

IV International Meeting on Plant Breeding

“Uncovering the power and the secrets behind DNA modifications”

PROCEEDINGS



October – 2020

THE INTERNATIONAL MEETING ON PLANT BREEDING

Plant breeding is one of the fundamental strategies for the development of more adapted and productive cultivars, particularly in a world surrounded by uncertainty on food security. Given this scenario, scientists have been putting great efforts into the development of tools that could help to overcome challenges faced every day in the field. Therefore, we feel that discussions around these new tools are important to build next-generation breeders who will make them useful and effective, ensuring its correct application within research and breeding.

The International Meeting on Plant Breeding is one of several events that compose the "Corteva Agriscience Plant Science Symposia Series". In this fourth edition, the topic we intended to address was the world of DNA modifications and gene editing, which has been widely discussed within breeders, geneticists, biotechnologists in the last years. Recent advances have been done regarding genome manipulation, showing itself as a potential tool for many breeding programs. That said, we wanted to go deeper into that discussion and provide a propitious environment for updates and propagation of information.

GVENCK

The Genetics and Plant Breeding Group “Prof. Roland Vencovsky” (GVENCK) is composed by graduate and undergraduate students in Genetics and Plant Breeding at “Luiz de Queiroz” College of Agriculture (ESALQ/USP), under coordination of Professor Dr. José Baldin Pinheiro. Our mission is to integrate academics, professors and professionals with the goal of improving the training of future breeders and geneticists.

The main activities of the group are:

- Organization of scientific and training events;
- Promotion of discussion on relevant topics in genetic and plant breeding;
- Technical visits to companies and public research institutions;
- Promote the guidance of young talents under training from the “alumni voice”, in which the alumni with consolidated careers will share professional experiences;
- Promote moments and opportunities for interaction between students, professors and researchers outside the university;
- Establishment of partnerships with companies and public institutions.

ORGANIZATION

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ACKNOWLEDGEMENTS

The organizing committee is thankful for all the support provided by Corteva Agriscience, ESALQ (“Luiz de Queiroz” College of Agriculture), Department of Genetics, The graduate program in Genetics and Plant Breeding.

PROGRAM:

September 30th, 2020 - Wednesday:

13:30 - 14:00 → **Jason Rauscher** - Opening session (Corteva)

14:00 - 15:00 → **Agustin Zsögön** - "De novo domestication of wild species: a platform to create more nutritious and resilient crops" (Federal University of Viçosa - UFV)

15:00 - 16:00 → **Daniel Voytas** - "Overcoming Bottlenecks in Editing Plant Genomes" (University of Minnesota)

16:00 - 16:20 → Research Presentation – Alexandre Aono “Assessing the potential of deep learning for sugarcane yellow leaf symptom severity phenotyping”

16:20 - 16:40 → Break – Poster presentation

16:40 - 17:40 → **Yinong Yang** - "CRISPR/Cas-enabled plant genome editing and precision breeding" (Pennsylvania State University)

17:40 - 18:00 → Research Presentation – Iara Gonçalves dos Campos “Major locus for spontaneous haploid genome doubling in exotic maize germplasm detected by a case-control GWAS”

October 1st, 2020 - Thursday

13:30 - 14:30 → **Antonio Costa de Oliveira** - “New Challenges for Cereal Breeding in Southern Brazil” (Federal University of Pelotas - UFPel)

14:30 - 15:30 → **Sandra Milach** - “Predictive Breeding in the Era of Genome Editing” (Corteva)

15:30 - 15:50 → Research Presentation – Marcos Antonio de Godoy Filho “*De novo* assembly of contrasting soybean genomes for the study of resistance to the stink bug complex”

15:50 - 16:20 → Break – Poster presentation

16:20 - 17:20 → **Lázaro Eustáquio Pereira Peres** - “Domestication and *de novo* domestication: tomato as a model.” (Luiz de Queiroz College of Agriculture - ESALQ/USP)

17:20 - 17:40 → Award “Roland Vencovsky”

17:40 - 18:00 → Closing remarks

SPEAKERS

Dr. Agustín Zsögön



Dr. Agustín Zsögön is a geneticist and holds a Masters degree in Plant Physiology and Biochemistry from the University of São Paulo (2006). In 2011 he received a PhD in Plant Biology from the Australian National University, and subsequently conducted post-doctoral research at the University of Sydney and the University of São Paulo. He has been tenured as Assistant Professor of Molecular Plant Physiology at the UFV since 2015. His main research interest is the genetic and functional analysis of domestication and breeding traits in Solanaceae. His work is funded by the Royal Society, UK Research and Innovation and the Alexander von Humboldt Foundation.

Dr. Antonio Costa de Oliveira

Dr Antonio Costa de Oliveira is an agronomist, with a Master of Science degree in Genetics and Plant Breeding from University of São Paulo and a PhD in Genetics (Purdue University - 1996), and also a Post-Doctorate from University of Georgia – 2007. Dr Antonio is currently a Professor of Genetics and Plant Breeding at FAEM / UFPel-Brazil, as well as PQ- Researcher 1A. He was President of the International Crop Science Society (2008-2012) and is currently the President of the Brazilian Society of Plant Breeding (SBMP). Dr Antonio has expertise in the field of Agronomy, with emphasis in Vegetal Breeding, working in: genomics applied to cereals, quantitative traits, variability and breeding. He has various international collaborations with groups from USA, Italy, Spain, France, Portugal, UK and Mexico.



Dr. Dan Voytas



Dr. Daniel Voytas is a Professor in the Department of Genetics, Cell Biology and Development and the Director of the Center for Precision Plant Genomics at the University of Minnesota. Dr. Voytas graduated from Harvard College in 1984 and received his Ph.D. from Harvard Medical School in 1990. He conducted postdoctoral research at Johns Hopkins University School of Medicine where he was a fellow of the Life Science Research Foundation. Prior to joining the University of Minnesota in 2008, Dr. Voytas was a professor at Iowa State University. Dr. Voytas' research focuses on developing methods to edit plant genomes. His laboratory developed a powerful genome editing reagent – Transcription Activator-Like Effector Nucleases (TALENs) – which was heralded by *Science* magazine as one of the top ten scientific breakthroughs of 2012. Dr. Voytas' lab is currently optimizing methods for efficiently making targeted genome modifications in a variety of plant species to advance basic biology and develop new crop varieties.

Dr. Lázaro Peres



Dr. Lázaro Eustáquio Pereira Peres is a Professor of Plant Physiology at the University of São Paulo (USP), Brazil, where he also received his PhD. His main research interest is plant development and its interaction with the environment, using tomato as a model. Dr. Peres has established and curates a large collection of induced mutants, natural genetic variation and transgenic plants in the model system tomato cv. Micro-Tom, which are being used worldwide for the study of physiological mechanisms and the genes behind them. Some of the genes studied in the Dr. Peres's lab have alleles that are exclusive from tomato related wild species, representing valuable genetic resources for fundamental studies on plant adaptation, domestication and breeding.

Dra. Sandra Milach



Sandra Milach had a Bachelor of Science degree in Agronomy from Federal University of Pelotas, Brazil, followed by a Master of Science degree in Genetics and Plant Breeding from the Federal University of Rio Grande do Sul, Brazil. She then earned her doctorate in Genetics and Plant Breeding from the University of Minnesota. Sandra leads Systems & Innovations for Breeding and Seed Products within the Research & Development organization for Corteva Agrisciences. Sandra is responsible for enabling and accelerating breeding and seed product development systems through applied science and technologies. Since January 2016, she leads the Global Breeding and Marker Technologies and now to Systems and Innovations for Breeding and Seed Products. Prior to joining the organization, Sandra was a Professor of Plant Genetics and Breeding at Federal University of Rio Grande do Sul and also worked at EMBRAPA, leading molecular breeding efforts in wheat and barley.

Dr. Yinong Yang



Yinong Yang is a Professor in the Department of Plant Pathology and Environmental Microbiology and the Huck Institutes of Life Sciences at the Pennsylvania State University. He received a BS in biology from Hangzhou University in 1982, an MS in botany from University of South Florida in 1990, and a PhD in plant molecular and cellular biology from University of Florida in 1994. After a postdoctoral position in Waksman Institute at Rutgers University, he became an assistant and associate professor at the University of Arkansas. He joined the faculty at Penn State

in 2006 and has been working in the areas of molecular plant-microbe interactions and functional genomics. His recent studies focus on improving CRISPR/Cas genome editing technology and its broad applications in genome engineering and precision breeding of agricultural crops.

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ASSESSING THE POTENTIAL OF DEEP LEARNING FOR SUGARCANE YELLOW LEAF SYMPTOM SEVERITY PHENOTYPING

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The proper identification of plant disease symptoms is crucial to avoid associated crop losses. Sugarcane yellow leaf (SCYL), caused by the SCYL virus (SCYLV), is a major disease of sugarcane. Its symptoms are easily mistaken for other stresses, and grading SCYL severity is troublesome. We present a novel, image-based proposal for automating the assessment of SCYL severity, using a panel of 97 sugarcane genotypes planted in a randomized complete block design with three replicates and infected with SCYLV. Plants were assessed for SCYL severity by three evaluators using a four-level scale, and one top visible dewlap leaf per row was collected and had its abaxial and adaxial surfaces photographed on a black background with a Nikon D3100 camera. 48 additional leaves representing severity levels were randomly collected among different plants and photographed the same way. All the images were preprocessed with the following steps using OpenCV library: median blur and Otsu's binarization; identification of connected components and selection of the region of interest; image segmentation; abaxial and adaxial surfaces' joining. Using Python sklearn and keras libraries, we compared six state-of-the-art convolutional neural network architectures for feature extraction (ResNet50, MobileNet, InceptionV3, VGG16, VGG19 and InceptionResNetV2), coupled with eight machine learning classification models (K-nearest neighbors, support vector machine, Gaussian process, decision tree, random forest, multilayer perceptron neural network, adaptive boosting and Gaussian naive Bayes) and three feature selection (FS) strategies (gradient tree boosting, L1-based FS through a linear support vector classification system and univariate FS using ANOVA) for predicting SCYL symptom severity. Predictive accuracies were assessed using leave-one-out and 10-fold cross validation strategies. We found ResNet-50 as the most promising deep learning strategy for feature extraction, and coupled with gradient tree boosting for FS and Gaussian naive Bayes algorithm enabled the achievement of accuracies up to 90%. This represents the first attempt of predicting SCYL symptom severity based on images and has a high potential to be expanded to other diseases that severely affect sugarcane.

Keywords: Image processing; machine learning; SCYLV.



4th IMPB INTERNATIONAL MEETING ON PLANT BREEDING UNCOVERING THE POWER AND THE SECRETS BEHIND DNA MODIFICATIONS

ASSESSING THE POTENTIAL OF DEEP LEARNING FOR SUGARCANE YELLOW LEAF SYMPTOM SEVERITY PHENOTYPING

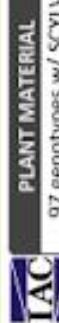
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INTRODUCTION

Plant diseases are a major threat in agriculture. Sugarcane yellow leaf (SCYL), caused by the sugarcane yellow leaf virus (SCYLV), is an important disease leading to considerable yield losses. Identifying SCYL symptoms and grading their severity is troublesome and requires trained personnel.

MATERIAL AND METHODS



PLANT MATERIAL
97 genotypes w/ SCYL

Randomized complete block design
Plots with 3 rows, 3 replicates

TRADITIONAL PHENOTYPING

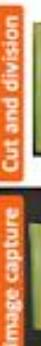


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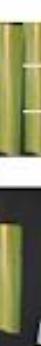
IMAGE PROCESSING



Image capture



Cut and division



Segmentation



Abaxial/Adaxial



Feature selection techniques



Gradient tree boosting (FS1), L1-SVM based (FS2), ANOVA (FS3)

Python OpenCV library

Python scikit-learn libraries

COMPUTER BASED CLASSIFICATION



Deep learning feature extraction
ResNet50, MobileNet, InceptionV3, VGG16, VGG19, InceptionRNv2



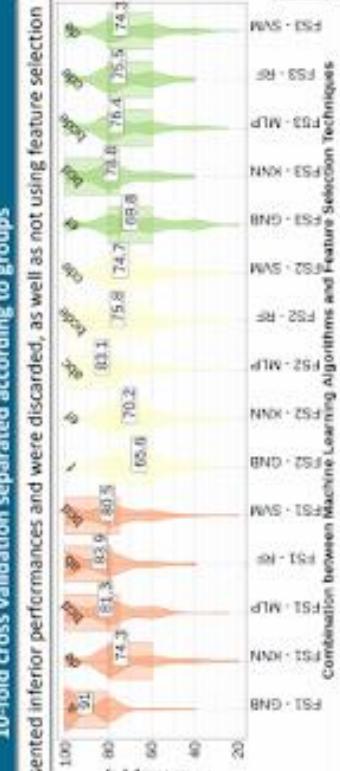
Machine learning (ML) classification
K-nearest neighbors (KNN), Gaussian process (GP), random forest (RF), AdaBoost (AD), support vector machine (SVM), decision tree (DT), multilayer perceptron (MLP), Gaussian naive Bayes (GNB)



Phenotype

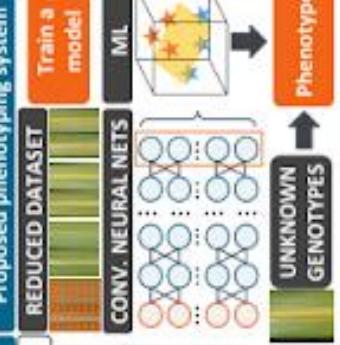
RESULTS

10-fold cross validation separated according to groups



Leave-one-out cross validation

ResNet50 established as the most promising strategy



Proposed phenotyping system
REDUCED DATASET
ResNet50 images
Conv. Neural Nets
UNKNOWN GENOTYPES
Phenotype

Reduced dataset
ResNet50 images
Conv. Neural Nets
UNKNOWN GENOTYPES
Phenotype

Reduced dataset
ResNet50 images
Conv. Neural Nets
UNKNOWN GENOTYPES
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Reduced dataset
ResNet50 images
Conv. Neural Nets
UNKNOWN GENOTYPES
Phenotype

Comparison between Machine Learning Algorithms and Feature Selection Techniques

F51 - GNB

F52 - MLP

F53 - KNN

F54 - RFN

F55 - SVM

F56 - RF

F57 - FS1

F58 - FS2

F59 - FS3

F60 - FS4

F61 - FS5

F62 - FS6

F63 - FS7

F64 - FS8

2

COMPATIBILITY OF INTER-SPECIFIC CROSSINGS IN *PHYSALIS* SPECIES

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The *Physalis* L. (Solanaceae) is a fruit plant that contains more than 100 species with high added value fruits, which have relevant physicochemical properties. Species of this genus have so far been little explored in studies aimed at the development of superior genotypes. Thus, it is necessary to start breeding programs that aim in the near future to develop more productive and competitive genotypes. In view of the aforementioned information and in order to provide technical and scientific bases for breeding programs, the objective was to determine the rate of artificial crossbreeding fixings between seven species of *Physalis*. All species were crossed with each other acting as pollen donors and receivers, thus carrying out bidirectional crossings. In addition to the crossings, a self-pollination of the parents was also performed, totaling 49 combinations. An accession of each species was used: *Physalis peruviana*, *Physalis ixocarpa*, *Physalis minima*, *Physalis pubescens*, *Physalis angulata*, *Physalis pruinosa* and *Physalis daturaefolia*. Ten plants from each accession were conducted in a greenhouse and in pots with a volume of 08 dm³. From the beginning of the reproductive stage, for the plants that acted as receptors, emasculation was carried out before the dehiscence of the anthers, avoiding self-pollination. For the pollen collection, freshly opened flowers were used and the artificial crossing was performed immediately, identifying each crossing with line marking and covering with paper. The fixation of artificial crosses was determined by counting the fruits formed from pollination. There was fruit fixation in most interspecific combinations. The greatest highlights were for the species *P. peruviana* and *P. pubescens*, as pollen receptors, showed high rates of fruit fixation in combination with all other species, except when they received pollen from *P. minimum* and *P. daturaefolia*. When the species *P. ixocarpa* and *P. angulata* were used as female parents, there was compatibility to fix fruits with all the other species, however, the fruit fixation index was mostly less than 40%. The *P. daturaefolia* as a pollen donor in combination with *P. minimum* and *P. pubescens*, did not provide fruit fixation. The same aspect occurs when pollen from *P. peruviana* was combined with *P. pruinosa* is also the combination of pollen from *P. pruinosa* in *P. daturaefolia*. And finally, it had self-compatibility for all *Physalis* species explored, with highlights for *P. peruviana* and *P. pubescens*, which obtained 100 and 94% of fruit fixation, respectively. The biggest problems of incompatibility between *Physalis* species are related to *P. daturaefolia* as a pollen donor, which had fruit fixation in only a few combinations. All *Physalis* species explored were self-compatible and there was fixation fruits for most interspecific combinations.

