHEIRADAS, MEDIADAS POR RNA INTERFERENTE

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**Abstract:**

Tomato is one of the main vegetables consumed globally, but its cultivation is complex because it is affected by numerous pathogens and pests. Among them, viruses have caused considerable damage to tomato crops, especially those transmitted by vector insects, particularly the whitefly. Whiteflies are difficult to manage, and the use of chemicals is still the primary method of control, which causes the development of populations resistant to the main active principles of insecticides. Thus, there is an interest in developing transgenic tomato plants that are resistant to *Bemisia tabaci*. This study used the RNA interference approach to obtain transgenic tomato plants, modified to express siRNA molecules corresponding to the *B. tabaci* V-type proton ATPase gene (*vATPase).* Genetically modified plants were generated with an intron-spliced hpRNA vector into which 420bp fragments from the vATPase gene were directionally cloned to generate sense and antisense arms flaking the *Flaveria trinervia pdk* intron. A total of five (1B7, 1B9, 1B12, 1B15 E 2B2) transgenic lines were generated and evaluated for molecular studies. PCR analysis revealed the presence of both *vATPase2* and *nptII* transgenes in all transgenic lines. A study on the silencing of *vATPase2* was carried out through a bioassay to test resistance to whiteflies. The mortality rate and the change in development at different stages of the life cycle of the fly's life cycle are being evaluated. Results have revealed a mortality rate of up to 5o% in the transgenic lines challenged so far, in comparison to the control lines.

**Key-words:** Insect resistance; whitefly; RNAi; tomato; vATPase2

## Acknowledgement

FAPDF

# EXPLORANDO A CONTRIBUIÇÃO DA AUXINA DERIVADA DE FUNGO NO DESENVOLVIMENTO DA DOENÇA VASSOURA-DE-BRUXA NO CACAUEIRO

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**Abstract:**
Witches' broom disease (WBD), caused by the fungus *Moniliophthora perniciosa*, is a devastating disease that greatly limits cocoa production in the Americas. Infected plants develop abnormal shoots, flowers, and fruits, suggesting hormonal imbalances during the infection. However, the specific plant hormones involved in the disease development remain unclear. Using dual-RNA-seq, we found a remarkable up-regulation of auxin-responsive genes in infected cacao plants, indicating that auxin may play a role in WBD development. Interestingly, we did not detect the activation of cacao genes involved in auxin biosynthesis, suggesting that the observed auxin response is triggered by the pathogen. Our analysis of the *M. perniciosa* genome revealed putative pathways for the production of indole-3-acetic acid (IAA), a key plant hormone. More importantly, we demonstrated that the supplementation of *M. perniciosa* cultures with tryptophan amino acid (the IAA precursor) results in the accumulation of IAA and other indoles. Using CRISPR/Cas9 to generate mutants, we are currently investigating the role of fungal-derived IAA in *M. perniciosa* virulence and witches' broom disease symptom development. Our findings may not only help us understand how *M. perniciosa* induces morphological changes in cacao but also expand our knowledge of how phytohormone production by fungal pathogens promotes plant susceptibility.
**Key-words:** Auxin; Hormone; Fungus; Cacao; Pathogen

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# PIRAMIDAÇÃO DE ESTRATÉGIAS BIOTECNOLÓGICAS PARA O CONTROLE DE NEMATOIDES DAS GALHAS NA CULTURA DA SOJA

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**Abstract:**
Soybeans are the most important crop grown in Brazil due to their economic profit and the multifunctional nature of its grain. Among the challenges in soybean production, the parasitism by root-knot nematodes (RKN) of the genus *Meloidogyne* stands out. This endoparasite feeds on the roots of economic crops and produces giant cell clusters called galls that interfere with the plant's uptake of water and soil nutrients. RKN is controlled by nematicides, which are inefficient and highly toxic, crop rotation, and moderately resistant cultivars derived from a single genetic source in soybean. Therefore, the use of biotech approaches to incorporate new sources of resistance into elite cultivars is promising. Here, two biotechnological strategies were used simultaneously to RKN control in soybean: (1) Overexpression of the *AdEXLB8* gene, which encodes the *Arachis duranensis* expansin-like protein B, involved in cell wall loosening. The *AdEXLB8* overexpression in model plants showed increased resistance to RKN; (2) RNAi-mediated silencing of nematode genes involved in its primary metabolism or plant infection process, such as those encoding cysteine protease, isocitrate lyase, splicing factor, and the effector 16D10. Soybean genetically modified (GM) plants were obtained using the *Agrobacterium*-based transformation method. These plants were screened by transgene amplification by PCR and enzyme-linked immunosorbent assays to detect phosphinothricin N-acetyltransferase protein. Plants from three independent transformation events at T2 generation were selected for challenge assays against *Meloidogyne incognita*. The experiment was carried out twice in a completely randomized design with 10 replicates in a greenhouse. Fifteen-day-old plants were inoculated with 1,000 J2 juveniles of *M. incognita*. After 60 days, the GM plants showed a significant reduction in the number of galls per gram of root (22.0-34.0%), in the number of egg mass per gram of roots (46.0-50.0%), in the number of eggs per gram of roots (59.0-59.6%), and in nematode reproduction factor (30.0-50.0%) compared to wild-type plants. So far, the pyramiding strategy appears effective in controlling *M. incognita* and can be applied to soybean breeding programs as a complementary source of resistance to RKN.
**Key-words:** *Glycine max*; RNAi; *Meloidogyne incognita*; ;

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# OSMOTIN1 IS INVOLVED IN RICE TOLERANCE TO *SCHIZOTETRANYCHUS ORYZAE* (ACARI: TETRANYCHIDAE) MITE INFESTATION

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**Abstract:**
Rice is one of the most consumed cereals in the world. The growing demand for this cereal leads researchers to seek new technologies for rice production without increasing agricultural frontiers. The southernmost state of Brazil, Rio Grande do Sul, is responsible for 70% of the rice grown in Brazil. Nevertheless, productivity losses are caused by different biotic stresses. In Brazil, one of the most common is the phytophagous mite *Schizotetranychus oryzae* (Acari: Tetranychidae), which damages leaf tissue and alters the metabolism of amino acids/carbohydrates, inhibiting plant development and seed production. The identification of defense proteins is extremely important for a better understanding of the mite-plant interaction. In a previous work, we detected a high expression of Osmotin1 protein (belonging to the pathogenesis-related protein 5 - PR5) in mite-tolerant rice cultivars, under infested conditions, suggesting that this protein may be involved in plant defense mechanisms. Therefore, we aimed to evaluate the mite tolerance/sensitivity of rice plants overexpressing *Osmotin1* gene (*OSM1_OE*) and lacking *Osmotin1* gene (*osm1_mut-ko*), which would allow us to infer whether Osmotin1 has an important role in plant defense. Thirty-day-old plants (WT, *OSM1_OE*, and *osm1_mut-ko* lines) were infested with four female mites. Shoot and root length and mite number per plant were evaluated 60 days after infestation (DAI). While no difference has been detected in shoot and root length of *OSM1_OE* lines under control or infested conditions, the three *osm1_mut-ko* lines presented higher shoot length under infested condition when compared with WT plants. At the same condition, two out of the three *osm1_mut-ko* lines also presented higher root length than WT plants. Interestingly, two out of the three *osm1_mut-ko* lines presented higher shoot length than WT plants even under control condition. The number of adult and immature mites, and mite eggs per leaf were lower in *OSM1_OE* lines when compared with WT plants. On the other hand, the lack of a functional *OsOSM1* gene in *osm1_mut-ko* lines resulted in higher presence of mites per leaf than WT plants. These data suggest that Osmotin1 is much likely involved in rice tolerance to *S. oryzae* infestation. The development of rice lines resistant to *S. oryzae* infestation can contribute to the maintenance of productivity in rice plantations subjected to mite attack.
**Key-words:** Osmotin; *Schizotetranychus oryzae*; mite infestation; rice;

# LIMYB INDUZ ACÚMULO DE LIGNINA: ISSO AFETARIA A DEFESA CONTRA PATÓGENO BACTERIANO?

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**Abstract:**
The L10-INTERACTING MYB DOMAIN-CONTAINING PROTEIN is a transcription factor involved in antiviral immunity mediated by the NSP-INTERACTING KINASE (NIK1) membrane co-receptor. After the elicitor perception by an unknown receptor, NIK1 is activated by phosphorylation. Activated NIK1 phosphorylates the ribosomal protein L10 (RPL10), which moves to the nucleus and interacts with LIMYB; the complex binds to ribosomal protein (RB) gene promoters and represses RB expression and the expression of other translational machinery-related genes. This phosphorylation cascade inhibits translation and promotes virus resistance. In addition, some MYB factors have been described as regulators of lignin precursor enzymes through the phenylpropanoid pathway, which also plays an essential role against pathogens. The cell wall is the first line of defense against pathogen invasion in plants, and increased lignification promotes the thickness of the cell wall strengthening the barrier against pathogen invaders. The LIMYB transcription factor is a strong candidate for regulating the expression of genes related to lignin synthesis and defense against other pathogens. Using Arabidopsis plants as a model organism and the genotypes wild-type (Col0) and LIMYB-overexpressing lines, we identified the LIMYB transcriptional landscape via interactive RNA-seq and CHIP-seq analyses. We found that LIMYB interacts with promoters of the lignin synthesis genes and positively regulates the expression of these genes. The LIMYB-induced *4-COUMARATE-COA-LIGASE 2 (4CL2)* and *PEROXIDASE (POD)* gene expression was confirmed by real-time RT-PCR. A transcriptional promoter analysis by luciferase activity assay in leaves of benthamiana confirmed that 4CL2 and POD promoters were functional LIMYB targets. Consistent with these results, 4CL2 and PEROXIDASE enzyme activities were higher in LIMYB-overexpressing lines and in the T474D-6 line, expressing a constitutively activated NIK1 mutant (NIK1-T474D), compared to the wild-type control, Col-0. These genotypes also displayed a higher lignin content and leaf mesophyll thickness than control, wild-type lines, which were consistent with gene expression and enzyme activity of the LIMYB targets. In contrast, the *limyb* knockout line displayed lower 4CL2 and PEROXIDASE expression and enzyme activity, decreased lignin content and leaf mesophyll thickness compared to Col-0. These results suggest that LIMYB-mediated regulation of lignin accumulation may serve as a defense strategy against viral and non-viral pathogens. Experiments are currently underway to challenge LIMYB and NIK mutants against bacterial pathogens.
**Key-words:** RNA-seq; CHIP-seq; Transcription Factor; AT5G05800; Phenylpropanoid

## Acknowledgement

Capes, CNPq, Fapemig e Finep

# RESPOSTAS DE *CARICA PAPAYA* L. AO COMPLEXO DO PAPAYA MELEIRA VIRUS EM FASES DIFERENTES DO DESENVOLVIMENTO DA PLANTA

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**Abstract:**
The Papaya Sticky Disease (PSD) in Carica papaya L. plants is associated with the mixed infection of a toti-like virus, papaya meleira virus (PMeV), and an umbra-like virus, papaya meleira virus 2 (PMeV2), named PMeV-complex. PSD is characterized by a spontaneous exudation of latex and a sticky aspect on fruits and leaf petioles only after the plant flowering, suggesting that a tolerance mechanism(s) operate(s) prior to C. papaya juvenile-adult transition. In this study, field-grown plants at different development phases (3-, 4-, 7- and 9- months post-germination, MPG) were analyzed by LC-MS/MS-based proteomics which identified 1,609 C. papaya leaf proteins. Using a label-free strategy, the differently accumulated proteins, i.e. 38, 130, 160, and 17 proteins at 3-, 4-, 7- and 9-MPG, respectively, were determined. The biological processes modulated mostly involved photosynthesis, proteasome, cell wall remodeling, and plant defense. In general, photosynthesis-related proteins were up- and down-accumulated prior to (4MPG) and after (7MPG) C. papaya flowering, respectively. Chlorophyll fluorescence parameters were collected from green-house grown PMeV-complex infected and control C. papaya plants and submitted to JIP-Test. Consistent with the modulated proteins, the infected juvenile plants presented increased activity either at the oxygen-evolving complex and mostly at PSI electron acceptors' reducing terminal. The performance indexes, PIabs and PItotal, which reflect the conserved energy potential to the reduction of the intermediate electron acceptors between PSII and PSI, were also increased in PMeV-complex infected C. papaya. In parallel, the proteomic analysis also showed the down-regulation of cell-wall remodeling proteins, i.e. cellulose and pectic polysaccharides degradation enzymes, at 4MPG. After flowering (7MPG), the plants accumulated several nucleotide interconverting enzymes, which are involved in the synthesis of both hemicellulose and pectin. As these polymers are related to cell-wall strengthening, differences in C. papaya cell-wall structure would be expected. Using both bright-field (BFM) and scanning electron (SEM) microscopy, the foliar laticifers, the cells infected by the PMeV-complex, were found with no visible cellular contents. Also, transmission electron microscopy (TEM) analysis of the PMeV-complex infected laticifer showed disorganization and vacuolization in the walls, and signs of degradation of cellulose microfibrils. The thickness average of laticifers' cell walls was 662 nm, more than twice as thick as found in controls. The proteomic and ultrastructure data showed that PMeV strongly affects C. papaya cell wall biology, likely related to the spontaneous exudation of latex observed as the main disease symptom. The results presented here shed light on specific players of C. papaya and PMeV-complex interaction, and are crucial for guiding genetic improvement programs towards a virus-resistant C. papaya genotype.
**Key-words:** Label-free quantitative proteomics; Papaya meleira virus; Carica papaya; Photosynthesis; Ultrastructure

**Acknowledgement**

CNPq, FINEP, CAPES, FAPES e FAPERJ.

# O USO DO RNA DE INTERFERÊNCIA OBJETIVANDO O CONTROLE DE NEMATOIDES PARASITAS DE PLANTAS: NOVOS ALVOS PARA A PROTEÇÃO DE CULTIVOS

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**Abstract:**
Meloidogyne incognita is one of the most important plant-parasitic nematodes (PPNs) causing severe crop losses worldwide. Plants have evolved complex defense mechanisms to respond to PPNs attacks. Conversely, PPNs have evolved infection mechanisms that involve the secretion of effector proteins into host plants to suppress immune responses and facilitate parasitism. Therefore, effector genes are attractive targets for the genetic improvement of plant resistance to M. incognita. In this study, we functionally characterized the putative Minc03328 (Minc3s00020g01299) and Minc16803 (Minc3s00746g16803) effector gene to evaluate its role during plant-nematode interaction. Herein, we characterized the Minc03328 effector gene, confirmed its higher expression in the early stages of M. incognita parasitism in plants, as well as the accumulation of the Minc03328 effector protein in subventral glands and its secretion. In turn, Minc16803 gene is expressed in all nematode life stages and encodes a protein with a characteristic N-terminal signal-peptide and the absence of transmembrane domains. Our in silico sequence analysis suggest that both effector genes are distant at the sequence and phylogenetic levels from other nematodes effector, but has remarkable characteristics closely associated with M. incognita parasitism in plants. Using the in planta RNA interference strategy, Arabidopsis thaliana plants overexpressing double-stranded RNA (dsRNA) were generated to specifically targeting and downregulating the Minc03328 and Minc16803 gene during nematode parasitism. Gall number and egg mass were reduced by up to 81% and 93% in the transgenic Minc03328- dsRNA lines, whereas the Minc16803 gene silencing assay showed 76% and 87% reduction for these same parameters. Histological analysis revealed giant cells without cytoplasm, disordered neighboring cells, and malformed maturing nematodes in transgenic galls. All findings strongly suggest that both effectors gene represent valuable promising target to engineer agricultural crops for M. incognita resistance through host-induced gene silencing.
**Key-words:** *Crop-protection*; *Effector protein*; *In planta RNAi*; *New biotechnological tools*; Plant-nematode interaction