Keywords: Solanaceae; artificial crosses; interspecific hybrids; genetic pre-breeding.

Acknowledgments: FAPESP- (process number 2019/15378-5).

Introduction

The *Physalis* L. (Solanaceae) is a fruit plant that contains more than 100 species with high added value fruits, which have relevant physiochemical properties. Species of this genus have so far been little explored in studies aimed at the development of superior genotypes.

Objective

The objective was to determine the rate of artificial crossbreeding fixings between seven species of *Physalis*.

Materials and methods

All species were crossed with each other acting as pollen donors and recipients. In addition to the crossings, a self-pollination of the parents was also performed. An accession of each species was used: *Physalis peruviana*, *P. ixocarpa*, *P. minima*, *P. pubescens*, *P. angulata*, *P. pruinosa* e *P. daturaefolia*. Since the beginning of the reproductive phase, emasculation was performed before dehiscence of the anthers, avoiding self-pollination. For the pollen collection, freshly opened flowers were used and the artificial crossing was performed immediately. The fixation of artificial crosses was determined by counting the fruits formed from pollination.

Results and discussion

Table 1. Fruit fixation index to interspecific crosses of *Physalis* sp.

Male parent	Female parent					Conclusion	
	<i>P. peruviana</i>	<i>P. ixocarpa</i>	<i>P. minima</i>	<i>P. pubescens</i>	<i>P. angulata</i>	<i>P. pruinosa</i>	
<i>P. peruviana</i>	94.00	48.00	24.00	64.00	72.00	0.00	16.00
<i>P. ixocarpa</i>	80.00	70.00	24.00	100.00	28.00	16.00	4.00
<i>P. minima</i>	8.00	12.00	58.00	16.00	28.00	8.00	8.00
<i>P. pubescens</i>	76.00	12.00	8.00	100.00	20.00	10.00	8.00
<i>P. angulata</i>	76.00	40.00	12.00	84.00	64.00	32.00	12.00
<i>P. pruinosa</i>	76.00	4.00	4.00	72.00	24.00	68.00	0.00
<i>P. daturaefolia</i>	4.00	8.00	0.00	0.00	8.00	4.00	76.00

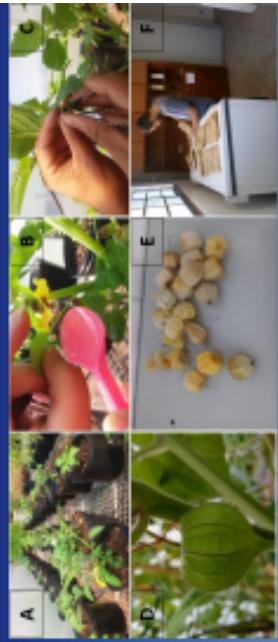


Figure 1. *Physalis* plants kept in a greenhouse (A), collecting pollen from flowers (B), emasculation of flower buds (C), fruit of interspecific crossings successfully fixed (D) and counting and separating the fruits of the cross (E and F).

The biggest problems of incompatibility between *Physalis* species are related to *P. daturaefolia* as a pollen donor, which obtained fruit fixation in only a few combinations.

All *Physalis* species explored were self-compatible and there was fixation fruits for most interspecific combinations.

Acknowledgments

FAPESP- (process number 2019/15378-5)

DE NOVO ASSEMBLY OF CONTRASTING SOYBEAN GENOMES FOR THE STUDY OF RESISTANCE TO THE STINK BUG COMPLEX

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Wei Wei²; Steven J. Clough^{2,4} and José Baldin Pinheiro¹

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Soybean is an important commodity in Brazil. In 2019, the soybean and its derivatives accounted for \$ 34,78 billion in exports. However, there are some obstacles to achieving greater soybean yield. Insect infestation in soybean fields, particularly by stink bug complexes, reduces crop yield and can transmit pathogens. An alternative to the use of insecticides is the development of resistant cultivars. For a better understanding of the soybean resistance to the stink bug complex, the genomes of soybean cultivar IAC-100 (tolerant) and cultivar CD-215 (susceptible) were sequenced and *de novo* assembled. Additionally, a genotyping-by-sequencing (GBS) analysis was conducted of a soybean population of 236 recombinant inbred lines derived from the crossing of the two cultivars (CD-215 x IAC-100). Together, we used these sequence data to identify variants in the population that were associated with the inheritance of resistance. For the genome sequencing, we used the 10X Genomics' Chromium technology. The *de novo* genome assemblies were accomplished using the software Supernova 2.1.1, 1.0.17, Ragoo 1.1, and REAPR. It was possible to generate assemblies with N50 scaffolds near 54 MB for both genomes, and account or approximately 1 GB of the genomes. Using NUCmer, we could see that our genomes have high synteny with the soybean reference Williams 82 and that our genomes also have gene numbers and repetitive elements very close to these numbers found in Wiliams 82 (approximately 58 thousand genes and 44% repetitive elements). To identify polymorphisms, we used GATK, found 290.189 unique SNPs in IAC-100 when compared to CD-215 and 335.229 unique INDELs. Analyzing the GBS data for the 25 most resistant genotypes, selected from past experiments involving resistance to the stink bug complex, we found 852 SNPs, shared between the selected genotypes and with the IAC-100. Several SNPs putative associated with resistance were found, revealing haplotypes with QTLs reported in studies not yet published by our group. We analyzed 3 haplotypes with QTLs, where we found large structural variants (>1Kb) putative, present only in the resistant cultivar, next to these variants we find candidate genes that are part of the route of resistance related compounds, such as phenylpropanoid/lignin and terpenes, we also found R genes and a possible gene related to a number of pods in resistant genotypes. In addition to providing two Brazilian soybean genomes with the best N50 scaffolds reported to date, these results open the door to several other studies and applications in plant breeding, such as fine mapping, marker-assisted selection, genome selection, expression of candidate genes, and genetic modifications using methods such as CRISPR/Cas9 technology.

Keywords: 10x chromium; variants; recombinant inbred lines; Supernova; linked-reads.

4th IMPB INTERNATIONAL MEETING ON PLANT BREEDING
DE NOVO ASSEMBLY OF CONTRASTING SOYBEAN GENOMES FOR THE STUDY OF RESISTANCE TO THE STINK BUG COMPLEX



Marcos Antônio de Oliveira Lobo^a, André Luiz Costa^a, Luciano dos Santos^a, São Paulo State University, São Paulo, Brazil; and José Augusto Vazquez^b, UFG do Oeste, Quiririm, Outubrasal Agriculture, University of Minas Gerais, Belo Horizonte, Brazil; and José Augusto Vazquez^c, USP Department of Agriculture, Piracicaba, São Paulo, Brazil.

Introduction

Soybean is the main commodity in Brazil. In 2018, soybean and its derivatives accounted for US\$40.9 billion in exports. However, there are some obstacles to obtaining a higher yield, such as attack by the complex of stink bugs. These insects diminish the yield of the crop and can transmit pathogens, and it stands out as a major source of yield loss. The development of resistant cultivars would therefore be beneficial.

Objective

For a better understanding of the genetic architecture involved in the resistance of soybean to the stink bug complex, the genomes of soybean cultivar IAC-100 (tolerant) and CD-215 (susceptible) were sequenced and assembled. SNPs found were compared with genotypes from a RILS population originated from the cross between IAC-100 and CD-215.

Materials and methods

- 10X Platform and illumina NovaSeq-6000
- Supernova - Assembly
 - Anchoring, ordering and breaking chimeric contigs using Ragoo. Error correction with REAPR and new ordering
 - Evaluation genome assembly with single-copy orthologs genes using Busco
 - Evaluation of assembly synteny with Williams82 using NUCmer
 - Annotation using repeatmasker and AUGUSTUS
 - Calling variants with GATK for genomes and 236 Genotypes of a RILs population genotyping with GBS5

UNCOVERING THE POWER AND THE SECRETS BEHIND DNA MODIFICATIONS

DE NOVO ASSEMBLY OF CONTRASTING SOYBEAN GENOMES FOR THE STUDY OF RESISTANCE TO THE STINK BUG COMPLEX

Marcos Antônio de Oliveira Lobo^a, André Luiz Costa^a, Luciano dos Santos^a, São Paulo State University, São Paulo, Brazil; and José Augusto Vazquez^b, UFG do Oeste, Quiririm, Outubrasal Agriculture, University of Minas Gerais, Belo Horizonte, Brazil; and José Augusto Vazquez^c, USP Department of Agriculture, Piracicaba, São Paulo, Brazil.

Identification of variants shared between the resistant

- Genome and the 25 most resistant RILs
- Manual identification of large structural variants (> 1 Mb) unique in IAC-100, in haplotypes with QTLs [3 selected QTLs]

Results and discussion

Genome	IAC-100	CD-215	Williams 82
Assembly	1,053 Mb	1,061 Mb	979 Mb
Scaffold N50	53.6 Mb	54.2 Mb	46.7 Mb
CG content	~34%	~33%	~35%
Scaffolds number	296	314	1192
Gene Number	58,444	57,946	59,847
Repetitive content	44%	44%	43%
Busco -C	97.74	97.33	98.18

Supernova - Assembly

- Anchoring, ordering and breaking chimeric contigs using Ragoo. Error correction with REAPR and new ordering
- Evaluation genome assembly with single-copy orthologs genes using Busco
- Evaluation of assembly synteny with Williams82 using NUCmer
- Annotation using repeatmasker and AUGUSTUS
- Calling variants with GATK for genomes and 236 Genotypes of a RILs population genotyping with GBS5



Conclusion

Among the variants found in this work, some may be associated with resistance to the stink bug complex. These variants will be used to genotype populations for association studies with the goal of identifying resistance associated regions. The use of 10x Chromium technology with this pipeline can be a great alternative to obtain genomes at a low cost, allowing a great advance for studies of specific characteristics. In addition, we provide two Brazilian soybean genomes, with the best scaffolding N50 reported to date.

EFFECT OF THE HETEROtic GROUP ON THE HAPLOID INDUCTION RATE IN TROPICAL MAIZE

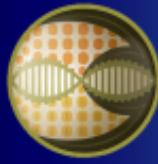
Gabriela Romêro Campos¹; Pedro Henrique de Souza¹; José Felipe Gonzaga Sabadin¹; Roberto Fritsche Neto¹

¹Universidade de São Paulo, Escola Superior de Agricultura “Luiz de Queiroz”, Piracicaba, SP, Brazil. E-mail: campos.gab@usp.br.

The double haploid (DH) technology can significantly reduce the time and cost of obtaining maize lines, which *in vivo* haploid inducers being the principal method in this context. The identification of haploids occurs mainly through morphological markers, with *R1-Navajo* (*R1-nj*) being the most used, responsible for the expression of anthocyanin in the endosperm and embryo of the seeds. So far, there are no inductors adapted to tropical climatic conditions and open to the scientific community and small companies. Besides, significant differences in induction rates between the flint and dent groups are reported in the literature. Therefore, we aimed to verify haploidy induction differences by the LI-ESALQ population in the flint and dent heterotic groups. We evaluated individuals from two cycles of intrapopulation recurrent selection between the LI-ESALQ inducer, which has the *R1-nj* gene. As external testers, we used two simple-crosses, those from different heterotic groups. The selection was made using an index, considering the putative and real haploid induction rate and the expressiveness of the *R1-nj* marker, where the phenotypic values of each characteristic were standardized and weighted according to their importance (0.25, 0.5, and 0.25 respectively). There were differences in the expressiveness of *R1-nj* and the induction of haploid regarding the dent and flint groups. The dent group obtained a higher average rate of putative haploid seeds in cycles 0 and 1 (5.59% to 0.71% and 3.86% to 1.16%, respectively), as well as greater expressiveness of *R1-nj* (98.25% compared to 50.15% in cycle 0 and 99.04% compared to 54.52% in cycle 1). However, the flint group obtained a higher average rate of real haploid seeds (0.67% to 0.38% in cycle 0 and 2.79 to 1.10% in cycle 1). Because of these variations, differences may also occur in selecting the best inducing genotypes, if targeted to a specific heterotic group. As the inducer's demand is not straightforward, the improvement must be carried out considering both groups into account, avoiding distortions in the efficiency of the inducing materials, which can occur in segregating populations from crosses between lines from different groups.

Keywords: Double haploid; *R1-navajo*; recurrent selection; heterotic group.

UNCOVERING THE POWER AND THE SECRETS BEHIND DNA MODIFICATIONS



Introduction

The doubled haploid (DH) technology can significantly reduce the time and cost of obtaining maize lines, which *in vivo* haploid inducers being the principal method in this context. The identification of haploids occurs mainly through morphological markers, with *R1-Navarro (R1-n)* being the most used, responsible for the expression of anthocyanin in the endosperm and embryo of the seeds when fertilization is complete (diploid seed). However, when it is haploid, anthocyanin production occurs only in the endosperm, which allows identifying among the progeny seeds which are haploid (putative). So far, there are no inducers adapted to tropical climatic conditions and open to the scientific community and small companies. Besides, significant differences in haploidy induction rates between the flint and dent groups are reported in the literature.