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SOCIEDADE BRASILEIRA DE GENÉTICA

VIII Simpósio Brasileiro de Genética Molecular de PLANTAS

# Estrutura e Função de Proteínas

# PROTEÔMICA COMPARATIVA ENTRE GENÓTIPOS DE CACAU COM NÍVEIS DE RESISTÊNCIA CONTRASTANTES À PODRIDÃO-PARDA

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**Abstract:**

Black pod, caused by the hemibiotrophic pathogen *Phytophthora* spp., is one of the most prevalent and destructive diseases, affecting all parts of cacao (*Theobroma cacao* L.). Present in all countries in the world where the crop is cultivated, it can lead to a 40% loss in production if control measures are not adopted. Among the species that cause brown rot in southern Bahia, *P. palmivora* is considered the most prevalent and the second most virulent. Understanding, at the molecular level, the relationship of susceptibility and resistance in the Phytophthora - host interaction becomes increasingly relevant. Therefore, the present work aims to characterize the protein profile of two cocoa genotypes with contrasting levels of resistance to black pod: CO2003 (susceptible) and CCN-51 (resistant), under control conditions and inoculated with *P. palmivora*. For this, the leaves were collected at 6 h, 24 h and 48 h after inoculation (hpi). Then, the proteins were extracted and identified using gel-free proteomics. In the control condition, 388 proteins were identified in the CO2003 genotype and 520 proteins in CCN-51. In the inoculated plants, 357 proteins were identified in the CO2003 genotype and 490 in CCN-51. A statistical significance analysis ($p \leq 0.05$ and fold-change $\geq 1.5$) on the abundance of common proteins between the inoculated treatment and the control, of each genotype at each collection time was performed. Comparing the genotypes in the inoculated treatment, 101 proteins were differentially abundant. Being 3 exclusive to the susceptible genotype and 19 exclusive to the resistant genotype. In both genotypes, the highest accumulation proteins were found at 6 hpi. At that time of collection, 12 of the 19 proteins exclusive to the CCN-51 genotype were also found, some of which are involved in the process of response to stress and defense, such as catalase, which is an essential enzyme for the detoxification of plant cells; protein with NB-ARC domain that acts as a regulator of resistance proteins (R) and a protein of the family of cysteine proteases with granulin repeat, involved in elicitor-stimulated programmed cell death. These results allow a better understanding of plant defense, in different cacao genotypes, in the initial phase of infection (biotrophic). Thus contributing with more information that may lead to pathogen control and improvement in cocoa productivity.

**Key-words:** *Theobroma cacao*; *Phytophthora palmivora*; Gel-free proteomics; ;

# INVESTIGANDO VIA PROTEÔMICA O IMPACTO DA SUPEREXPRESSÃO DE BIP EM PLANTAS NA RESPOSTA VEGETAL AO ATAQUE DE MONILIOPHTHORA PERNICIOSA

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**Abstract:**
*Moniliophthora perciciosa* is one of the main pathogens affecting the cocoa crop. The control of this phytopathogen is generally carried out by means of resistant genotypes accompanied by phytosanitary pruning. The identification of plant genes related to the plant defense mechanism is important to unravel the molecular basis of plant-pathogen interaction. One potential gene studied is the binding protein (BiP), a molecular chaperone located in the lumen of the endoplasmic reticulum that binds to newly synthesized proteins as they are translocated into the ER and maintains it in a competent state for subsequent folding and oligomerisation. BiP is an abundant protein under all growth conditions, but its synthesis is markedly induced under conditions that lead to accumulation of unbound polypeptides in the ER, such as when plants are under pathogen attack. In a recent study, we overexpressed the BiP gene in *Solanum lycorperscum* plants and inoculated them with *M. perniciosa*. The control (untransformed) plants showed severe symptoms of witches' broom disease, while the symptoms of the transgenic strains varied from severe to mild according to the level of BiP in transgenic lineages. In the present study, we applied the gel-free proteomics technique to unravel how the overexpression of BiP affects plant response to *M. perniciosa* attack. We inoculated BiP-plant (tomato superexpressing BiP) and control plants (Non transgenic) with a mix of *M. perniciosa* spores. After protein extraction, purification and tryptic digestion, peptides were analyzed by liquid chromatography-mass spectrometry. A total of 171 proteins were identified in BiP-plant inoculated treatment, 30 of which were unique. In NT inoculated plants 178 proteins were identified, 37 of which were unique. The cluster analysis revealed that defense-related proteins had their regulation greatly increased in overexpressing BiP-plant in response to *M. perniciosa* attack. Among them: Peroxidase, PR10, PR1, catalases and pathogeneses- related protein P2. The proteomic analysis of not inoculated plants identified 214 proteins in BiP-plant, of which 33 were exclusive proteins and 196 proteins in NT plants, 15 of which were exclusive. 181 proteins were identified in both treatments. The analyses showed that even without inoculation, the transgenic plants maintained a higher level of proteins known to be involved in the defense mechanism such as PR10, Proteinase inhibitor II, wound-induced Proteinase inhibitor I and peroxidase. These findings suggests that BiP overexpression in plants provide them with an arsenal of proteins that gives a molecular advantage to the plant that support a prompt response/ defense under an pathogen infection.
**Key-words:** Witches' broom disease; defense mechanism; biotic stress; response proteins; molecular chaperone

# CRIANDO UM INIBIDOR DE CISTEÍNO PROTEASE VEGETAL

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**Abstract:**
Proteases are enzymes that catalyze the hydrolysis of proteins and polypeptides. The regulation of protease activity is crucial in several metabolic and physiological processes. It can be affected by different factors such as pH, redox potential, glycosylation and enzymatic inhibitors. This work focuses on phytocystatins, a group of proteins that reversibly inhibit papain and legumain cysteine proteases. Phytocystatins can be identified by the consensus sequences [LVI]-[AGT]-[RKE]-[FY]-[AS]-[VI]-X-[EDQV]-[HYFQ]-N and Q-x-V-x-G. In addition, they do not present disulfide bridges and glycosylation. They are classified into 3 types according to their molecular weight and structural similarities. Phytocystatins type I are low molecular weight proteins (about 10-kDa) and can inhibit papain cysteine protease. Phytocystatins type II are bifunctional, inhibit papain and have a carboxy-terminal extension with an inhibitory domain [SNSL] for legumain cysteine protease. The phytocystatins type III have multiple inhibitory domains for papain and have high molecular weight. The overexpression of phytocystatins in plants is a known strategy to make plants more resistant to abiotic stresses such as drought, salinization and cold. Furthermore, the potential use of these inhibitors to affect digestive proteases of phytophagous arthropods opens up possibilities for biotechnological insecticidal-acaricide compounds. Among the phytocystatins, only type II phytocystatins are able to inhibit legumains. We designed a mutant inhibitor, called 4xSNSL, containing four legumain inhibitory motives [SNSL] in the loop regions from the rice cystatin type I backbone. The mutations promoted larger fluctuations in the loop domains compared to the original rice cystatin-I. Fortunately, such mutations did not affect the general secondary structural nor the three-dimensional structure, suggesting the mutant is structurally stable. In addition, it was observed that inhibitory residues were favorably arranged in all mutated domains. The cystatin mutant 4xSNSL coding sequence was introduced in the pETDuet-1 vector. The inhibitor was expressed in E. coli, purified from the bacterial soluble fraction using Ni-NTA agarose and purification confirmed by SDS-PAGE. The purified 4xSNSL will be used to determine its thermal stability, inhibitory constant against papain and legumain, stability against cysteine proteinases digestion and also to identify potential targets in plant systems.
**Key-words:** Cystatin; Proteinase inhibitor; Legumain; Molecular dynamics;

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# HORMÔNIOS VEGETAIS COMO DOADORES DE GRUPAMENTOS ACIL PARA MODIFICAÇÕES PÓS-TRADUCIONAIS DE PROTEÍNAS

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**Abstract:**
Protein post-translational modification is a universal process and occurs in most proteins. Protein lysine residues are considered hotspots for the addition of different chemical groups, such as acylation, methylation, phosphorylation and hydroxylation. Lysine acylation encompasses the covalent addition of a wide range of acyl donors to the ε-amino group as a mechanism involved in biological signaling. Lysine acetylation is well studied in histones due to its impact as an epigenetic hallmark that promotes changes in chromatin structure and availability. Other acyl groups derived from molecules from carbohydrate and lipid metabolisms (e.g., lactate, succinate, hydroxybutyrate) were identified as lysine modifications of histones and of other proteins. Plant hormones harboring acyl groups often form conjugates with free amino acids to regulate their activity and function during plant physiological processes and responses. Besides forming low-molecular weight conjugates, auxin was shown to covalently modify proteins from bean seeds,strawberry and pea. In addition to auxin, other phytohormones with acyl groups are unexplored potential substrates for post-translational acylation of proteins. Using mass spectrometry libraries available at the Proteomics Identification Database (PRIDE), we performed searches for tryptic peptides derived from proteins modified by phytohormones containing acyl groups to verify the existence of such post-translational modifications. MS/MS spectra were searched against the proteome of Arabidopsis, rice and soybean available on Phytozome V13 with the MaxQuant software (version 2.0.3.0). We set new variable modifications corresponding to the mass shift resulting from the amide linkage of a lysine with indole-3-acetic acid (K-IAA, 158.061 Da), abscisic acid (K-ABA, 247.133 Da), jasmonic acid (K-JA, 193.123 Da), gibberellic acid (K-GA, 329.139 Da), and salicylic acid (K-SA, 121.028 Da). The minimum score threshold for peptides identification was set to 40. Carbamidomethylation of cysteines was defined as a fixed modification and methionine oxidation was selected as a variable modification, along with lysines modification with plant hormones. We identified proteins containing lysines modified with all acyl-containing phytohormones in the three analyzed species. Our findings support that plant hormones with carboxyl groups can be linked as amides to protein lysine ε-amino residues. Several of the identified proteins can be associated with abiotic stress responses, such as soybean MCA1 (K-ABA), Arabidopsis NST1-like protein (K-IAA and K-SA), rice Topless2 (K-GA) and KAS (K-JA). The functional implications of hormone covalent association to non-histone proteins remains to be investigated, as well as the identification of the enzymes involved in this covalent linkage.
**Key-words:** post-translational modifications; phytohormones; acylation; ;

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# Genômica Aplicada de Plantas

# DESENVOLVIMENTO E OTIMIZAÇÂO DE MÈTODO DE EDIÇÃO DE GENOMA LIVRE DE DNA EM SOJA VIA CRISPR/CAS9

**Abstract:**
In the past few years, CRISPR/Cas9 gene editing system has allowed fast improvement of several traits of agronomic importance in plants. For soybean (*Glycine max*), one of the world's most important commodities, DNA-free approaches are scarce, mostly been use in protoplasts solely for gene screening purposes. Thus, considering its potential advantages in terms of cost and time, the aim of this study is to present a CRISPR/Cas9-based biolistic ribonucleoprotein (RNP) delivery protocol for genome editing of soybean embryonic axis. sgRNAs targeting the two copies of phytoene desaturase (*GmPDS*) genes, a key enzyme in carotenogenic pathway, presented in chromosomes 11 and 18, were designed in CRISPR-P2.0 to be compatible with both PAM's and scaffolds required for Cas9 nucleases from *Streptococcus pyogenes* (*SpCas9*) and *Staphylococcus aureus* (*SaCas9*), to compare its editing efficiency. For the RNP-mediated genome editing, the choice of *GmPDS* gene is desirable because the albino genotype provided by gene knockout is easier to detect. After target selection and sgRNA design, the main challenges faced by DNA-free genome editing in plants are synthesize sgRNA efficiently and inexpensively, as well as and optimize nuclease expression conditions. For sgRNA, oligonucleotides were synthesized containing the CRISPR RNA (crRNA) fused with the trans-activated crRNA (or scaffold RNA), portions of the sgRNA, which provided a high yield of in vitro transcription, ranging from 15 to 60 µg. In parallel, the heterologous expression of SaCas9 nuclease was optimized in four *Escherichia coli* type B-derived strains. *E. coli* BL21 and pLysS presented the better expression capacity, with a maximum of 15 and 10mg/L, respectively. In contrast, for Rosetta and ArcticExpress expression systems, SaCas9 production was only possible to identify through (His)tag western blotting. It was observed that the expression conditions related to nutritional and metabolic control influence Cas9 expression. An average of 1.2 mg/100mL was obtained using modified Terrific Broth (TB) media over 0.2 mg/100mL in Super broth (SB) media and no yield was observed in Luria-Bertani (LB) media with any E. coli strain. Post induction temperature of 28°C resulted in more protein in soluble fraction than 37°C and 18°C when expressed overnight. IPTG final concentration ranging from 1mM and 0.5mM gave no significant variation in Cas9 expression, while higher or lower results in no expression. Activity of RNP comprising both SpCas9 and in home produced SaCas9 was assessed through in vitro cleavage assay. Moreover, this study will procced to in vivo RNP delivery using gold particle biolistic to embryonic axis in Williams 82 and BRS537 soybean genotypes for validation through albino phenotype generation by *GmPDS* gene knockout.
**Key-words:** Ribonucleoprotein; Glycine Max; SaCas9; SpCas9; GmPDS

# COMPONENTES DE RENDIMENTO E RESPOSTAS DE EXCESSO DE FERRO EM PLANTAS DE ARROZ EDITADAS PARA OS GENES DE TRANSPORTE VACUOLAR OSVIT1 E OSVIT2

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**Abstract:**
Rice (*Oryza sativa* L.) is one of the most consumed staple foods in the world. However, grains are low in essential elements such as iron (Fe) and zinc (Zn), commonly lacking in humans' diets. Biofortification of rice grains is an excellent strategy to supply both nutrients. Various strategies were used to accumulate Fe into the seed endosperm, commonly consumed as white rice, but we still have not achieved the necessary concentration. The rice *vacuolar iron transporter* (VIT) genes *OsVIT1* and *OsVIT2* are expressed in flag leaves, nodes and aleurone layer, and regulate Fe transport into the vacuole. Loss-of-function mutants for each gene showed higher accumulation of Fe in the endosperm, suggesting that these transporters are good target genes for Fe biofortification. OsVIT1 and OsVIT2 are also involved in Fe accumulation into the vacuoles, but their role in Fe excess responses is not clear. Previously, our group generated two independent double knockout mutants (*osvit1/2.*1 and *osvit1/2.2*) using the CRISPR/Cas9 system, which resulted in increased accumulation of Fe in the seeds. Here we analyzed morphological and agronomic traits of double knockout lines cultivated in greenhouse conditions until full maturity, and performed experiments exposing mutant plants to Fe excess conditions. The percentage of filled seeds was reduced in all five lines. Also, all five genotypes presented more spikelets compared with WT, but lower spikelet weight. Three lines presented higher shoot dry weight, while four lines presented reduced shoot height compared to the WT, suggesting a general trend of decreased growth and seed set. When exposed to Fe excess, we observed a general trend of decreased SPAD values in double mutant lines exposed to 5 mM Fe compared to WT. These partial results suggest that knockout of both *OsVIT1* and *OsVIT2* may lead to decreased performance of rice plants. Among the five lines, one was more similar to WT, and non-transgenic seeds from this line (null-segregant for the transgene) were generated. Further genetic engineering of biofortification genes will be performed using this selected line, aiming at the generation of plants with high Fe concentration in their seeds. Moreover, we will further explore the role of VIT transporters in rice plants subjected to Fe overload.
**Key-words:** Biofortification; CRISPR/Cas9 ; Iron; endosperm;