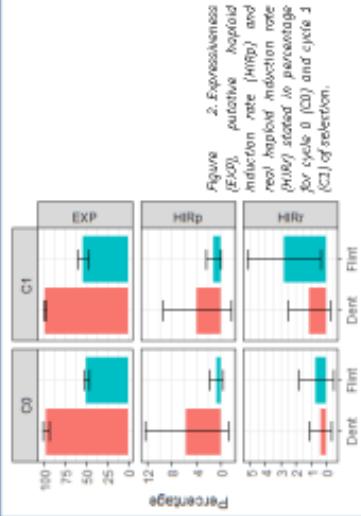
Then we calculated the putative haploid induction rate ($H1Pp$), the expressiveness of *R1-n* ($[EXP]$) and the real haploid induction rate ($H1Rr$). The selection was made using a selection index (I) (equation 1), where the phenotypic values of each trait were standardized and weighted according to their importance.

$$I = 0.5 \times H1Rr + 0.25 \times H1Pp + 0.25 \times EXP \quad (1)$$



Figure 1. C1 (yellow), putative haploid and inhibited seed, respectively.

Results and discussion



Objective

Aimed to verify haploid induction differences by the Li-ESALQ population in the flint and dent heterotic groups.

Materials and methods

We evaluated individuals from two cycles of intrapopulation recurrent selection between the Li-ESALQ inducer. As external testers, we used two single-crosses, those from different heterotic groups. Seeds were classified by an expert researcher according to *R1-n* (Figure 1), and the seeds classified as putative haploids were taken to the field for validation.

There were differences in the expressiveness of *R1-n* and the induction of haploid regarding the dent and flint groups (Figure 2). The expressiveness of *R1-n* is mainly due to the genetic differences between the two germplasms, mainly, *R1-n* inhibition genes present in the flint group, had already reported in the literature, which decreases the marker's expressiveness. There was also a large variation in the rates of haploid induction between and within the different germplasms, as a consequence of the genetic variability existing in the Li-ESALQ population. As a consequence of these variations, there are differences in the selection of the most suited inducing genotypes. Admitting only the flint group, the genotypes selected to compose the next cycle are not the same as those selected for the dent group. So, the index must select genotypes with good performance in both germplasms.

Conclusion

As the inducer's demand is not straightforward, the improvement must be carried out considering both groups into account, avoiding distortions in the efficiency of the inducing materials, which can occur in segregating populations from crosses between lines from different groups.

Acknowledgments

To all from the Laboratory of Allotogenous Plant Breeding who participated and assisted in the development of the project.

FROM *IN SILICO* DATA TO GENOME EDITING: IDENTIFYING RESISTANCE GENES AGAINST *Sporisorium scitamineum* IN ENERGY CANE

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Sugarcane (*Saccharum* sp.) present great economic, environmental, and social importance in the worldwide scenario. Brazil is the largest producer and exporter of the crop. Traditionally used for sugar and ethanol (1^a Generation) production, this scenario has been changing with the diversity of novel applications of its bio-products, such as a source for renewable bioenergy. New genotypes, called energy cane, have been developed by plant breeding programs focused on cellulosic ethanol (2^a Generation). Although productive, such genotypes are susceptible to various diseases and pests, generating yield losses depending on the variety and environmental conditions. Among the diseases that most affect sugarcane, sugarcane smut, caused by the biotrophic fungus *Sporisorium scitamineum*, is considered one of the most relevant and devastating currently. The disease's primarily characteristic symptom is the formation and emergence of a black apical structure covered with fungal spores, called smut-whip. Little information on the genetic resistance basis is available concerning this pathogen for energy cane genotypes, considered all moderately susceptible to the disease. This project aims to evaluate the global transcriptional profile of contrasting energy cane genotypes while infected with *S. scitamineum* and select a set of candidate genes related to genetic resistance against the pathogen to validate their biological function *in planta* via gene editing strategies. Using energy cane genotypes with contrasting levels of smut resistance, RNA-Seq libraries will be built and sequenced. Co-expression networks will be created to select a set of candidate genes related to host resistance against the disease. The expression profile of these genes will be validated using RT-qPCR, and they will be used for genome editing of energy cane plants via CRISPR system, for the first time in sugarcane. Genetically edited plants will be compared and phenotyped with non-transformed plants regarding the number of whips per plant (a), per treatment (b) and time of symptom development (whip emergence) (c). These results will serve as potential *input* for future functional studies on host resistance mechanisms and assist decision-making in plant breeding programs. Such approaches will enable identification and the understanding of mechanisms contributing to resistance in energy cane.

Keywords: Plant pathogen-interaction; Sugarcane; Genome editing; Smut disease.

Acknowledgements: CNPq (142289/2020-5); CAPES (88887.480825/2020-00); FAPESP.

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FROM IN SILICO DATA TO GENOME EDITING: IDENTIFYING RESISTANCE GENES AGAINST SPOROSORIUM SCITAMINEUM IN ENERGY-CANE

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Perspectives

Recently, Rody et al. (2020) (in preparation) generated co-expression networks for sugarcane genotypes with contrasting levels of resistance against *S. scitamineum*. Among all genes, those associated with TFs, RGAs, cell wall, flowering, and transporter were identified as contributors to resistance. A similar approach will be applied for energy cane but focusing on prospecting genes for genome editing in sugarcane, for the first time.

A: Transcription factors (TFs)
B: Transporters
C: Flowering genes
D: Cell wall genes
E: Resistance gene analogs (RGAs)

Materials and methods

1. Plant material and RNA-seq sampling
2. RNA sequencing and data analysis
3. Co-expression network analysis
4. Candidate genes selection and validation
5. Genome editing via CRISPR/Cas system
6. Phenotyping for symptom formation

Introduction

Sugarcane (*Saccharum sp.*) has great economic, social, and environmental importance worldwide. Traditionally used for sugar and ethanol production, sugarcane is currently used as a source of bioenergy. Although productive, bioenergy genotypes, called energy cane, are susceptible to various diseases, including the sugarcane smut disease, caused by the fungus *Sporisorium scitamineum*. Despite the importance of the disease, little information is known for energy cane genotypes.

Objective

Evaluate the global transcriptional profile of contrasting energy cane genotypes while infected with *S. scitamineum* and select a set of candidate genes related to genetic resistance against the pathogen to validate their biological function in plants via genome editing strategies (CRISPR/Cas system).

Preliminary results

Our research group (Genomic's Group) has invested in global approaches (genomics, transcriptomic, proteomics, metabolomics) to obtain information of the host's resistance mechanisms for the sugarcane vs *S. scitamineum* system. With the imminent need to integrate biological data, we assume that the application of concepts derived from Systems Biology for this pathosystem (now for energy cane) will provide specific candidate genes for future functional analysis.

Acknowledgments

FAPESP, CNPq, CAPES

Fig. 1. Sugarcane Bio- products. Life cycle of *S. scitamineum* (Monteiro-Vitorelli et al., 2018).

Fig. 2. Sugarcane smut disease. Life cycle of *S. scitamineum* (Monteiro-Vitorelli et al., 2018).

Fig. 3. Co-expression network. Selected candidate genes for contrasting genotypes of sugarcane against *S. scitamineum*. Such approaches will enable researchers to identify/understand mechanisms that contribute to genotype specialization, as resistant or susceptible, under biotic stress. Also, these results will serve as input for future functional studies as well as assist decision making in plant breeding programs.

GENOMIC REGIONS ASSOCIATED WITH FUSARIUM WILT RESISTANCE IN COMMON BEANS

Jean Fausto de Carvalho Paulino¹; Caléo Panhoca de Almeida¹; Sérgio Augusto Morais Carbonell²; Alisson Fernando Chiorato²; Roberto Fritsche-Neto³; Qijian Song⁴; César Júnior Bueno⁴; Luciana Lasry Bechimol-Reis¹.

¹Instituto Agronômico – CRGV. ²Instituto Agronômico – CGF. ³Universidade de São Paulo – ESALQ. ⁴USDA-ARS - Beltsville USA. ⁴Instituto Biológico – Campinas. E-mail: jeanbiotec@gmail.com.

Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *phaseoli* (*Fop*), is considered the main fungal soil disease in beans. The most economical and efficient method to control this disease is the use of resistant cultivars. The present study aimed to identify common bean genomic regions associated with resistance to *Fop* race 06 through genome wide association studies (GWAS) in a panel containing 205 accessions from the Germplasm Bank of the Agronomic Institute (IAC). The experiment was performed with a randomized block design of 6 replications. The accessions were genotyped with 5,398 high quality SNPs from the BeadChip BARCBean6K_3. The low quality and monomorphic SNPs were filtered with a Minimum Allele Frequency (MAF) of 0.05 (MAF > 0.05), and for the phenotypic analysis, the plants were inoculated by the root-dip method, evaluated after 21 days of inoculation on a scoring scale from 1 to 9 to disease severity and the area under the disease progress curve (AUDPC), and the average of the values obtained implemented in the mixed model REML/BLUE. The genotypic and phenotypic data were analyzed for genome-wide association using the FarmCPU model. The results demonstrated seven significant SNPs on chromosomes Pv01, Pv03, Pv04, Pv08, Pv09 and Pv11 were associated with AUDPC and three significant SNPs severity on chromosomes Pv08, Pv10 and Pv11 with disease severity. These SNPs explained between 10% to 26% of the phenotypic variation associated with AUDPC and disease severity. The markers ss715646647 (Pv08) and ss715648096 (Pv11) were significant for disease severity and AUDPC indicating the possibility of candidate genes for *Fop* resistance. Subsequently, gene annotation will be performed based on the reference genome of *P. vulgaris*, to identify regions containing candidate genes resistance to fusarium wilt in common beans.

Key Words: *Phaseolus vulgaris* L; *Fusarium oxysporum* f. sp. *phaseoli*; Disease resistance; SNPs; GWAS.

Acknowledgements: FAPESP (Grant 2017/24711-4) and CAPES (Grant 88882.444193/2019-01).



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GENOMIC REGIONS ASSOCIATED WITH FUSARIUM WILT RESISTANCE IN COMMON BEANS

Jean Faustio de Carvalho Paulino¹; Caleó Panhoca de Almeida²; Sérgio Augusto Moraes Carbonell³; Alisson Fernando Chiorato⁴; Roberto Fritsche-Neto⁵; Qi Jian Song⁶; César Júnior Bueno⁷; Luciana Lásy Bechimol-Reis⁸.

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Introduction

Fusarium wilt, caused by *Fusarium oxysporum* f. *phaseoli* (*Fop*), is a major disease of common bean, causing large economic losses. Genetic resistance is one of the main mechanisms of pathogen control and it is fundamental in breeding for resistant cultivars. In this scenario, molecular markers have been extensively used to define the genetic architecture of agronomic traits by genome wide associations studies (GWAS) and estimating how many and which QTL are responsible for the phenotypic variation in the populations studied.

Objectives

The present study aimed to identify common bean genomic regions associated with resistance to *Fop* race 06 through genome wide association studies (GWAS) in a panel containing 205 accessions from the Germplasm Bank of the Agronomic Institute (IAC) with 5,393 high quality SNPs genotyped by BeadChip (BARCBean6K_3-Illumina).

Conclusions

The ss715646647 (Pv08) and ss715648096 (Pv11) markers were significant for disease severity and AUDPC indicating the possibility of candidate genes for *Fop* resistance. The results provide valuable information about the mechanism in the genetic control of *Fop* resistance in common beans and developed genetic and genomic resources that can assist in the development of new cultivars with high levels of resistance to *Fop*.

Acknowledgments

FAPESP (Grant 2017/24711-4) and CAPES (Grant 888882-444193/2019-01).



Results and Discussion

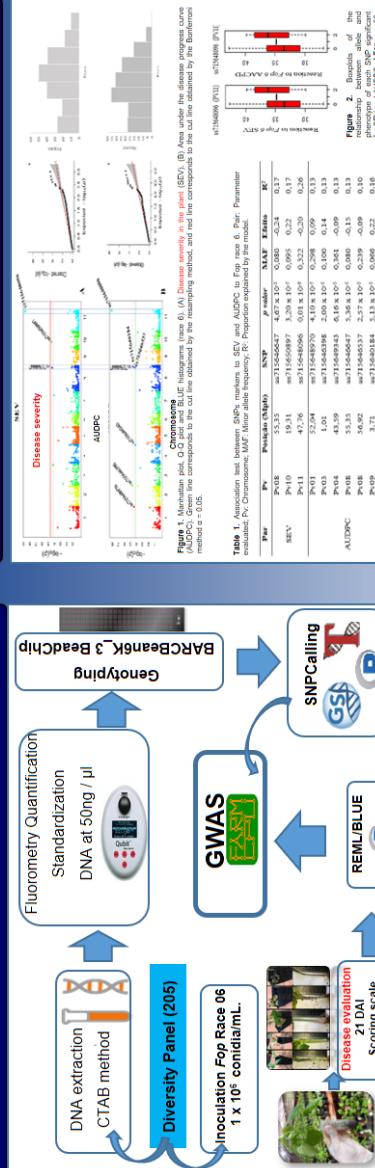
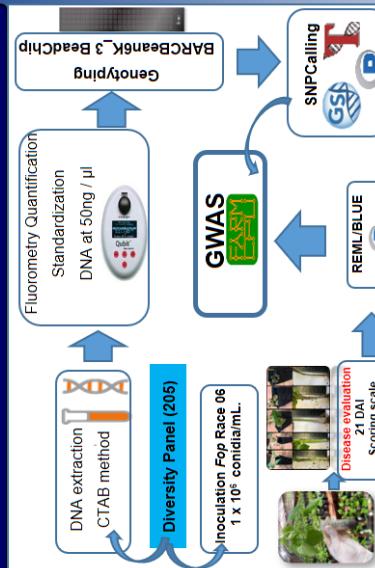


Table 1. Association test between SNPs markers to SEV and AUDPC to Fop race 06. Pair: Parameter evaluated; Pv: Chromosome; MAF: Minor allele frequency; R²: Proportion explained by the model.

Table 1. Association test between SNPs markers to SEV and AUDPC to Fop race 06. Pair: Parameter evaluated; Pv: Chromosome; MAF: Minor allele frequency; R²: Proportion explained by the model.

Materials and Methods



Results and Discussion

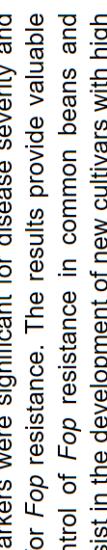


Table 2. Boxplots of the association test for each marker.

Table 2. Boxplots of the association test for each marker.