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CNPq, CAPES, FAPERGS, PPGBCM, PPGBM

# DESENVOLVIMENTO DE UM SISTEMA PARA SELEÇÃO DE NOVOS GENES CAS PARA EDIÇÃO GENÔMICA EM PLANTAS

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**Abstract:**
Genome editing using the CRISPR/Cas system is a powerful tool for precisely manipulating genes in various organisms, including plants. This technique uses a guide RNA to target specific DNA sequences, and a Cas protein to cut or edit the DNA at that location, resulting in a desired phenotype. In agriculture, it has been widely used to enhance productivity and quality in various crops due to its efficiency, simplicity, and low cost. To develop a system for selecting new Cas genes, the complete genomes of 210 bacteria were sequenced, and potential new genes for genome editing were identified through bioinformatic analysis. The potential of these new Cas genes will be further analyzed by validating their nuclease activity *in vitro* and i*n vivo* using *Arabidopsis thalia*na and *Nicotiana benthamiana*. The target gene for this study is the PDS gene, which encodes the enzyme phytoene desaturase. This enzyme plays a critical role in the biosynthesis of terpenoids, which are pigments important for photosynthesis and plant growth. Disruption of the PDS gene can lead to a decrease in carotenoid production, resulting in photobleaching with loss of leaf coloration. Furthermore, the Cas protein will be fused with a CPP cell-penetrating peptide to improve the efficiency of CRISPR/Cas system delivery in plants. The Cas protein needs to enter plant cells to perform gene editing, but the plant cell wall makes it difficult for it to penetrate and enter the cells. CPP can overcome this barrier and facilitate the delivery of the Cas protein into the cells. The Cas gene sequence will be introduced into a modified pETDuet vector, and the Cas protein and single-guide RNA will be co-expressed in Escherichia coli, streamlining production of Cas ribonucleoproteins. The purification of ribonucleoproteins from the bacterial soluble fraction will be carried out using immobilized metal affinity chromatography. The purity of the ribonucleoproteins will then be confirmed by analyzing them on a SDS-PAGE. The nuclease activity of the ribonucleoprotein complexes will be analyzed, and the selected and validated Cas genes could be used to edit relevant agricultural crops, such as rice, soybean, strawberry, tobacco, apple, and eucalyptus. This could lead to the development of more productive, pest- and disease-resistant crops and advance genome editing technology.
**Key-words:** gene editing; CRISPR/Cas; phytoene desaturase; CPP;

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# VISÃO DA ORIGEM DO PARASITISMO EM PLANTAS A PARTIR DE UMA ANÁLISE DE GENÔMICA COMPARATIVA.

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**Abstract:**
Parasite plants have evolved at least 12 times within the angiosperms independently. The haustorium is a key organ that unifies this group. It allows the establishment of a parasitic vascular pipe that can invade host tissues. The acknowledgment of the host's presence is the first step in establishing the haustorium. It is followed by cell proliferation, volume expansion, and re-differentiation into vascular system cells. The high occurrence of parasitism in the evolution of angiosperms and the coincidence of the stages of haustorium formation in distant species suggest that the steps leading to the emergence of a heterotrophic lifestyle are not particularly difficult to accomplish. Recent research has demonstrated that the establishment of the haustorium in facultative parasitic plants appeared after the readjustments of already existing pathways linked to the development of lateral roots. Therefore, we conducted a deep genomic comparative analysis to understand the origins of parasitic lifestyles in angiosperms. Using the software Orthofinder (version 2.5.4) we unraveled the shared orthogroups between the parasitic species of the family Orobanchaceae, *Phtheirospermum japonicum* and *Striga asiatica*, and of the family Convulvulaceae, *Cuscuta campestris* and *Cuscuta australis*. Five other non-parasitic species were included in the analysis: *Ipomoea cairica* and *Lindenbergia philippensis* as non-parasitic members of the families Convulvulaceae and Orobanchaceae, respectively; *Solanum lycopersicum* and *Mimulus guttatus* as outgroup representatives of both families; and *Arabidopsis thaliana*. The analysis placed 90% of genes in 25750 orthogroups, with 6861 species-specific orthogroups, containing 32952 genes, and 28212 genes not assigned to any orthogroup. To investigate sequences with parasitic functions, we focused on annotating the genes assigned to shared orthogroups between the four parasitic plants and testing for enrichment and depletion of significant functional bin terms using the web tool Mercator 4.0. Orthogroups exclusively shared between the four parasitic species were enriched for bin terms like RNA biosynthesis in C. campestris and S. asiatica, and protein homeostasis and enzyme classification in P. japonicum. Generally, the shared orthogroups between the parasitic and non-parasitic species studied showed enrichment for constitutive biological processes. We then identified functional enrichment bins in the specific and unassigned parasitic plant sequences. The data showed a possible enriched function related to parasitism, warranting further investigation of the expression profiles of these genes. In this manner, we hope to further understand the molecular tools parasite plants have used in their evolutionary history and, in this way, have a clearer view of how parasitism has appeared in plants.
**Key-words:** Haustorium; Parasitic plants; Genomic analysis ; Orthogroups;

# DEPLEÇÃO DO QUADRO DE LEITURA *UPSTREAM* COMO NOVA ESTRATÉGIA PARA MANIPULAR A TRADUÇÃO DE *GMPR10* USANDO CRISPR/CAS9 PARA AUMENTAR A TOLERÂNCIA DA SOJA A FITONEMATOIDES.

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**Abstract:**
The root-knot nematode (RKN), *Meloidogyne spp.*, is considered one of the most economically important plant pathogens, impacting both the yield and quality of soybean. In previous transcriptomic and proteomic studies on contrasting soybean genotypes (BRS133- susceptible and PI595099- highly tolerant), we searched for candidate genes that might be directly related to the increased tolerance of soybean to RKNs. Among these candidates, we identified genes encoding proteins that inhibit or degrade the digestive tract and cuticle enzymes of these pathogens, such as the pathogenesis-related class 10 protein (*GmPR10*). Overexpression of *GmPR10* in transgenic tobacco plants resulted in a reduction in the number of galls per gram of root (51.6- 57.8%), number of eggs per gram of root (41.9-43.5%), and in nematode reproduction factor (40.4-48.7%) compared to wild-type plants. Likewise, tests on transformed hairy soybean roots showed a 40 % reduction in galls. These results suggest that the *GmPR10* gene is a promising candidate for engineering modification. Editing upstream open reading frames (uORFs) has recently emerged as a strategy to increase mRNA translation using CRISPR/Cas9 technology. uORFs affect the translation of associated downstream primary ORFs (pORFs), not always in a positive way. Therefore, their identification and editing could improve the translation of the *GmPR10* gene. In this study, we identified two uORFs in the 5'-UTR sequence of this gene. The entire 5' leader sequence was amplified, cloned, and site-directed mutated to delete its uORFs start codons (-ATG). The validation of these predicted uORFs was carried out in transformed *Nicotiana benthamiana* protoplasts using a dual-luciferase reporter vector, in which it is possible to analyze the effect of mutated and non-mutated uORFs by dividing the expression of luciferase by that of Renilla-luciferase (LUC/REN). As expected, expression analyses of mRNA levels showed no statistically significant differences between mutated and non-mutated uORF variants. As for the results of the expression of the reporter proteins (LUC/REN), we found that single mutations at the uORF 1 and 2 did not result in significant differences compared to non-mutated sequences. However, double mutations (uORFs 1 and 2) increased LUC/REN activity by approximately 3.5-fold, suggesting that both uORFs should be edited using CRISPR/Cas9 to increase *GmPR10* protein levels in soybean plants. The uORF depletion strategy using CRISPR/Cas9 for gene translation is innovative in soybean and enables the production of non-transgenic plants tolerant to |RKNs.
**Key-words:** *Glycine max*; *Meloidogyne incognita*; uORF; genome editing;

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# O NOCAUTE DE PROTEÍNAS ABSCISIC ACID/STRESS/RIPENING (ASR) CAUSA AUMENTO DA SENSIBILIDADE À DEFICIÊNCIA DE FERRO EM ARROZ (*ORYZA SATIVA* L.)