GENETIC DIVERSITY AND POPULATION STRUCTURE IN COMMON BEAN GERMPLASM FROM AGRONOMIC INSTITUTE (IAC)

Jean Fausto de Carvalho Paulino¹; Caléo Panhoca de Almeida¹; Sérgio Augusto Morais Carbonell¹; Alisson Fernando Chiorato¹; Roberto Fritsche-Neto⁵; Qijian Song⁶; Luciana Lasry Bechimol-Reis¹

¹Instituto Agronômico – CRGV. ²Universidade de São Paulo – ESALQ. ³USDA-ARS - Beltsville USA. E-mail: luciana.reis@sp.gov.br.

The present study aimed to evaluate the genetic diversity, population structure and the relationship among a panel with 288 common bean accessions (PDF-288) from the germplasm of the Agronomic Institute (IAC, Campinas). The panel was genotyped by BeadChip (BARCBean6K_3-Illumina) with 4,042 high quality SNPs. The diversity was evaluated with genetic distance, and population structure was estimated with the STRUCTURE software. The results demonstrated the distinction of Mesoamerican and Andean accessions and evidenced the majority presence of Mesoamerican accessions in the evaluated group, with greater genetic variability. The structuring by gene pool ($K = 2$) was predominant and the high value obtained by the fixation index (F_{ST}), demonstrating that there is genetic structuring between the accessions from different gene pools, followed by another subdivision ($K = 4$) which was divided according to the type of grain, origin and time of release. To obtain a core collection for association mapping from PDF-288, a low level of genetic differentiation was observed by the fixation index (F_{ST}). Similar values without significant differences were also observed by the t test for the Shannon Diversity Index, observed heterozygosity (H_o), expected heterozygosity (H_e) and inbreeding coefficient (F). The evaluated panel (PDF-288) maybe represented in terms of diversity through a smaller set, constituting a core collection (PDF-Core) of 205 Mesoamerican genotypes based on desirable agronomic traits for Brazilian breeding programs. Analyses were also carried out based on the linkage disequilibrium (LD), where better LD decay was observed based on the r^2 value observed for the PDF-Core demonstrating the possibility of future use of this panel in other genome wide association studies (GWAS).

Key Words: *Phaseolus vulgaris* L; Variability; SNPs; Linkage Disequilibrium; GWAS.

Acknowledgements: FAPESP (Grant nº 2017/24711-4) and CAPES (Scholarship nº 88882.444193/2019-01).



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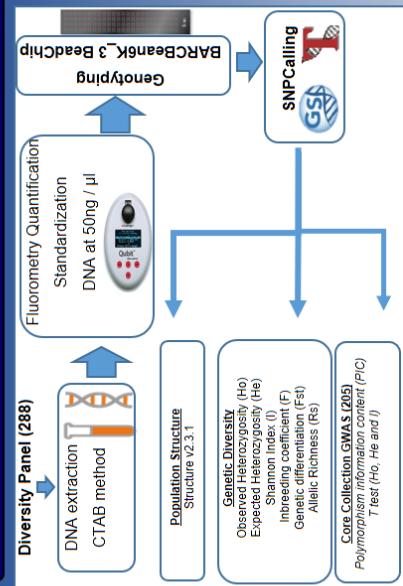
Jean Fausto de Carvalho Paulino¹; Caiélio Panhoca de Almeida²; Sérgio Augusto Morais Carbonell³; Alison Fernando Chiorato⁴; Roberto Fritzsche-Neto⁵; Cijian Song⁶; Luciana Lasty Bechimol-Reis⁷.

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Introduction

Common bean (*Phaseolus vulgaris* L.) is the most important source of protein in the Brazilian diet. The species has been domesticated independently in Mesoamerica and the southern Andes, and several research groups around the world have been studying the common bean in order to improve the crop, with the aim of developing more productive cultivars that are tolerant to biotic and abiotic stresses. In this scenario, molecular markers have been extensively used to define the genetic architecture of agronomic traits by genome wide associations studies (GWAS) and estimating how many and which QTL are responsible for the phenotypic variation in the populations studied.

Materials and Methods



Results and Discussion

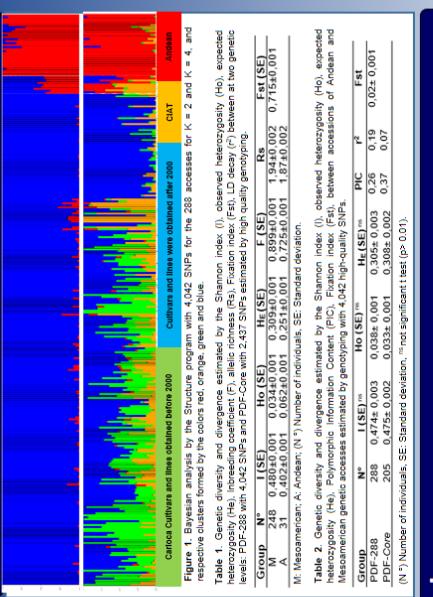


Figure 1. Bayesian analysis by the Structure program with 4,042 SNPs for 288 accessions for $K = 2, 3, 4$, and respective clusters formed by the colors red, orange, green and blue.

Table 1. Genetic diversity and divergence, estimated by the Shannon index (I), observed heterozygosity (Ho), expected heterozygosity (He), increasing coefficient (F), allelic richness (Rs), Fixation index (FST) and PDF-Cave with 4,042 SNPs estimated by high quality genotyping.					
Group	N	I (SE)	Ho (SE)	F (SE)	Rs (SE)
M	248	0.38(0.01)	0.34(0.01)	0.859(0.002	1.34(0.002
A	31	0.42(0.01)	0.30(0.01)	0.725(0.001	1.87(0.002
M. Mesoamerican - Andean (N*)	Number of individuals (N)	SE	Standard deviation.		

Table 2. Genetic diversity and divergence, estimated by the Shannon index (I), observed heterozygosity (Ho), expected heterozygosity (He), increasing coefficient (F), allelic richness (Rs), Fixation index (FST) and PDF-Cave with 4,042 high quality SNPs. M. Mesoamerican - Andean (N*) Number of individuals (N) SE Standard deviation.

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Conclusion

The panel presents diversity between Andean and Mesoamerican accesses and the core collection composed of Mesoamerican accesses showed better LD decay pattern with more polymorphic markers that may be used for other GWAS studies.

Acknowledgments

FAPESP (Grant no 2017/24711-4) and CAPES (Scholarship no 88882.444193/2019-01)

GNOTOBIOTIC SYSTEM AS AN EARLY INDICATOR OF MAIZE GROWTH PROMOTION

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The use of Plant Growth Promoting Rhizobacteria (PGPR) into crop field application is increasing due to microbial contributions to host sanity, plant architecture and resources usage. The search for novel inoculants demanded faster means to evaluate success of host-microbe interaction, therefore the *in vitro* cultivation of the plant harboring a single bacterium, might facilitate comprehension of its contribution to host physiology and yield. PGPR *Bacillus thuringiensis* RZ2MS9 isolated from guarana plant *Paullinia cupanea* promoted maize and soybeans growth under greenhouse conditions, however the knowledge of such benefic interaction at a molecular level is scarce. In this work, we proposed a maize-PGPR gnotobiotic system settled *in vitro* to access host-microbial transcriptional changes using RT-qPCR expression according to MIQE guidelines toward understanding of RZ2MS9 contribution to maize agronomic parameters quantified *in vitro* and greenhouse cultivation conditions. Remarkably, *in vitro* maize leaves and roots gene expression profile reproduced greenhouse counterpart results for most of analyzed genes, and phytostimulatory effect in roots of bacterized plants was observed for both cultivation systems, validating proposed *in vitro* system for further studies. Higher chlorophyll contents were observed in leaves of bacterized maize seedlings cultivated in greenhouse collected at V2 stage. RZ2MS9 favored roots higher sink strength and growth at early V2 stage expressed by increase of fresh matter, dry matter and soluble sugars and higher expression of auxin-responsive gene *iaa14*, sucrose synthase *susy* than control. RZ2MS9 contribution to increased chlorophyll content in leaves of greenhouse cultivated maize seedlings might be related to interference into host hormonal balance in which release of hormone bound forms in roots and leaves by the host, shown for the first time in this work might participate. The proposed gnotobiotic system was capable to reproduce RZ2MS9 growth promotion effect in maize observed in greenhouse for most evaluated parameters, which corroborates its use as an early indicator of host-microbe interaction success.

Keywords: Host-microbe interaction; phytostimulation; tropical rhizobacteria; invertases; sink strength.

Acknowledgments: To CNPq - process 140590/2017 and also to CAPES for conceded scholarships.

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GNOTOBIOtic SYSTEM AS AN EARLY INDICATOR OF MAIZE GROWTH PROMOTION

UNCOVERING THE POWER AND THE SECRETS BEHIND DNA MODIFICATIONS

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Introduction

Inoculation of Plant Growth Promoting Rhizobacteria (PGPR) such as *Bacillus* sp. R22MS9 benefits agricultural yield through enhancement of plant crop host physiological processes.



Few studies addressed transcriptional changes and precise growth promotion mechanisms activated during host-microbial interaction. The search for novel inoculants demanded faster and reproducible strategies to evaluate success of early host-microbe interaction, therefore the proposition of *in vitro* gnotobiotic cultivation, that is, the inoculation of a single bacterium into plants cultured under axenic conditions might facilitate comprehension of contribution of each microbe to host growth and development.

Materials and methods

Proposed gnotobiotic system: V2 stage Pioneer Pa285H plantlets inoculated with R22MS9 (10^8 CFU.mL⁻¹) and control cultivated until *in vitro* (conic tubes with MS, 15h light/9h)

Eight treatments:

- Proposed
- gnotobiotic *in vitro* and greenhouse
- R22MS9 and control-treated roots
- Leaves and roots

Agronomic features:

- Fresh weight, dry weight, chlorophyll content (Spectrophotometry)

Maize genes

- Biotic interaction and defense
- Growth
- Sucrose metabolism
- Photosynthesis

Genic expression

- MDE guidelines RNA → cDNA → RT-qPCR (Pfeffl Method)
- REST

Results and discussion



The objective of this study was to propose a novel gnotobiotic system as a fast and reproducible way to investigate the contribution of R22MS9 to maize growth promotion and gene expression at early interaction (V2 stage) *in vitro* and under greenhouse conditions.

Conclusion

The proposed gnotobiotic system was capable to reproduce R22MS9 growth promotion effect in maize observed in greenhouse for most evaluated parameters, which corroborates its use as an early indicator of host-microbe interaction success.

Acknowledgments

CNPq - process 140590/2017 and also CAPES for conceded scholarships, and Laboratory of Microorganisms Genetics.

SELEÇÃO DE GENÓTIPOS DE TOMATEIRO F₂RC₁ COM CARACTERÍSTICAS PARA PROCESSAMENTO INDUSTRIAL TOLERANTES AO DÉFICIT HÍDRICO INDUZIDO POR MANITOL

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O tomateiro (*Solanum lycopersicum* L.) é considerado uma das hortaliças de maior importância econômica em escala mundial. No entanto, as atuais cultivares comerciais não são tolerantes a estresses abióticos e constantemente são acometidas por déficits hídricos. O manejo do déficit hídrico, especialmente quando se refere ao tomateiro de indústria é de difícil manejo a nível de campo. Com isso, torna-se relevante a introgressão de genes de tolerância/resistência. Para isso, o acesso LA 716 pertencente a espécie *Solanum pennellii* é uma importante fonte de resistência. Objetivou-se selecionar *in vitro* genótipos da segunda geração do primeiro retrocruzamento (F₂RC₁) obtidos a partir do cruzamento interestípico da linhagem BRS Tospodoro com o acesso LA-716 (genitor doador), com tolerância ao déficit hídrico. Utilizou-se 20 sementes tolerantes ao déficit hídrico do genitor doador acesso LA-716, 20 sementes do genitor recorrente 'BRS tospodoro' e 500 sementes dos genótipos segregantes F₂RC₁. As sementes foram colocadas para germinar em meio de cultura MS, o qual foi adicionado 22,29 g.L⁻¹ de manitol P.A (C₆H₁₄O₆) caracterizado como álcool hexanídrico que quando em solução com água é absorvido e proporciona diferença aos níveis hídricos disponíveis à embebição das sementes. Na distribuição do material experimental foi adotada uma derivação do delineamento de blocos aumentados, denominado de delineamento experimental de testemunhas intercaladas, em que as testemunhas se equivaleram aos tratamentos comuns e os genótipos segregantes aos tratamentos regulares. Avaliou-se porcentagem, tempo médio de germinação, comprimento da maior raiz, altura e número de folhas das plântulas. Os dados foram submetidos à análise de variância, utilizando-se a matriz de variância e covariância residual para a realização do teste de comparação de médias de Dunett ($p \leq 0,05$ e $\leq 0,01$). Em condições de estresse hídrico induzido por manitol, nenhuma semente de 'BRS Tospodoro' germinou. As sementes da espécie *S. pennellii* germinaram 74% em 7 dias, resultando em 10,9 mm no comprimento de raiz, 22,1 mm na altura da planta e 6 folhas. Os genótipos F₂RC₁ que germinaram e se destacaram foram UZTI-80, UZTI-72, UZTI-149, UZTI-92, UZTI-14, UZTI-192, UZTI-51, UZTI-55, UZTI-98, UZTI-44, UZTI-157, UZTI-169 e UZTI-76. Esses genótipos tiveram maior comprimento raiz e número de folhas quando comparados ao acesso LA-716 (padrão de tolerância ao déficit hídrico). Adicionalmente esses genótipos, exceto UZTI-80, tiveram número de folhas igual ou superior a LA-716. Os genótipos F₂RC₁, UZTI-80, UZTI-72, UZTI-149, UZTI-92, UZTI-14, UZTI-192, UZTI-51, UZTI-55, UZTI-98, UZTI-44, UZTI-157, UZTI-169 e UZTI-76 demonstraram boa tolerância ao déficit hídrico e que podem contribuir para o desenvolvimento de cultivares de tomateiro com característica para processamento industrial.

Palavras-chave: *Solanum lycopersicum*; *Solanum pennellii*; estresse hídrico; tolerância à seca.

Agradecimentos: FAPESP (Processo 2019/05829-0).

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Introduction

The tomato (*Solanum lycopersicum*) is considered one of the most economically important vegetables on a world scale. However, current commercial cultivars are generally tolerant to abiotic stresses and are constantly affected by water deficit. Thus, the introgression of tolerance / resistance genes becomes relevant.

Objective

The objective was to select *in vitro* genotypes of the second generation of the first backcrossing (BC₁F₂) from the interspecific cross between BRS Tospodoro strain with the LA-716 accession (donor parent), with tolerance to water deficit.

Materials and methods

The research compared 20 seeds tolerant to the water stress of the donor parent access LA-716, 20 seeds of the recurrent parent 'BRS Tospodoro' and 500 seeds of the segregating genotypes BC₁F₂. The seeds were placed to germinate in MS culture medium, where 22.29 g·L⁻¹ of mannitol P.A. (C₆H₁₂O₆) was added. A derivation of the augmented block design was adopted, called the experimental design of interleaved controls, in which the controls were equivalent to common treatments and the segregating genotypes to regular treatments. Percentage, average germination time, length of the largest root, height and number of seedling leaves were evaluated. The data were submitted to analysis of variance.

Results and discussion

Under conditions of water stress induced by mannitol, no 'BRS Tospodoro' seeds germinated. The seeds of the species *S. pennellii* germinated 74% in 7 days, resulting in 10.9 mm in root length, 22.1 mm in plant height and 6 leaves. The BC₁F₂ genotypes that germinated and stood out were UZT1-80, UZT1-72, UZT1-149, UZT1-92, UZT1-192, UZT1-51, UZT1-55, UZT1-98, UZT1-44, UZT1-44, UZT1-157, UZT1-169 and UZT1-76.

Conclusion

The genotypes BC₁F₂ UZT1-80, UZT1-72, UZT1-149, UZT1-92, UZT1-14, UZT1-192, UZT1-51, UZT1-55, UZT1-98, UZT1-44, UZT1-157, UZT1-169 and UZT1-76 demonstrated good tolerance to water stress and could contribute to the development of tomato cultivars with characteristics for industrial processing.



Figure 1. Selection process of tolerant genotypes

Figure 2. Tomato seedlings from access La-716 *in vitro* submitted to water stress induced by mannitol.

Acknowledgments

FAPESP (Process 2019/05829-0)

Table 1. Length of the largest root (LR), height (HT) and number of leaves (NL).

Genotypes	LR – [mm]	HT – [mm]	NL
'LA-716' (P1)	10,9	22,1	6,0
'BRS Tospodoro' (P2)	-	-	-
UZT1-80 (BC ₁ F ₂)	24,0*	55,0*	4,0*
UZT1-72 (BC ₁ F ₂)	23,0*	48,0*	6,0
UZT1-149 (BC ₁ F ₂)	24,0*	48,0*	5,0
UZT1-92 (BC ₁ F ₂)	25,0*	57,0*	5,0
UZT1-14 (BC ₁ F ₂)	24,0*	52,0*	6,0
UZT1-192 (BC ₁ F ₂)	23,0*	50,0*	5,0
UZT1-51 (BC ₁ F ₂)	21,0*	56,5*	5,0
UZT1-55 (BC ₁ F ₂)	23,0*	53,0*	7,0
UZT1-98 (BC ₁ F ₂)	23,0*	55,0*	5,0
UZT1-44 (BC ₁ F ₂)	23,0*	58,0*	5,0
UZT1-157 (BC ₁ F ₂)	23,0*	40,0*	7,0
UZT1-169 (BC ₁ F ₂) differ from Stolbium pennei†‡§	23,0*	39,0*	6,0
UZT1-76 (BC ₁ F ₂)	25,0*	39,0*	

MAJOR LOCUS FOR SPONTANEOUS HAPLOID GENOME DOUBLING IN EXOTIC MAIZE GERMPLASM DETECTED BY A CASE-CONTROL GWAS

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Maize (*Zea mays* L.) breeding programs often rely on limited genetic diversity, which can be expanded by incorporating exotic germplasm. A major barrier to take advantage of exotic germplasm is the lengthy process of developing inbred lines. The production of doubled haploids (DH) can shorten the timeframe of inbred line development considerably. The aims of this study were to perform genotypic characterization of inbred lines derived from the tropical BS39 population using different breeding methods, to identify genomic regions showing segregation distortion in inbred lines derived by the DH process using spontaneous haploid genome doubling (SHGD), and use case-control association mapping to identify loci controlling SHGD. Four different inbred line derivation processes were created: BS39_DH and BS39_SSD were derived from the BS39 population by doubled haploid (DH) and single-seed descent (SSD) methods, and BS39xA427_DH and BS39xA427_SSD from the cross between BS39 and A427, used as a SHGD donor. A total of 663 inbred lines were genotyped using Genotype-by-Sequencing (GBS) and Diversity Array Technology Sequencing (DArtSeq). Gene diversity (HS), genetic differentiation (F_{ST}), haplotype sharing, and case-control GWAS analyses were performed. HS and F_{ST} for the DH sets provided evidence of the presence of a SHGD locus near the centromere of chromosome 5. The case-control GWAS for the DH set pinpointed this locus more precisely. Our results further indicate that the DH process captures genetic variability from the source population comparable to the SSD process. Haplotype sharing analysis showed almost 100% exclusive contribution of the A427 genome in the region near the centromere on chromosome 5 of BS39xA427_DH, presumably due to an allele in this region affecting SHGD. This locus enables DH line production in exotic populations without artificial haploid genome doubling.

Keywords: maize; doubled haploid; exotic germplasm; single seed descent.

Acknowledgements: USDA's National Institute of Food and Agriculture, Plant Sciences Institute, Crop Bioengineering Center, R.F. Baker Center for Plant Breeding, K.J. Frey Chair in Agronomy at Iowa State University; CNPq, CAPES.

¹Federal University of Viçosa. ²Iowa State University

Introduction

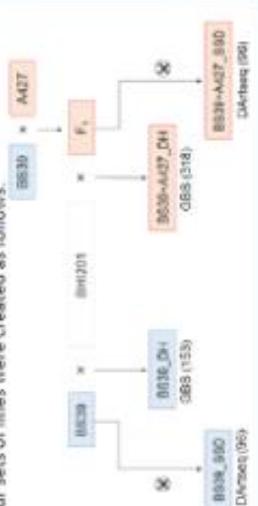
Maize (*Zea mays* L.) breeding programs often rely on limited genetic diversity, which can be expanded by incorporating exotic germplasm. A major barrier to take advantage of exotic germplasm is the lengthy process of developing inbred lines. The production of doubled haploids (DH) can shorten the timeframe of inbred line development considerably.

Objectives

To perform genotypic characterization of inbred lines derived from the tropical B539 population using SHGD and DH breeding methods; To identify genomic regions showing segregation distortion in lines derived by the DH process using spontaneous haploid genome doubling (SHGD); Use a case-control GWAS to identify loci controlling SHGD.

Materials and methods

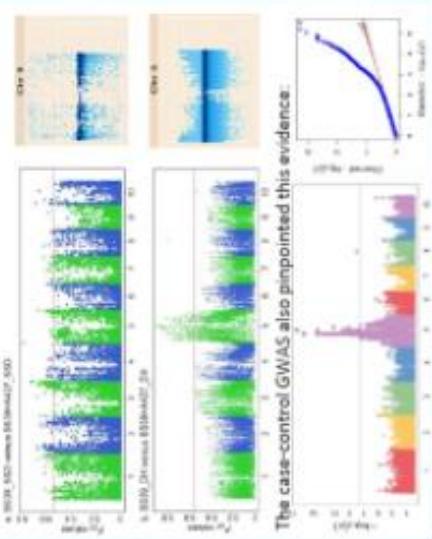
Four sets of lines were created as follows:



- Gene diversity (H_S) and genetic differentiation (F_{ST}) were estimated contrasting sets that carried A427 alleles with sets with no A427 contribution;
- Haplotype sharing between B539×A427_DH and versus A427 inbred line was obtained;
- Case-control GWAS was performed using the FarmCPU model contrasting B539_DH and B539×A427_DH, scored as "1" and "0", respectively.

Results and discussion

H_S and F_{ST} for the DH sets provided evidence of the presence of a SHGD locus near the centromere of chromosome 5.



Conclusion

Haplotype sharing analysis showed almost 100% exclusive contribution of the A427 genome in the region close to the centromere on chromosome 5, probably caused by an allele affecting SHGD

Acknowledgments

USDA's National Institute of Food and Agriculture, Plant Sciences Institute, R. F. Baker Center for Plant Breeding, K. J. Frey Chair in Agronomy at ISU, CNPq, and CAPES.

ANÁLISE MULTIVARIADA PARA ESTUDOS DE ATRIBUTOS EDAFOLIMÁTICOS INFLUENTES NA CULTURA DA SOJA

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A ACP é uma técnica estatística de análise multivariada que transforma linearmente um conjunto original de variáveis, inicialmente correlacionadas entre si, num conjunto substancialmente menor de variáveis não correlacionadas que contém a maior parte da informação do conjunto original. A ACP é a técnica mais conhecida e está associada à ideia de redução de massa de dados, com menor perda possível da informação. Procura-se redistribuir a variação observada nos eixos originais de forma a se obter um conjunto de eixos ortogonais não correlacionados. A seleção de cultivares adaptadas a diferentes regiões dentro de um programa de melhoramento depende da compreensão dos efeitos ambientais que caracterizam cada região. Para isso é necessário compreender a estrutura da variância e covariância das variáveis influentes na cultura, e assim, conhecer a importância relativa de cada variável. Objetivou-se com este trabalho explicar a estrutura da variância e covariância de um conjunto de dados ambientais por meio de combinações lineares das variáveis originais. Foram avaliados dados edafoclimáticos de 11 cidades do Mato Grosso, dentre elas a temperatura (°C), umidade relativa média (%), pressão atmosférica (mbar), altitude (m), precipitação total (mm), classe de solo e classificação climática segundo Köppen, os dados climáticos foram obtidos através do Banco de Dados Meteorológicos para Ensino e Pesquisa (BDMEP). Foi avaliada uma série histórica de dez anos, de setembro de 2009 a fevereiro de 2019, fazendo uma média para cada mês. Os meses utilizados para o estudo foram de setembro a fevereiro. Todas as análises foram feitas no software Rstudio, utilizando o pacote "psych". Com base nos resultados obtidos pela técnica, os dois primeiros PCs foram responsáveis por 66,55% da variação total dos dados ambientais estudados, em que o PC1 foi responsável por 47,25% e o segundo, PC2, por 14,30% das variações dos dados. Portanto, dois primeiros componentes principais resumem efetivamente a variância amostral total e podem ser utilizados para o estudo do conjunto de dados. Com a seleção de dois componentes principais, a redução da dimensão de 12 variáveis originais para 2 componentes principais é eficiente.

Palavras-chave: Análise multivariada; Componentes Principais; edafoclima.



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MULTIVARIATE ANALYSIS FOR EDAPHOCLIMATIC STUDIES IN SOYBEAN CROP

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Introduction

PCA is a multivariate statistical analysis technique that linearly transforms an original set of variables, initially correlated with each other, into a substantially smaller set of uncorrelated variables that contains most of the information in the original set. PCA is the best known technique and is associated with the idea of reducing data mass, with the least possible loss of information. We seek to redistribute the variation observed in the original axes in order to obtain a set of unrelated orthogonal axes. The selection of cultivars adapted to different regions within a breeding program depends on an understanding of the environmental effects that characterize each region. For this it is necessary to understand the structure of the variance and covariance of the influential variables in the culture, and thus, to know the relative importance of each variable.

Objective

The objective of this work was to explain the structure of the variance and covariance of a set of environmental data through linear combinations of the original variables.

Materials and methods

Edaphoclimatic data from 11 cities in Mato Grosso were evaluated, including temperature (°C), average relative humidity (%), atmospheric pressure (mbar), altitude (m), total precipitation (mm), soil class and climatic classification according to Köppen, the climatic data were obtained through the Meteorological Database for Teaching and Research (BDMEP). A ten-year historical series from September 2009 to February 2019 was evaluated, averaging for each month. The months used for the study were from September to February. All analyzes were performed using the Rstudio software, using the 'psych' package.



Results and discussion

Based on the results obtained by the technique, the first two PCs were responsible for 66.55% of the total variation of the studied environmental data, in which PC1 was responsible for 47.25% and the second, PC2, for 14.30% of variations of the data.

Conclusion

Therefore, the first two main components effectively summarize the total sample variance and can be used to study the data set. With the selection of two main components, reducing the dimension from 12 original variables to 2 main components is efficient.

NEW ANGULAR LEAF SPOT RESISTANCE QTLs FOR CARIOCA COMMON BEAN

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The angular leaf spot (ALS), caused by the fungus *Pseudocercospora griseola* (Sacc.) Crous & U. Braun is one of the most important diseases of common bean (*Phaseolus vulgaris* L.), which may result in losses of up to 80% of production. Recent studies have demonstrated that bean cultivars show different degrees of resistance throughout plant development and due to the quantitative profile of the disease, several resistance *loci* are necessary to guarantee a lasting resistance. Associated molecular markers may be used as an important tool for the early selection of resistant genotypes. For this purpose, the AM genetic map estimated from 1,091 SNPs genotyped via GBS in a segregating inter-pool genetic population (IAC-Milênio x AND 277) composed of 91 BC₂F₃ carioca lines was used to identify markers linked to ALS resistance QTLs. The AM population was evaluated for resistance to ALS at three different phenological stages (PS), been PS V2 and V3 under controlled conditions of infection and PS R8 under natural conditions (field). Using the multiple interval mapping approach (MIM), four QTLs were identified, showing that in addition to the *Phg-1* locus, identified on chromosome Pv01 (ALS1.1^{AM}) and associated with PS V3 in the current study, the AND 277 cultivar portrayed the QTL *Phg-5* reported for the first time in the descending cultivar CAL143 as ALS10.1^{UC}. The QTL ALS10.1^{AM} associated with PS V2 was the one with the greatest effect (34%) and a new QTL named ALS11.1^{AM} was identified at the beginning of Pv11 associated with PS R8. A second QTL for PS R8 (ALS3.1^{AM}) was mapped on chromosome Pv03 and appears to be the same QTL ALS3.1^{UC} also identified in the cultivar CAL 143. Finally, several putative resistance genes involved in the ALS resistance response were identified, which have great potential to be used in development of molecular markers for marker-assisted selection.

Keywords: *P. griseola*; Linkage mapping; Carioca bean.

Acknowledgements: FAPESP (Grants 2017/01753-3; 2014/11145-2; 2019/19670-2).

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Introduction

The angular leaf spot (ALS), caused by the fungus *Pseudocercospora griseola* (Sacc.) Crous & U. Braun is one of the most important diseases of common bean (*Phaseolus vulgaris* L.), which may result in losses of up to 80% of production. Recent studies have demonstrated that bean cultivars show different degrees of resistance throughout plant development and due to the quantitative profile of the diseases, several resistance loci are necessary to guarantee lasting resistance. The identification of new QTLs associated with resistance to ALS make it possible to select candidate genes for the defense response and to obtain associated molecular markers for early molecular selection and pyramiding of favorable alleles.

Objective

The aim of the present study was to identify QTLs associated with genetic resistance to ALS at different phenological stages of common bean through linkage mapping using a segregating inter-pool genetic population.