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**Abstract:**
Rice (*Oryza sativa*) is central to the diet of half of the world's population. However, its seeds contain low iron (Fe) concentrations, which is critical for human nutrition. Fe deficiency is common for plants, as Fe is not readily available for uptake by roots. The Abscisic Acid/Stress/Ripening (ASR) proteins, which are responsive to different types of stresses, were previously shown to be involved in mechanisms that confer tolerance to aluminum (Al) in rice, especially OsASR5. However, their involvement with Fe homeostasis was never explored. Thus, seeds of OsASR5-RNAi plants and its respective wild-type (WT) background (cv. Nipponbare) were analyzed in a hydroponic system under control (CC) or Fe deficiency (-Fe) treatments. After 21 days of treatment, OsASR5-RNAi plants showed reduced shoot height and lower dry weight of shoot and roots under -Fe compared with the WT plants. OsASR5-RNAi plants showed increased root length under both treatments, which indicates a greater partition of photosynthates for the root growth in this genotype. Visually, the fifth leaf of OsASR5-RNAi plants showed to be more chlorotic than those of WT plants under -Fe, an observation confirmed with SPAD. We observed decreased net photosynthesis, stomatal conductance, water use and carboxylation efficiencies between 7 and 14 days of -Fe treatment in OsASR5-RNAi plants. These parameters showed a slight recovery between 14 and 21 days of treatment in the OsASR5-RNAi plants, while the WT plants continued to decline. Altogether, these results indicate that OsASR5-RNAi plants are more sensitive to Fe deficiency, resulting in an earlier impaired photosynthetic activity. Therefore, to better understand the impact of ASR protein knockdown in rice, we performed a transcriptomic analysis of roots from WT and OsASR5-RNAi plants under CC and -Fe. Among the key genes involved in the Fe homeostasis in rice, only *OsYSL2* showed to be strongly upregulated in OsASR5-RNAi plants under -Fe, in relation to the WT under CC. So, these results showed that the knockdown of the ASR proteins affects the growth of rice plants under Fe deficiency, and that these proteins are involved in Fe homeostasis.
**Key-words:** Rice; Iron; Deficiency; Knockdown; ASR

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# SELEÇÃO E AGRUPAMENTO DE LINHAGENS $S_2$ DE MILHO UTILIZANDO MARCADORES AFLP

**Maria Angélica Marçola [1]; Gabriela Inocente [2]; Deoclecio Domingos Garbuglio [3]; Pedro Mário de Araújo [3]; João Candido de Souza [4]**

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**Abstract:**
Maize breeding programs are guided by the search for superior genotypes in the shortest possible time. In this way, information regarding the combining ability of lines still in partial inbreeding stages can accelerate the process of obtaining superior hybrids, increasing the probability of maximizing heterosis or hybrid vigor when in crosses. The use of molecular markers can help selection, directing towards identifying heterotic groups or patterns. Thus, this work sought to access information regarding the heterotic patterns in divergent groups of $S_2$ lines and their applications in the composition of intermediate maize hybrids, using AFLP markers. The result was compared with field data from the evaluated lines and their crosses *per se* (82 intermediate hybrids) in three different environments in the State of Paraná, Londrina, Guarapuava and Santa Tereza do Oeste. A joint analysis and the Scott-Knott test at 5% probability were performed to group the means. A performance pattern was observed for certain hybrids associated with common parents that were repeated throughout the environments, even if inserted in different hybrids, with male parents (PC0201): 204, 215, 220 and 223, and female parents (PC0202): 103, 107, 109, 111, 113, 118 and 119. Through the Bayesian analysis it was evident that the ancestry of LPE0202 was superior in relation to LPE0201 in the intermediate hybrids. It was observed through the neighbor-Net that crosses of genetically contrasting lines resulted in hybrids with high grain yield, confirming the positive relationship between productivity of the evaluated materials and genetic distance obtained by AFLP molecular markers, which were efficient in the identification and clustering of the heterotic patterns even in stages of partial inbreeding ($S_2$). The conservation of heterotic patterns in the $S_2$ stage allows an efficient discard or selection of promising lines, for advanced stages of inbreeding. A grouping of genotypes distinct from the other clusters was observed, as commercial hybrids from a common genetic pool were used to form the original populations.
**Key-words:** AFLP Marker; Incomplete imbreeding; Intermediate hybrids; ;

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# FERRAMENTA (RGESY) DE ANÁLISE DE GENES DE REFERÊNCIA PARA ESTUDOS DE EXPRESSÃO VIA RT-QPCR

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**Abstract:**
Gene expression through RT-qPCR can be performed by the relative quantification method, which requires the expression normalization through reference genes. Therefore, it is essential to validate, experimentally, the candidate reference genes. Thus, although there are several studies that are conducted to identify the most stable reference genes, most of these studies validate genes for very specific conditions, not exploring the whole potential of the research. It turns out that new experiments must be conducted by researchers that have interest in analyzing gene expression of treatments and/or conditions present, but not explored, in these studies. In this study, we present the *RGeasy* tool, which aims to facilitate the selection of reference genes, allowing the user to choose genes for a greater number of combinations of treatments/conditions, compared to the ones present in the original articles, with just a few clicks. *RGeasy* was validated with RT-qPCR data from gene expression studies performed in two coffee species, *Coffea arabica* and *Coffea canephora*, and it can be used for any animal, plant or microorganism species. In addition to displaying a rank of the most stable reference genes for each condition or treatment, the user also has access to the primer pairs for the selected reference genes.
**Key-words:** Endogenous genes; Relative expression; Normalization; ;

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# TRANSLATIONAL RESEARCH FOCUSED ON HD-ZIP I TRANSCRIPTION FACTORS. FROM A MODEL PLANT GROWN IN A CULTURE CHAMBER TO CROPS IN FIELD CONDITIONS AND BACK TO FUNDAMENTAL SCIENCE.