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NEW ANGULAR LEAF SPOT RESISTANCE QTLs FOR CARIOLA COMMON BEAN

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Results and discussion

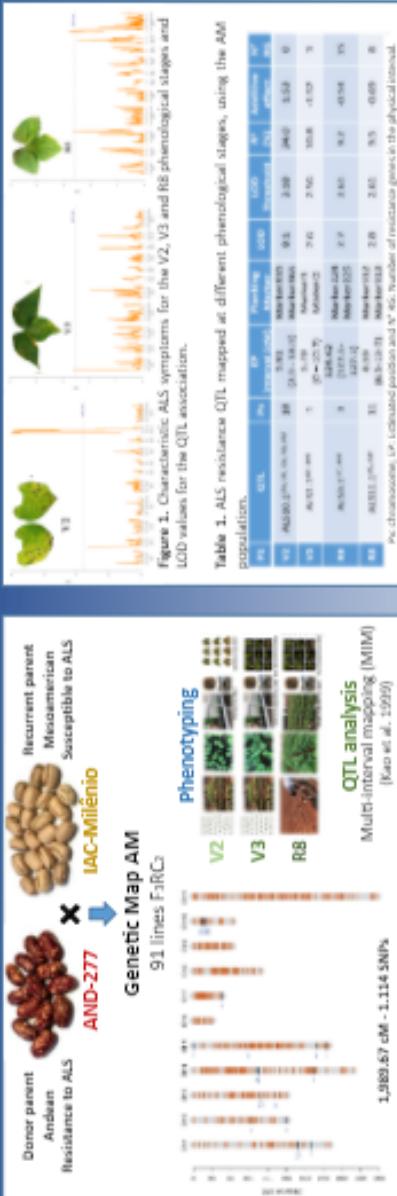
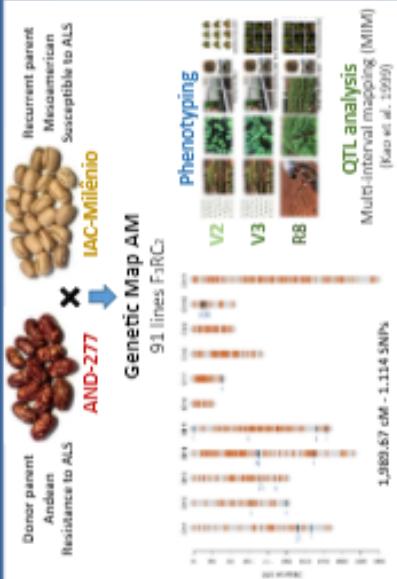


Figure 1. Characteristic ALS symptoms for the V2, V3 and R3 phenological stages and LOD values for the QTL association.

Materials and methods



Conclusion

The quantitative inheritance of the disease was again confirmed by the identification of loci associated with different phenological phases and different environmental conditions. Besides the *Rhg-1 locus*, the AND277 cultivar has the *Rhg-5* reported for the first time in the CAL143 cultivar (derived from AND 277) as ALS10.1 and a new QTL named ALS11.1 was identified at the beginning of Pv11.

Acknowledgments

FAPESP (Grants 2017/01753-3; 2014/11145-2; 2019/19670-2), CAPES and CNPQ.

PR-1 GENES COULD PLAY AN IMPORTANT ROLE IN THE RESPONSE MECHANISM TO HYDRIC STRESS IN TOMATO cv. MICRO-TOM

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The PR-1 genes encode proteins that activate defense mechanisms against pathogens. Recent studies suggest that these genes may also be associated with mechanisms of response to abiotic stress. This work aimed to evaluate the behavior of two transgenic lines of tomato *Solanum lycopersicum* cv Micro-Tom (*TcPR-1f*, *TcPR-1g*) submitted to three levels of soil moisture: 100%, 60%, 30% of the field capacity (CC). Physiological parameters such as stomatal conductance, photosynthetic rate, transpiration, chlorophyll content and biomass were evaluated. Severe drought significantly affected ($p<0.05$) all physiological variables in the evaluated plants. The *TcPR-1f* genotypes are able to overcome the moderate dryness level (60%) producing biomass values similar to the control treatment in 100% CC. In addition, the *TcPR-1f* mutants showed higher values ($p<0.05$) of chlorophyll SPAD, leaf area and number of leaves in comparison to the control genotypes under the same soil moisture conditions. Although the genes (*TcPR-1f* and *TcPR-1g*) belong to the same family, they have different structures. The *TcPR-1f* gene has an additional 110 base pair sequence located at the beginning of the 3' side of the sequence. This segment could encode a signal peptide or functional domain in proteins, contributing to the improved performance of transgenic *TcPR-1f* plants under water stress conditions, whose molecular bases are still unknown. Additional studies are being developed to decipher the role of *TcPR1* genes and their structure in water stress response/tolerance.

Keywords: PR-1; Pathogenesis; climatic change; oxidative stress.

4th IMPB INTERNATIONAL MEETING ON PLANT BREEDING

UNCOVERING THE POWER AND THE SECRETS BEHIND DNA MODIFICATIONS

PR-1 GENES COULD PLAY AN IMPORTANT ROLE IN THE RESPONSE MECHANISM TO DROUGHT STRESS IN TOMATO CV. MICRO TOM

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¹Instituto Agronômico de Campinas^{*}; Mariana.gote085@hotmail.com

²Escola Superior de Agricultura Luiz de Queiroz.

Introduction

PR-1 genes encode proteins that activate defense mechanisms against pathogens. Recent studies suggest that these genes may also be associated with mechanisms of response to abiotic stress.

Objective

This study aims to evaluate the behavior of two strains of transgenic tomato (*Solanum lycopersicum* cv Micro-Tom (TcPR-1f, TcPR-1g) submitted to three soil moisture levels: 100%, 60%, 30% of the field capacity (CC).

Materials and methods

Seeds of the genotypes were sown (A), after 15 DAS were transplanted in plastic pots (B), a week before tasseling the IRGA physiological analyses were taken, after 30 DAS the SPAD 45 DAS was analyzed and destructive analyzes were performed to evaluate Biomass (C & D).

Results and discussion

Figure 1. Seeds of the genotypes were sown (A), after 15 DAS were transplanted in plastic pots (B), a week before tasseling the IRGA physiological analyses were taken, after 30 DAS the SPAD 45 DAS was analyzed and destructive analyzes were performed to evaluate Biomass (C & D).

Figure 2. Effects of drought, Transpiration and conductance. Severe drought significantly affected ($p<0.05$) all physiological variables in the evaluated plants (Figure 1).

Figure 3. Effect of three levels of soil moisture on the leaf number. Red brown and brown indicate or W/T.

Figure 4. Effect of three levels of soil moisture in the stem biomass - Red and brown coffee indicates the W/T, the green colors indicates of the PR-1, the blue colors of the PR-1-1 and the purple color indicates of the PR-1-g.

Conclusion

The TcPR-1f mutants showed higher values ($p<0.05$) of SPAD chlorophyll, leaf area (Figure 2) and number of leaves (Figure 3) in relation to the control genotypes under the same soil moisture conditions. Although the genes (TcPR-1f and TcPR-1g) belong to the same family, have a different structure.

Acknowledgments

We are grateful to the Laboratory of Molecular Biology of Plants of the Higher School of Agriculture "Luiz de Queiroz" (ESALQ), University of São Paulo (USP). This work was funded by the National Council for Scientific and Technological Development.

PROTEIN INTERACTION BETWEEN TRANSCRIPTION FACTORS OF *Arabidopsis thaliana* AND EFFECTORS OF *Sporisorium scitamineum*

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A common disease in sugarcane is smut, caused by the biotrophic fungus *Sporisorium scitamineum*. The susceptible plants infected by this pathogen show the formation of a whip-shaped structure, which occupies the position of the host's reproductive organs. Smut disease can cause losses of up to 62% in sugarcane production and reduce the quality of the juice in susceptible varieties. However, the emergence of smut epidemics in resistant varieties can persist in stressful environmental conditions. Thus, it is necessary to investigate at a molecular level the interaction between this pathogen and its host, in order to understand the pathogenesis process, and establish more effective methods for controlling the disease. Studies of interaction between proteins can elucidate cellular functions that impact plant development and growth, such as the flowering pathway. Therefore, it is important to understand the biochemical mechanisms that occur during the sugarcane-smut interaction. Nevertheless, analysis using the hybrid sugarcane genome remain a challenge for the scientific community, due to its complex, extensive genome, with a high level of polyploidy and the presence of transposons. As an alternative, research with model plants is being applied in order to have a better understanding of this genome and the functioning of its genes, such as those involved in the process of defense. The *Arabidopsis thaliana* model has been used to study fungus pathosystems, such as *Ustilago maydis* and *Ustilaginoidea virens*, in corn and rice, respectively. The objective of this work is to establish bimolecular fluorescence (BiFC) complementation tests to analyze protein interactions between effectors of the fungus *S. Scitamineum* and transcription factors of the Squamosa family that are involved in the development of flowering of *Arabidopsis thaliana*. The BiFC assay is based on the presence of fluorescence signals when there is a protein interaction, which can facilitate the understanding of the modulation of the plant's physiology during infection of the pathogen. Thus, since knowledge of this scenario for the sugarcane-smut pathosystem is scarce, this project aims to investigate the protein interaction between transcription factors of the Squamosa family of *Arabidopsis thaliana* and candidates for effectors of *S. scitamineum*, with the purpose of generate data for future studies of the sugarcane pathosystem, as well as assist genetic improvement programs for varieties resistant to this disease in sugar cane.

Keywords: Sugarcane; Smut; Disease.

**INTERNATIONAL MEETING
ON PLANT BREEDING**

4th IMPB

PROTEIN INTERACTION BETWEEN TRANSCRIPTION FACTORS OF ARABIDOPSIS THALIANA AND EFFECTORS OF SPORISORIUM SCITAMINEUM

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INTRODUCION

A common disease in sugarcane is smut, caused by the biotrophic fungus *Sporisorium scitamineum*. The susceptible plants infected by this pathogen show the formation of a whip-shaped structure, which occupies the position of the host's reproductive organs. Smut disease can cause losses of up to 60% in sugarcane production. The emergence of smut epidemics in resistant varieties can persist in stressful environmental conditions. Thus, it is necessary to investigate at the interaction between this pathogen and its host to understand the pathogenesis process, and establish effective methods for controlling the disease.

MATERIALS AND METHODS

BiFC and Gateway

Cloning N C + X Y → GFP → SPLASH → Arabidopsis → Fluorescence → Image analysis → Preliminary Results

PERSPECTIVES

Analysis of each step of the construction of vectors through sequencing, in order to confirm the correct constructions, that is, with the amplicons and the other sequences included in the vectors that are important for the expression of the genes.

In order to verify the protein interaction between the two proteins, the results will be analyzed through images of *N. benthamiana* leaves under fluorescence microscopy. It is expected to observe signs of fluorescence (GFP) in cells, nucleus or cytoplasm. Thus, the interaction between proteins will be confirmed.

ACKNOWLEDGMENTS

CAPES **FAPESP** **CNPq**

PRIMER3 **Uniprot**

Figure 1. Symptoms of smut disease in sugarcane (Monteiro-Vitorino et al., 2018).

Figure 2. Bioluminescence (BiFC) complementation tests to analyze protein interactions between effectors of the fungus *S. scitamineum* and transcription factors of the *Squamosa* family that are involved in the development of flowering of *Arabidopsis thaliana*.

SELECTION OF EXPERIMENTAL PURPLE-FLESHED SWEET-POTATO GENOTYPES

Leal, M.H.S¹, Zeist, A.R¹, Silva Junior, A. D.¹, Rodrigues Júnior N.¹, Pieri, J. R. S¹, Arantes, J.H.V¹, Garcia Neto J.¹, Taroco B. R.¹

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The sweet potato (*Ipomoea batatas* L.) is a vegetable belonging to the *Convolvulaceae* family. Its roots contain a high amount of carbohydrates, potassium and minerals, making it an excellent alternative against malnutrition and in favor of a healthy diet. The world population has increased in the same way as the demand for nutritious and healthy food. Considering the aforementioned information, the aim herein was to develop and select experimental purple sweet potato genotypes in terms of agronomic, physical root characteristics and resistance to *Euscepes postfasciatus*. Genotypes were obtained in polycross blocks. All related genotypes flourished and pollination was carried out at random by insects. For the scarification process, the seeds were dipped in Sulfuric Acid 98% for 50 min, washed in distilled water and sown in expanded 127-cell polystyrene trays containing substrate based on bio-stabilized pine bark and maintained in a greenhouse. The seedlings were cloned when reaching 5-6 true leaves. Then, the main branch of each genotype was used to conduct the field experiments. An experimental design comprising augmented blocks with interleaved controls was adopted. They were then evaluated regarding the number of commercial roots per plant and production of commercial roots, as g plant⁻¹. The appearance of the tuberous roots was determined by means of a scale, where 1- non-standard, with a very irregular shape, the presence of large veins and deep cracks, 2- very uneven, with the presence of large veins and cracks, 3- non-uniform, with large veins and cracks, 4- slightly non-uniform with the presence of veins, and 5- regular fusiform shape, without veins or cracks. Resistance to *E. postfasciatus* was also determined by a scale, where 5- roots free from damage, 4- roots with low damage, 3- few damaged commercial roots, 2- most damaged commercial roots and, 1- unacceptable commercial roots for human and animal consumption. The experimental genotypes obtained for the characteristics of number of commercial roots, production of commercial roots, root appearance and resistance to *E. postfasciatus* averaged 1.46, 0.72 kg, 2.79 and 2.92, respectively. Concerning the same parameters, 24.59, 24.59, 41.82 and 17.21% of the experimental genotypes presented a greater effect than the control. Of the 125 experimental purple pulp genotypes, a total of 28, 32, 28 and 31 were superior to the control commercial cultivar 'SCS 370 Luiza' for number of commercial roots, production of commercial roots, root appearance and resistance to *E. postfasciatus*, respectively. The genotypes UZBD-K-09, UZBD-K-56 and UZBD-K-78 were superior to 'SCS 370 Luiza' for all explored parameters. Among the parameters evaluated, the genotypes UZBD-K-09, UZBD-K-56 and UZBD-K-78 were superior.

Keywords: *Ipomoea batatas*, *Euscepes postfasciatus*, breeding, root appearance.

Acknowledgments: FAPESP (Process 2019/16730-4).

Leal, M.H.S.¹, Zeist, A.R.¹, Silva Junior, A.D.¹, Rodrigues Júnior N.¹, Pieri, J.R.S.¹, Arantes, J.H.V.¹, Garcia Neto J.¹, Taroco B.R.¹
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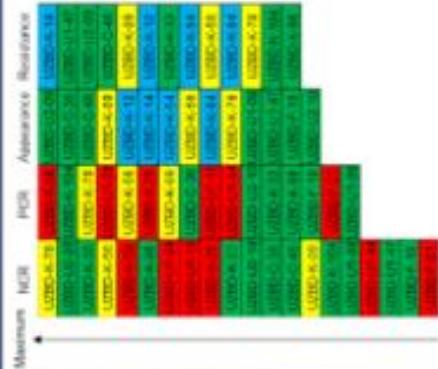
Introduction

The sweet potato (*Ipomoea batatas*) is a vegetable belonging to the Convolvulaceae family. Its roots contain a high amount of carbohydrates, potassium and minerals.

Objective

Considering the aforementioned information, the aim herein was to develop and select experimental purple sweet potato genotypes in terms of agronomic, physical root characteristics and resistance to *E. postfasciatu*s.

Results and discussion



SELECTION OF TOLERANT TOMATOES GENOTYPES F₂ FOR HIGH AIR TEMPERATURES

Nilson Rodrigues Júnior¹; André Ricardo Zeist¹; Murilo Henrique Souza Leal¹; André Dutra Silva Júnior¹; Jair Garcia Neto¹; José Henrique Verhalem Arantes¹; Julia Roberta Sanches De Pieri¹; Amanda Carvalho Perrud¹

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Tomatoes (*Solanum lycopersicum* L.) are originally from the Andean region, encompassing Peru, Northern Chile and Ecuador. It is currently the second most economically important vegetable in agribusiness. It requires amount adequate light and temperature with averages close to 21 °C to obtain high productivity, and adverse climatic conditions significantly hinder plant processes such as flowering and fruiting. Climate change, especially with regard to rising temperatures in the medium to long term, threatens world agricultural production. The temperature is expected to increase between 1.4 and 5.8 °C over the next 100 years. This could cause a 30% drop in agricultural productivity by 2080. The objective of this work was to select F₂ tomato plants tolerant to high air temperatures. The segregating experimental genotypes F₂ were UZT 188, UZT 139, UZT 151, UZT 148, UZT 150 and UZT 145. Genotypes Santa Clara and Redenção (*Solanum lycopersicum*) and the wild accession *S. habrochaites* var. *hirsutum* PI-127826 were used as controls. An experimental design of augmented blocks with interleaved controls was adopted. Thirty seeds of each segregated population F₂ and controls were planted in expanded polystyrene trays, containing commercial substrate. The trays were placed in a humid chamber type *floating*. The chamber was placed exposed to sunlight during summer in the west region of São Paulo state, with daytime temperatures reaching above 50 °C and nighttime temperatures close to 35 °C. After three weeks of seedlings exposure to high temperatures, the evaluation was carried out based on seed germination percentage, number of leaves, seedlings height and leaf damage by using the scale of grades: 0 (no injuries); 1 (1-5% of lesions on the leaf surface); 2 (6-25%); 3 (26-50%); 4 (> 50%). The data were subjected to analysis of variance and means comparison using Dunnett's test ($p \geq 0.05$). The accession PI 127826 was the most tolerant to heat stress (grade 1), followed by genotypes UZT 150-1, UZT 150-2, UZT 150-3, UZT 150-4, UZT 150-5, UZT 145-1, UZT 145-2 and UZT 145-3 (grade 2), while the control cultivars Redenção and Santa Clara did not tolerate high temperatures. The F₂ segregating genotypes UZT 150-1, UZT 150-2, UZT 150-3, UZT 150-4, UZT 150-5, UZT 145-1, UZT 145-2 and UZT 145-3 are the most relevant to continue the breeding process aiming at cultivation in adverse conditions provided by global warming.

Keywords: *Solanum lycopersicum*; breeding; thermal stress.

Acknowledgments: FAPESP (Processo 2019/17804-1).

SELECTION OF TOLERANT TOMATOES GENOTYPES F₂ AT HIGH TEMPERATURES

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Introduction

The tomato (*Solanum lycopersicum*) it is currently the second largest vegetable of importance in climatic conditions significantly hinder plant agriculture. It is a plant that needs good temperature and light conditions to obtain good productivity, where adverse processes such as flowering and fruiting. Climate change, especially with regard to rising temperatures in the medium to long term, threatens world agricultural production. The temperature is expected to increase between 1.4 and 5.8 °C over the next 100 years.

Objective

The objective was to select F₂ tomato genotypes tolerant of high air temperatures

Materials and methods

The segregating experimental genotypes F₂ UZT 188; UZT 139; UZT 151; UZT 148; UZT 150; UZT 145. How controls Santa Clara and Redenção (*Solanum lycopersicum*) and the wild *S. habrochaites* var. *hirsutum* accession PI-127826 were used.

UNCOVERING THE POWER AND THE SECRETS BEHIND DNA MODIFICATIONS

Unoeste



TEMPERATURES GENOTYPES F₂ AT HIGH AIR

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Results and discussion

Table 1. Evaluation of tomato genotypes after 15 days under heat stress

Genotype	Live plants	Injury	Height
UZT 150	5	2	4
UZT 145	3	2	3.4
Santa Clara	0	0	0
Redenção	0	0	0
PI-127826	8	1	7.5

The access PI-127826 was the most tolerant to thermal stress (note 1), followed by the genotypes UZT 150-1, UZT 150-2, UZT 150-3, UZT150-4, UZT 150-5, UZT 145-1, UZT 145-2 and UZT 145-3 (note 2). However, the controls Redenção and Santa Clara cultivars did not support the high temperatures.



Figure 1. High temperature chamber exposed to the sun (A) and expanded polystyrene trays containing seeds and the sprouts of the wild *S. habrochaites* var. *hirsutum* accession PI-127826 (B).

Acknowledgment

FAPESP (Processo 2019/17804-1)

THE SMUT PATHOGEN *SPORISORIUM SCITAMINEUM* USES DIFFERENT STRATEGIES TO INFECT RESISTANT AND SUSCEPTIBLE SUGARCANE GENOTYPES

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The sugarcane smut pathogen *Sporisorium scitamineum* is a biotrophic fungus, host-dependent for sexual reproduction. The disease spreads across all the sugarcane producing countries, except in Fiji, the group of volcanic islands in the South Pacific in Oceania, causing economic losses and reducing the crop yield. During the later years, the Genomics Group at ESALQ/USP has been extensively studying the sugarcane-smut interaction, considering its various aspects, including the pathogen attack mechanisms and plant defense responses. The pathogens secrete molecules to modulate plant physiology, counterattack, and protect themselves against the plant host's defense barriers. In this work, we used the RNA-Seq technique to determine the expression profile and compare the colonization of two sugarcane genotypes with contrasting resistance levels (smut-resistant, SP80-3280, and susceptible, IAC66-6) at the early stage of infection, 48 hours after inoculation. Additionally, we re-analyzed previous data of the axenic growth of *S. scitamineum*. We found compelling results considering genes' expression profile of the fungus infecting resistant and susceptible sugarcane genotypes. The pathogen expressed more peroxidases, including the catalases KatE and KatG, when infecting the resistant genotype, consistent with the early defense response involving reactive oxygen species (ROS) production by the SP80-3280 genotype. Furthermore, we predicted the putative fungal secretome, including candidate effectors, secreted proteases, and hydrolases, revealing their expression patterns among the three treatments. Herein we provide insights into the differential infection of resistant and susceptible sugarcane genotypes infected with the pathogen, suggesting potential candidates for selecting resistant sugarcane genotypes that trigger the host immune system.

Keywords: RNA-Seq; Transcriptional profile; *Saccharum* spp.

4th IMPB INTERNATIONAL MEETING ON PLANT BREEDING

THE SMUT PATHOGEN *SPORISORIUM SCITAMINEUM* USES DIFFERENT STRATEGIES TO INFECT RESISTANT AND SUSCEPTIBLE SUGARCANE GENOTYPES

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Introduction

The pathogen *Sporisorium scitamineum* causes sugarcane smut disease. The characteristic symptom of the disease is the development of a whip-like structure from the apex of the plant responsible for fungal sporogenesis and dissemination. The fungus can colonize resistant plants but forming no whips. This work aimed to compare the transcriptional profiles (RNA-Seq) of the fungus infecting plants of two different sugarcane genotypes (resistant and susceptible) 48 hours after inoculation. We also reanalyzed previously published data from *S. scitamineum* growth in axenic culture [Taniiguti et al., 2015].

Objective

The use of dual transcriptome data analysis can reveal candidate genes associated with *S. scitamineum* expression profile differentiation when infecting smut-resistant vs. -susceptible plants.

Acknowledgments



UNCOVERING THE POWER AND THE SECRETS BEHIND DNA MODIFICATIONS

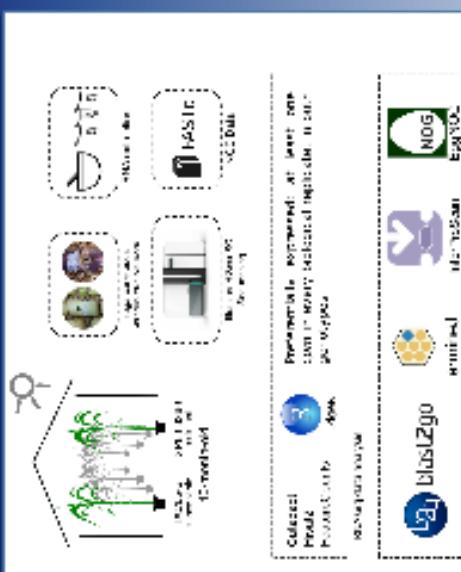


THE SMUT PATHOGEN *SPORISORIUM SCITAMINEUM* USES DIFFERENT STRATEGIES TO INFECT RESISTANT AND SUSCEPTIBLE SUGARCANE GENOTYPES

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Materials and methods



Results and discussion

In the comparison between SP8032-80 (resistant) and IAC66-6 (susceptible) genotypes, 1,076 genes were considered as preferentially expressed. Out of those, were 113 differential expressed genes (DEGs) with $P < 0.05$, 63 downregulated and 50 up-regulated. Peroxidases up-regulated KatG and KatG, also when comparing with *S. scitamineum* growth in axenic culture. Related with the detoxification of the H_2O_2 produced by the resistant plant.

Figure 1 – Distribution of transcriptome reads mapped to *S. scitamineum* chromosomes.

Conclusion

The sugarcane smut pathogen (*S. scitamineum*) uses different strategies when infecting the two contrasting genotypes SP8032-80 (resistant) and IAC66-6 (susceptible).

TOLERANCE OF PROCESSING TOMATOES BC₃F₂ GENOTYPES TO WATER DEFICIT

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Tomatoes are considered to be widespread, positioning themselves in the agro-industrial chain as one of the most important in the agribusiness complex, as it is one of the most consumed vegetables in the world, both fresh and processed. The water consumption by the tomato crop is quite high, standing at around 880 mm (or 8800 m³/ha) throughout the crop cycle. Aiming at the lack of water in the world, genotypes with greater efficiency in the use of water and/or with greater tolerance to drought may allow a substantial increase in the area planted with the crop, with less impact on the use of natural resources. The objective was to select genotypes of second-generation industrial tomato from the third backcross (BC₃F₂) with tolerance for water deficit. The genotypes of the second generation of the third backcross (BC₃F₂) were obtained by crossing the commercial cultivar Redenção with *Solanum pennellii* accession LA 716 (donor parent). The genotypes BC₃F₂ RVTA pl#138, RVTA pl#54, RVTA pl#248, RVTA pl#290, RVTA pl#138, RVTA pl#135, RVTA pl#301, RVTA pl#292, RVTA pl#133, RVTA pl#325, RVTA pl#59 and RVTA pl#291 were explored. These genotypes were initially selected for resistance to pest arthropods. As controls, the donor parent LA 716 (tolerant to water deficit) and the commercial cultivar Redenção (susceptible to water deficit) were used. The seedlings were transplanted into 10 dm³ pots after presenting 4-5 defined and fully expanded leaves. The pots contained commercial substrate based on bio-stabilized pine bark. The experimental design was in randomized blocks, containing three replications with eight plants each. The plants were irrigated without distinction until 30 days after transplantation, using micro-drippers to standardize the development of the plants. Since then, two levels of water supply have been adopted, 0% (water stress) and 100% (control) of water needs per 19 days. In the condition of 100% supply, a maximum water tension in the soil of 25 kPa was adopted. Plants were evaluated by means of a rating scale based on leaf wilt. The genotypes RVTA pl#290, RVTA pl#291, RVTA pl#301, RVTA pl#325, RVTA pl#54, RVTA pl#135, RVTA pl#138 e Redenção were the most affected by the water deficit. The genotypes RVTA pl#291, RVTA pl#59, RVTA pl#248, RVTA pl#133 and accession LA-716 stood out in relation to the other genotypes in conditions of water deficit. RVTA pl#292, RVTA pl#59, RVTA pl#248 and RVTA pl#133 BC₃F₂ genotypes are promising for the development of cultivars for industrial processing tolerant to water deficit.

Keywords: *Solanum pennellii*; breeding; drought tolerance; water stress.

TOLERANCE OF PROCESSING TOMATOES BC₃F₂ GENOTYPES TO WATER DEFICIT

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Introduction

Tomatoes are considered to be widespread, positioning themselves in the agro-industrial chain as one of the most important in the agribusiness complex, as it is one of the most consumed vegetables in the world, both fresh and processed. The water consumption by the tomato crop is quite high, standing at around 8800 mm (or 8500 m³/ha) throughout the crop cycle. Aiming at the lack of water in the world, genotypes with greater efficiency in the use of water and/or with greater tolerance to drought may allow a substantial increase in the area planted with the crop, with less impact on the use of natural resources.

As controls, the donor parent LA-716 (tolerant to water deficit) and the commercial cultivar Redenção (susceptible to water deficit) were used. The experimental design was in randomized blocks, containing three replications with eight plants each. The plants were irrigated without distinction until 30 days after transplantation, using micro-drippers to standardize the development of the plants. Since then, two levels of water supply have been adopted, 0% (water stress) and 100% (control) of water needs for 19 days. In the condition of 100% supply, a maximum water tension in the soil of 25 kPa was adopted. The plants were evaluated using a classification scale based on leaf wilt.



Figure 1. Conduction of tomatoes in pots

Results

Table 1. Comparison of tomato genotypes to water stress

Genotype	100% (control)	0% (water stress)
RVTA pl#250	5.00 A	4.00 B
RVTA pl#291	5.00 A	4.00 B
RVTA pl#301	5.00 A	4.33 B
RVTA pl#323	5.00 A	4.33 B
RVTA pl#292	5.00 A	4.66 A
RVTA pl#59	5.00 A	4.66 A
RVTA pl#54	5.00 A	4.33 B
RVTA pl#248	5.00 A	4.66 A
RVTA pl#133	5.00 A	5.00 A
RVTA pl#136	5.00 A	4.33 B
RVTA pl#138	5.00 A	3.66 B
Redenção	5.00 A	3.33 B
LA-716	5.00 A	5.00 A

Conclusion

RVTA pl # 292, RVTA pl # 59, RVTA pl # 248 and RVTA pl # 133, BC₃F₂ genotypes are promising for the development of cultivars for industrial processing tolerant to water deficit.