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**Abstract:**
Worldwide research on Plant Molecular Biology has often been performed working with model species like Arabidopsis thaliana, Nicotiana benthamiana, or Oryza sativa, grown in culture chambers or greenhouses. However, there is a long way to traverse from the model to the crop and from the laboratory to the field. Even though models are very useful to acquire knowledge faster, the truth is that more frequently than desired, the observations made with such species were not reproduced in field conditions where a complex stress mix occurs. Sunflower exhibits several divergent transcription factors (TFs). Among them, HaHB4 and HaHB11 belong to the HD-Zip I family. HaHB4 conferred drought and salinity tolerance to Arabidopsis, whereas HaHB11 improved yield and flooding tolerance in such a model. We did genetic constructs able to express these TFs and used them to transform soybean, maize, rice, and wheat. We conducted culture chamber, greenhouse, and field trials in different environments, and notably, the results indicated that crops partially conserved the beneficial traits observed in the model. After long regulatory processes in several countries, HaHB4 soybean and wheat became rare successful cases commercially released in 2022. In field assays, where a severe drought occurred, transgenic HaHB4 outyielded the commercial line by 26%-95%. HaHB11 was able to confer tolerance to flooding and defoliation and increased yield to the three crops in four campaigns. A careful analysis of these and other transformed plants exhibiting enhanced yield as a shared trait lead us to develop a mechanical treatment allowing to enlarge the stems, Conducting to improve the production and health of tomatoes, bell pepper, chia, strawberry, and other species. The development of this manual technique, useful in family agriculture and small farmers, exemplifies how science is only one, and the connection from fundamental research to its application is a two-way road. Even though failures are more frequent than successes, basic research is essential to reach innovative applications.
**Key-words:** transcription factor; HD-Zip I; drought; translational biotechnology; crop improvement

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Coastal Agrobiotechnology Institute CONICET-UNL

# ANÁLISE TRANSCRIPTÔMICA DE GENES RELACIONADOS À PAREDE CELULAR EM FOLHAS DE *SETARIA VIRIDIS* EM DIFERENTES ESTÁGIOS DE DESENVOLVIMENTO

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**Abstract:**
*Setaria viridis* is an important model for C4 grasses. Identification of genes associated with plant cell wall (PCW) over different plant developmental stages is promising for molecular breeding programs of plants phylogenetically related to S. viridis, such as sugarcane, an important bioenergy crop. Here, we employed comparative genomics methods and previous literature to identify PCW-related genes. Briefly, proteomes of two dicots and five monocot plants were downloaded from Phytozome, MaizeGDB, and Sol Genomics Network, and OrthoFinder2 was used to identify homologous sequences across different plants. We identified putative PCW genes in *Setaria viridis* based on homologs previously described in *A. thaliana* and maize. We recovered Gene Ontology (GO) and KEGG Ortholog (KO) annotations from Phytozome (*S. viridis* genome v2.1). Carbohydrate-Active Enzymes (CAZymes) and transcriptional regulators (TRs) were identified with HMMER, using HMM profiles based on dbCAN2 and MyTFDB (https://bitbucket.org/diriano/mytfdb), respectively. To study gene expression, we employed RNA-Sequencing data of leaves at different developmental stages. Briefly, plants were grown under 65% humidity, photoperiod of 16/08 light/dark and 450 mmol/light, at 26ºC. Leaves in three stages were collected: pre-booting [PB, transition from vegetative to reproductive], phase 1 [F4, high cell growth], and final phase [FF, complete deposition of secondary PCW and senescence]; for each stage, leaves from different plants were pooled and three technical replicates were generated from each pool. RNA extraction, quality check, and sequencing of paired-end, unstranded RNA-Seq data was carried out in a NovaSeq 6000 platform (Novogene Co, Ltd). Sequencing quality was analyzed using FastQC and ribosomal contamination was verified and cleaned with bbmap/bbduk. We quantified gene expression and identified differential expressed genes (DEGs) in contrasts between developmental stages employing Kallisto and Sleuth, respectively. We obtained a total of 3,810 DEGs (F4 vs. PB: 105, F4 vs. FF: 1,965, PB vs. FF: 3,419). GO enrichment analysis using GOATOOLS identified enriched (e) and purified (p) terms in two contrasts: PB vs. FF (up: 78e; 69p; down: 37e / 12p) and F4 vs. FF (up: 45e / 37p; down: 19e / 2p). Among differentially expressed genes, we identified 285 CAZymes (68 families) and 217 regulators (172 transcription factors, 23 other regulators, and 22 orphans). Based on the orthology analysis, we identified 1,211 putative PCW genes in *S. viridis*, including genes involved in biomass recalcitrance such as *BAHD* acyltransferases, xylan arabinosyltransferases (*XAT*), and caffeic acid O-methyltransferase (*COMT*). 174 PCW genes were differentially expressed in at least one contrast (fold-change > 2) and were associated with 28 different KEGG pathways, including seven *XAT* homologs, *COMT1*, TRs, and CAZymes. As main conclusions, we have raised candidate PCW genes for further comparative genomic and transcriptomic analyses, and planning functional wet lab experiments.
**Key-words:** Comparative genomics; Transcriptomics; Plant cell wall; Grasses; Leaf development

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# ANÁLISE EM ESCALA GENÔMICA DE PROTEÍNAS CONTENDO DOMÍNIO SKIP/SNW EM PLANTAS

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**Abstract:**
Since the beginning of mankind, it has enjoyed the benefits provided by plants. Despite the complex mechanisms used by plants to ensure their survival, many species still have their development impaired by various environmental stresses. Cowpea (Vigna unguiculata [L.] Walp) represents an important food and socioeconomic source for the Northeast region of Brazil, with its productivity affected in more 80% by Cowpea Severe Mosaic Virus (CPSMV), depending on the stage of development in which the plants are infected. In order to understand the molecular mechanisms involved in such plant-pathogen interaction, Paiva et al. (2016), through label-free proteomic analysis, observed that the success of CPSMV infection occurs due to a transient suppression in the synthesis of host proteins, among which, an ortholog of GAMYB-BINDING PROTEIN. The main objective of this research was to identify and characterize, on a genomic scale, proteins containing the SKIP/SNW domain, occurring in GAMYB-BINDING PROTEIN, in different plant species, including plants of agronomic interest. The *in silico* analyzes conducted in our study allowed us to reconstruct the molecular phylogeny of SKIP/SNW domain-containing proteins, to determine the structure of their corresponding coding genes and the architecture of conserved motifs, as well as to predict cis-elements present in gene promoters. Therefore, the findings of this work expand the knowledge about the structure and putative role of proteins containing the SKIP/SNW domain, which may help in the development of plants tolerant to a plethora of environmental adversities.
**Key-words:** Plant Genomics; GAMYB-BINDING PROTEIN; Synteny; Phylogeny;

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# ANÁLISE FUNCIONAL E PROSPECÇÃO DE MARCADORES MICROSSATÉLITES EM *BATIS MARITIMA*

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**Abstract:**
*Batis maritima* is an edible, shrubby halophyte, found in salty areas flooded by the Atlantic Ocean, measuring between 10 - 40cm in height, with woody stems and succulent leaves. *B. maritima* supports severe abiotic conditions, such as high temperatures and salinities. This work aimed to prospect microsatellite regions and the main domains of proteins in Batis maritima genome. DNA isolation was performed from leaves using the CTAB method. The Oxford nanopore platform was used for partial sequencing of the species genomes.Microsatellite regions and the main protein domains were prospected with specific pipelines. The data generated from the sequencing were used in the GMATA software to prospect dimer and trimer repeat motifs. The parameters employed to identify SSR loci were with at least 6 repeats for di and tri-nucleotides. This software generates files with the number of potential markers and their characteristics, such as expected size, primer pairs and repetition motives. 11237 di and trimer markers were found, in which 9704 it was possible to obtain the design of primers through the software. The mena repeated protein domains found werePentatricopeptide repeat, Leucine-rich repeat, E MOTIF, WD40 repeat, and Mitochondrial substrate/solute carrier. The main protein domains found were Reverse transcriptase zinc-binding domain, Reverse transcriptase domain, Reverse transcriptase, RNA-dependent DNA polymerase, Protein kinase domain, Integrase, catalytic core, while the main families were Endonuclease/exonuclease/phosphatase superfamily, Tetratricopeptide- such as helical domain superfamily, P-loop containing nucleoside triphosphate hydrolase, H-like ribonuclease superfamily, protein kinase-like domain superfamily. These proteins are linked to the resistence of *B. Maritima* to such harsh abiotic conditions. In this work, it was possible to prospect microsatellite markers (SSR), which may help in studies of genetic diversity, as well as in mapping and assisted selection by molecular markers, in addition to finding the main protein domains, families and repeated regions, distributed along of the genome.
**Key-words:** Genomics; Sequencing; Bioinformatics; ;

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VIII Simpósio Brasileiro de Genética Molecular de PLANTAS

# Transdução de Sinais em Plantas

# ATIVAÇÃO DO CIRCUITO DE SINALIZAÇÃO ANTIVIRAL NIK1-RPL10-LIMYB POR ESTRESSES ABIÓTICOS

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**Abstract:**
The nuclear shuttle protein-interacting kinase 1 (NIK1) is a leucine-rich repeat receptor-like kinase (LRR-LRK), which functions as a positive and negative regulator of antiviral and antibacterial immunity, respectively. Upon begomovirus infection, NIK1 is phosphorylated by interaction with an unknown pattern recognition receptor (PRR), which recognizes viral nucleic acids and activates NIK1 kinase. Activated NIK1 initiates a signaling cascade that induces the phosphorylation and translocation of the ribosomal protein RPL10A to the nucleus, where it interacts with the transcription factor L10-interacting Myb domain-containing protein (LIMYB). The complex LIMYB-RPL10 represses the expression of ribosomal protein genes and translation initiation factors, ultimately suppressing global translation as an antiviral immune mechanism. NIK1 downregulates PAMP-triggered immunity (PTI) by interacting with the flagellin receptor FLS2 and its co-receptor BRI1-associated kinase 1 (BAK1).As NIK1 interacts with several RLKs, it may function as a coreceptor for different stress-sensing receptors, activating different stress pathways in plants. To address this hypothesis, we monitored the activation of the NIK1/RPL10/LIMYB signaling module in response to abiotic stresses, such as heat and osmotic stress. After the induction of heat stress at 38°C in Arabidopsis, NIK1 the phosphorylation of immunoprecipitated NIK1 was confirmed 10 minutes after exposure to heat, whereas 10% PEG promoted NIK1 phosphorylation at 25 minutes after treatment. NIK1 phosphorylation was monitored using an anti-phosphoserine antibody and prepared anti-NIK1 serum. Following NIK1 activation, heat and osmotic stress promoted RPLL10A and LIMYB phosphorylation. The activation of these downstream components was confirmed by examining the expression of some target genes. Heat (1h) and PEG treatment (3h) repressed the expression of the ribosomal protein genes *L13* and *S25* and initiation factor genes *Ef1β* and *eIF2*-α in Col-0 but not in the *limyb-32* lines, which retained NIK1 phosphorylation induced by heat and osmotic stress. These results confirmed that the heat- and osmotic stress-induced NIK1 activation requires LIMYB to repress the expression of translation machinery-related genes As osmotic stress is a dehydration component, we also examined whether NIK1 activation would protect plants under drought. The wild type Col-0, null mutants *nik1-1*, *nik2-1* (functional paralog of NIK1), *nik1/nik2* double mutant, *limyb-32* and the superactive NIK1-T474D mutant-expressing line were grown for 30, and then subjected to drought. When reaching TRA of approximately 40%, rehydration was performed to100% of field capacity. The lowest plant survival rate was observed for the double knockout *nik1/nik2*, while100% of the NIK1-T474D mutant-expressing plants survived the stress period. The *nik1-1* and *nik2-1* mutants displayed a survival rate much lower than observed in Col-0, being more accentuated in *nik1-1*. These results suggest that the NIK1-mediated control of translation under persistent stress may confer a certain level of plant tolerance to drought. We are currently monitoring the effect of NIK1 signaling activation under heat stress.
**Key-words:** NIK1; Antiviral immunity ; Antibacterial immunity ; Abiotic stress;

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# GLUTATIONA PEROXIDASE-LIKE 8 (GPXL8) DE ARABIDOPSIS ATUA COMO UMA SENSORA DE $H_2O_2$ E OXIDA PROTEÍNAS ALVO

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**Abstract:**
Glutathione peroxidases (GPXs) are antioxidant enzymes that catalyze the reduction of hydrogen peroxide ($H_2O_2$) or organic peroxides to water or alcohols, using either glutathione (GSH) or thioredoxins (TRXs) as reducing agents. Although animal and non-animal GPXs share a common ancestor, they present different enzymatic mechanisms, structures and reducing agent preferences. Because plant and yeast GPXs use TRXs as reductants, they were termed GPX-like (GPXL). Yeast GPXL3p (*syn.* Orp1p) can oxidize the transcription factor Yap1 by reacting with $H_2O_2$. Ultimately, this increases the expression of genes encoding proteins involved in antioxidative defense. This suggests that GPXLs may have other functions besides peroxide reduction. *Arabidopsis thaliana* GPXL8 is localized in the cytosol and nucleus. Although its role in reactive oxygen species (ROS) detoxification has been described previously, little is known about its involvement in ROS sensing and signaling. Therefore, our main goals are to identify proteins interacting with GPXL8 and determine whether and how GPXL8 oxidizes such proteins. To evaluate the ability of GPXL8 to react with $H_2O_2$ and oxidize target proteins, we used a reduction-oxidation-sensitive GFP (roGFP2) as an artificial GPXL8 target protein. *In vitro* assays display that GPXL8 can oxidize roGFP2, showing that it possesses a thiol oxidase activity. Substitution of the resolving cysteine (C89S) or the central cysteine (C70S) close to the active site revealed that both are involved in regulating and limiting the oxidation of roGFP2, but only the peroxidatic cysteine (C41) is essential for the GPXL8 thiol oxidase activity. In contrast, both resolving and peroxidatic cysteines are necessary for the peroxidase activity, suggesting different catalytic mechanisms. GPXL8 putative interactors were identified by the TurboID-based proximity labeling method. Our preliminary findings indicate that GPXL8 can interact with proteins involved in several biological processes, but further studies are necessary to confirm these interactions and their biological relevance. Through this project, we expect to expand our understanding of how plants relay information from $H_2O_2$ to target proteins in response to various environmental stimuli.
**Key-words:** Glutathione peroxidase; ROS; roGFP2; TurboID; Redox relay

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# IDENTIFICAÇÃO GENÔMICA DOS COMPONENTES CENTRAIS DA SINALIZAÇÃO ABA E A ANÁLISE TRANSCRITÔMICA REVELAM CIRCUITOS GÊNICOS ENVOLVIDOS NA RESPOSTA À SECA EM MAMONA (RICINUS COMMUNIS L.).

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**Abstract:**
Castor bean (Ricinus communis L.) oil-content seeds have a high commercial value in the cosmetic, pharmaceutical, and even bioenergetics industries. Castor bean can survive long periods of water deficit and high temperatures. Therefore, it has been recognized as a drought-tolerant species, allowing the study of gene networks involved in drought response and tolerance. Gene families related to abscisic acid (ABA) signaling play a crucial role in the developmental and environmental adaptation processes of plants, being specially relevant to water stress response. However, the core components of ABA signaling, as well as gene networks related to drought response, are still not well understood in castor beans. This work aims to identify and characterize putative genes of ABA signaling pathways in *Ricinus communis L* and their general response to drought. Our analysis identified 7 *RcPYL*, 63 *RcPP2C*, and 6 *RcSnRK2* genes in castor bean genome, which was further characterized by chromosomal distribution with synteny regions, gene structure, evolutionary relationships, and conserved motif analyses. The castor bean general expression profile was investigated by RNAseq in root and leaf tissues in response to drought stress. These analyses allow the identification of genes differentially expressed, including genes from the central core ABA signaling, genes related to photosynthesis, cell wall, energy transduction, antioxidant response, and transcription factors. These analyses provide new insights into the core components of ABA signaling in castor beans, allowing the identification of several molecular responses associated with the high physiological adaptation of castor bean to drought stress. Besides that, it contributes to the identification of candidate genes for genetic improvement.
**Key-words:** Abscisic acid; Castor bean; PP2C; ABA; drought stress response

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# Author Index

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# VIII Simpósio Brasileiro de Genética Molecular de PLANTAS

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