Materials and methods

The BC₃F₂ genotypes RVTA pl # 138, RVTA pl # 54, RVTA pl # 248, RVTA pl # 290, RVTA pl # 136, RVTA pl # 135, RVTA pl # 301, RVTA pl # 292, RVTA pl # 133, RVTA pl # 325, RVTA pl # 59 and RVTA pl # 291 were explored.

VARIANCE COMPONENTS AND ADAPTABILITY AND STABILITY OF COTTON GENOTYPES USING GGE BI PLOT ANALYSIS

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The upland cotton (*Gossypium hirsutum* L.), originated at Central America, is considered one of greatest socioeconomic importance crops for Brazilian agricultural scenario. This study aimed to estimates the variance components and to apply the genotype + genotype-by-environment (GGE) biplot analyses in a multi-environmental trial (MET) data from cotton genotypes. Ten trials were conducted in the Midwest region of Brazil. The trials consisted of randomized complete block design with 12 cotton cultivars with four replicates each. The genotypes were evaluated for fiber yield (FY), fiber length (FL), fiber strength (FS), and micronaire (MIC). The estimation of variance components was made through restricted maximum likelihood (REML) and the prediction of genotypic values was made through best linear unbiased prediction (BLUP). The significance of the random effects of the model (genotypic and GxE interaction effects) were tested using the likelihood ratio test (LRT). The GGE biplot analysis was performed to find the best genotypes regarding adaptability and stability. The statistical analyses were performed using the following software: ASREML-R and Selegen-REML/BLUP; the GGE-biplot analysis were made at *GGEbiplotGUI* package in R. Significance of the genetic and GxE interaction effects ($p < 0.05$) were detected by the LRT for all traits. The variance components were significantly different from zero as well as from the coefficients of determination. The LRT values demonstrate the existence of genetic variability for all traits among the 12 cotton genotypes under study, which implies the possibility of selecting superior genotypes from the pool. The values of heritability were classified as low ($hg^2 < 0.29$) in FY (for all ten environments) and for MIC traits, and moderate ($0.30 < hg^2 < 0.50$) in FL and FS. The FY trait had the lowest estimated value of heritability, showing to be strongly affected by GxE interaction. Conversely, while FY and MIC had high ($0.70 < r_{gE} < 0.89$) values for selective accuracy, FS and FL values were higher ($0.90 < r_{gE}$). The values higher than 0.70 revealed the strong genetic control of the traits under study. The GGE-biplot indicated the formation of two mega-environment (ME) for FY trait and one ME for the traits FL, FS and MIC. For FY, the genotype G3 and G7 were the most adapted for each ME, while G5, G1 and G3 were the most adapted for ME formed for the traits FL, FS and MIC, respectively. Regarding stability, the genotypes G3, G12, G1, and G5 were the most stable ones, for the traits FY, MIC, FS, and FL, respectively. The GGE biplot indicated that the genotypes G3 and G5 were highly adaptable and stable for the main traits in cotton (FY and FL). Therefore, the GGE Biplot methodology proves to be an important tool for the identification of genotypes with high adaptability and stability in cotton breeding.

Keywords: Fiber quality; genotype-by-environment interaction; *Gossypium hirsutum*; principal component analysis.

Acknowledgements: CAPES, CNPq and FAPEMIG.

Introduction and Objective

The upland cotton (*Gossypium hirsutum* L.), originated at Central America, is considered one of greatest socioeconomic importance crops for Brazilian agricultural scenario. This study aimed to estimates the variance components and to apply the genotype + genotype-by-environment (GGE) biplot analyses in a multi-environmental trial (METT) data from cotton genotypes.

Materials and methods

Ten trials were evaluated for fiber yield (FY), fiber length (FL), fiber strength (FS), and micronaire (MIC). The estimation of variance components was made through REML and the prediction of genotypic values BLUP. The GGE biplot analysis was performed to find the best genotypes regarding adaptability and stability. The statistical analyses were performed using ASREML-R, Selegen-REML-BLUP and GGEbiplotGUI package in R.

Results and discussion

The values of heritability were classified as low ($H_g^2 < 0.29$) in FY (for all ten environments) and for MIC traits, and moderate ($0.30 < H_g^2 < 0.50$) in FL and FS. The FY trait had the lowest estimated value of heritability, showing to be strongly affected by GxE interaction. Conversely, while FY and MIC had high ($0.70 < r_{gE} < 0.89$) values for selective accuracy, FS and FL values were higher ($0.90 < r_{gE}$). The values higher than 0.70 revealed the strong genetic control of the traits under study.

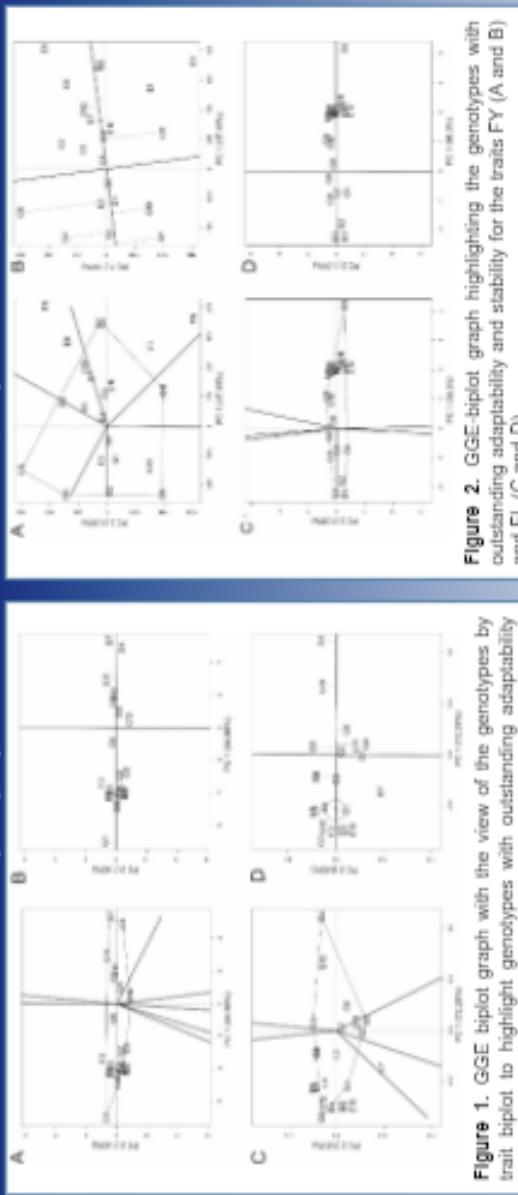


Figure 1. GGE-biplot graph with the view of the genotypes by trait biplot to highlight genotypes with outstanding adaptability and stability for FS (A and B) and MIC (C and D) traits.

The GGE-biplot indicated the formation of two mega-environment (ME) for FY trait and one ME for the traits FL, FS and MIC. For FY, the genotype G3 and G7 were the most adapted for each ME, while G5, G1 and G3 were the most adapted for ME formed for the traits FL, FS and MIC, respectively. Regarding stability, the genotypes G3, G12, G1, and G5 were the most stable ones, for the traits FY, MIC, FS, and FL, respectively.

Conclusion

Therefore, the GGE Biplot methodology proves to be an important tool for the identification of genotypes with high adaptability and stability in cotton breeding.

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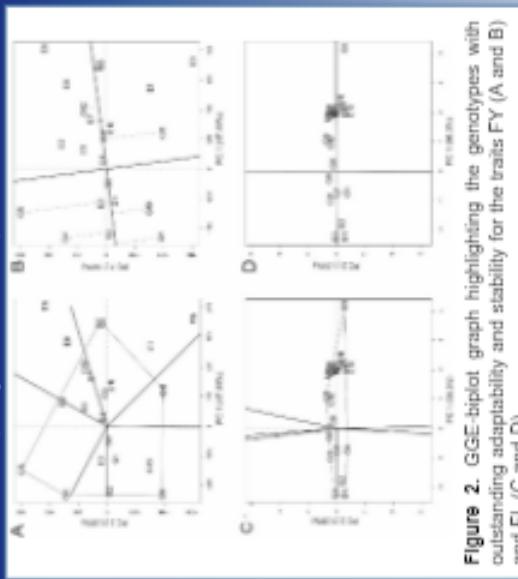


Figure 2. GGE-biplot graph highlighting the genotypes with outstanding adaptability and stability for the traits FY (A and B) and FL (C and D).

INFLUÊNCIA DA HETEROSE NA PRODUTIVIDADE DE GRÃOS EM POPULAÇÕES TROPICAIS E TEMPERADAS DE MILHO-PIPOCA

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Compreender a heterose é crucial para escolher populações potenciais de milho-pipoca (*Zea mays everta*) para serem utilizadas em programas de melhoramento. Com isso, o objetivo deste estudo foi avaliar a importância da heterose entre populações tropicais e temperadas de milho-pipoca sobre a produtividade de grãos. Nove populações de milho-pipoca sendo 3 tropicais e 6 temperadas, foram cruzadas em esquema dialélico. Os genótipos foram avaliados em um experimento disposto no delineamento alfa-lattes 9 x 10 com três repetições na safra 2017/18. A parcela experimental foi composta de duas linhas de 4 m, espaçadas por 0,8 m, com densidade de 5 plantas m⁻¹. No ponto de maturidade fisiológica todas as espigas de cada parcela foram colhidas e secas ao ar. O rendimento de grãos (kg ha⁻¹) foi estimado em 145 g kg⁻¹ de umidade. Para a produtividade de grãos, o resultado mostrou que a população ARZM 07-49 é superior às demais populações em termos de frequência de genes favoráveis. Essa população apresentou o maior valor para a média populacional 4480,1 kg ha⁻¹. Em geral, as populações temperadas apresentaram valores baixos para a produtividade média das populações, apresentando um rendimento médio de 2270 kg ha⁻¹, enquanto as populações tropicais apresentaram produtividade média de 3130 kg. ha⁻¹. A explicação mais provável para isso parece ser a adaptação superior deles aos ambientes testados. Como esperado, para a produtividade de grãos, a heterose média foi positiva e de alta magnitude (486,8 kg ha⁻¹), indicando dominância unidirecional positiva. A heterose varietal, em valor absoluto, indica divergência da população ARZM 07-49 em relação aos demais genitores para produtividade de grãos. As estimativas de heterose são um indicador das populações com maiores diferenças de frequência gênica. Visto isso, as maiores diferenças de frequências gênicas foram observadas entre as populações Sintético-UFV e UFV-MP1 (1092 kg ha⁻¹), e Sintético-UFV e ARZM 07-49 (1077 kg ha⁻¹). Para a produtividade de grãos, a combinação mais divergente entre si em relação ao grupo parental foi Sintético-UFV x UFV-MP1 (-505,43 kg ha⁻¹). Por fim, conclui-se que há variabilidade genética e dominância para produtividade de grãos. Um grupo heterótico com alta heterose para produtividade de grãos foi identificado a partir do cruzamento Sintético-UFV e UFV-MP5.

Palavras-chave: Grupo heterótico; melhoramento; população; dialelo; rendimento de grãos.

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Introduction

The maize breeding programs are fundamentally based on utilization of heterotic patterns and groups (Melani and Carena 2005). The establishment of maize germplasm into a known heterotic group allows the maximum exploitation of heterosis, which provides increase yield potential (Hochholdinger and Baldauf 2018). According to Lee (1995), a heterotic group is comprised of a set of germplasm that tends to display higher degree of heterosis when crossed with an external group than when crossed with a member of its own group. Whereas, heterotic pattern refers to specific pair of genotypes of two distinct heterotic groups. Based on phenotypic data, the heterotic groups have been classified by two main approaches, the diallel analysis (Soengas et al. 2003) and testcrosses (Barata and Carena 2006). However, due to environmental influence in the morphological traits, molecular markers has also been employed to classify heterotic groupings. Furthermore, understanding heterosis is crucial to choose the potential popcorn populations to be used in future hybrid programs.

Objective

The objective of this study was to evaluate the importance of heterosis between tropical and temperate popcorn populations on grain yield.

Materials and methods

Nine populations of popcorn, three of tropical origin and five of temperate origin, belonging to the popcorn germplasm bank of the UFV, and a temperate population from CIMMYT, were crossed in a diallel scheme.





UNCOVERING THE POWER AND THE SECRETS BEHIND DNA MODIFICATIONS IN TROPICAL AND TEMPERATED POPCORN POPULATIONS

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The genotypes were evaluated in an experiment arranged in a 9×10 alpha-lattice design with three replications in the 2017/18 crop in the experimental field of the UFV Corn Improvement Program. The experimental plot was composed of two lines of 4 m, spaced by 0.8 m, with a density of 5 plants m^{-2} . At the point of physiological maturity, all ears of each plot were harvested and air dried. When they reached 13% humidity, the ears were threshed and the beans were reserved in a cold chamber at $4^{\circ}C$ until the moment of being evaluated. Grain yield ($kg\ ha^{-1}$) was estimated at $145\ g\ kg^{-1}$ of moisture.

Results and discussion

For grain yield, the result showed that the ARZM 07-49 population is superior to the other populations in terms of the frequency of favorable genes. This population had the highest value for the population average $4480.1\ kg\ ha^{-1}$. In general, temperate populations showed low values for the average productivity of the populations, presenting an average yield of $2270\ kg\ ha^{-1}$, while the tropical populations presented average productivity of $3130\ kg\ ha^{-1}$. The most likely explanation for this appears to be their superior adaptation to the tested environments. As expected, for grain yield, the average heterosis was positive and of high magnitude ($486.8\ kg\ ha^{-1}$), indicating positive unidirectional dominance. Varietal heterosis, in absolute value, indicates divergence in the ARZM 07-49 population in relation to the other parents for grain productivity. Heterosis estimates are an indicator of populations with the greatest differences in gene frequency. In view of this, the greatest differences in gene frequencies were observed between the Synthetic-UFV

Conclusion

Finally, it is concluded that there is genetic variability and dominance for grain yield. A heterotic group with high heterosis for grain yield was identified from the crossing Synthetic-UFV x UFV-MP1.

References

- Barata C and Carena MJ (2006) Classification of North Dakota maize inbred lines into heterotic groups based on molecular and testcross data. *Euphytica* 151: 339–349.
- Hochholdinger F and Baldauf JA (2018) Heterosis in plants. *Current Biology* 28: R1089–R13.
- Lee M (1995) DNA markers and plant programs. *Advances in Agronomy* 55: 265–344.
- Melani MD and Carena MJ (2005) Alternative maize heterotic patterns for the northern Corn Belt. *Crop Science* 45: 2186–2194.
- Soengas P, Ordás B, Malvar RA, Revilla P and Ordás A (2003) Heterotic patterns among flint maize populations. *Crop Science* 43: 844–849.

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