

PROCEEDINGS

I International Meeting on Plant Breeding



October - 2017

THE INTERNATIONAL MEETING ON PLANT BREEDING

The growing demand for food makes breeders pursue more productive, stable and resistant genotypes. However, finding the best breeding strategy is not simple. It is a multidisciplinary and dynamic science and the combination of classical and modern approaches may constitute an important approach to promote higher genetic gains. Based on this idea, our event is focused on fostering discussions with key professionals in genetics and plant breeding in order to prepare stakeholders for challenges in agriculture.

The International Meeting on Plant Breeding is one of several events in the "DuPont Plant Sciences Symposia" series. Our aim is training new professionals by providing the opportunity to interact with researchers from public and private institutions through discussions of relevant scientific topics.

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 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 in training from the "voice of the graduates", in which the alumni with consolidated careers 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.

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ACKNOWLEDGEMENTS

The organizing committee is thankful for all the support provided by DuPont Pioneer, ESALQ -"Luiz de Queiroz" College of Agriculture, The Department of Genetics, The graduate program in Genetics and Plant Breeding and FEALQ (Foundation of Agrarian Studies Luiz de Queiroz)

PROGRAM

October 04, 2017 - (Wednesday)

07:00 – 08:00 – Registration

08:00 – 08:45 – Opening session – Tabare Abadie (DuPont Pioneer)

08:45 – 09:45 – Lecture 1 – Enhancing Yield Gains in Soybean
George Graef (University of Nebraska)

09:45 – 10:15 – Coffee break

10:15 – 11:15 – Lecture 2 – Improving phenotypic prediction through crop model-whole genome prediction integration
Charlie Messina (DuPont Pioneer)

11:15 – 12:00 – Poster session

12:00 – 14:00 – Lunch

14:00 – 15:00 – Lecture 3 – Advanced methods to study plant phenotyping of stress responses
Paulo Herrmann (EMBRAPA)

15:00 – 16:00 – Lecture 4 – Plant Breeding for Disease Resistance
Luís Eduardo Aranha Camargo (USP/ESALQ)

16:00- 16:30 – Coffee break

16:30 – 17:30 – Lecture 5 – Smart Breeding: tools and technologies to increase genetic gain in maize
Fabiani Rocha (DuPont Pioneer)

17:30 – 18:00 – Poster Session 2

19:00h – Dinner

October 05, 2017 - (Thursday)

08:30 – 09:30 – Lecture 6 – Genomic Selection in Plant Breeding: Why? and How?
José Crossa (CIMMYT)

09:30 – 09:45 – Student oral presentation

09:45 – 10:15 – Coffee break

10:15 – 11:15 – Lecture 7 – Applications of Genomics in Polyploid Crops
Gabriel Rodrigues Alves Margarido (USP/ESALQ)

11:15 – 11:30 – Closing remarks

SPEAKERS

Dr. Tabare Abadie



With 35 years of career in Plant Breeding, his areas of interest include Quantitative Genetics and Statistics as well as Technological Change in Large Organizations, Career Development, Education and Mentoring. Currently in DuPont Pioneer, Tabare leads the Research Effectiveness Team, responsible of building an educational and career development environment for research employees worldwide and to develop relations with Academia. Some of the programs under his supervision include the world wide DuPont Pioneer Plant Breeding Symposia, Fellowships and Internships. Before this assignment, he led a group responsible for the deployment of Molecular Breeding across species globally. Before coming to Iowa,

Tabare was a Wheat and Barley Breeder at La Estanzuela (Uruguay) and a Full Professor in Plant Breeding at the Universidad de la Republica (Uruguay), and served as consultant for EMBRAPA CENARGEM (Brazil).

Dr. George L. Graef

George Graef is Professor in the Department of Agronomy & Horticulture at the University of Nebraska –Lincoln, where he leads the soybean breeding program. He received his B.S. with honors in Environmental Horticulture at the University of Connecticut, and completed his M.S. and Ph.D. degrees at Iowa State University in Plant Breeding and Cytogenetics, with a minor in Genetics. His professional efforts have been focused on identifying constraints to soybean production for farmers in Nebraska and the north central USA, addressing those challenges through plant breeding. His applied research focus is on developing highly productive cultivars with resistance to important biotic and abiotic stresses, as well as improved compositional quality for feed, food, or industrial end uses. The two most important abiotic stresses addressed by the breeding program are iron-deficiency chlorosis on high-pH soils and drought tolerance.



Dr. Charlie Messina



Development of a successful strategy for prediction of crop yield that accounts for the effects of genotype, environment and management, creating many opportunities for enhancing the productivity of agricultural systems. Charlie research team at DuPont Pioneer focuses on two areas of research, 1) crop physiology and mechanistic modeling of plant growth and development, and 2) the integration of these with statistical learning algorithms to enable end-to-end prediction framework for yield.

Dr. Paulo Herrmann



Dr. Herrmann graduated in Electrical Engineering Electronics at Barretos Educational Foundation (1986), completed his master's degree in electrical engineering from the University of São Paulo (1993) and PhD in Physical Chemistry from the University of São Paulo (1999). He was a postdoctoral fellow at the University of Pennsylvania in the Department of Chemistry and in the period 2012 to 2015 participated as a researcher of virtual labs program of Embrapa in Germany. During this time, he was conducting research and organizing the international cooperation at "Forschungszentrum Juelich / Institute of Bio-Geosciences - 2: Plant Sciences, in new methods applied plant phenotyping.. He has experience in Electrical

Engineering with emphasis in Electronic instrumentation and Physical Chemistry of surface applied to agriculture issues, at the nanoscale. He has interest in the following topics: agricultural instrumentation, atomic force spectroscopy, atomic force microscopy, development of low cost sensors using conducting polymers and application of new methods to plant phenotyping.

Dr. Luis Eduardo. A. Camargo

Luis Camargo is an Associate Professor in the Department of Plant Pathology at USP and has expertise in the field of plant-microbe interactions. His main interest is to identify the genetic components involved in the reaction of plants to pathogens and their relationships with proteomic, metabolomic and phenotypic changes. The ultimate goal is to identify biological markers that can be used to discover resistant genotypes. He currently is studying the metabolomic changes in sugarcane in response to infection by Lxx in collaboration with Dr. Ana Paula Alonso (CAPS – OSU). He co-advises a student of the dual degree program in Translational Plant Sciences (OSU) and International Cell and Molecular Biology (USP).



Dr. Fabiani da Rocha



Fabiani da Rocha completed her Bachelor's Degree in Agronomy and MSc in Plant Production at the Santa Catarina State University; and her PhD degree in Genetics and Plant Breeding at Esalq - Luiz de Queiroz College of Agriculture. Since March 2015 she is working with Dupont Pioneer as a Corn Breeder based at the station in Passo Fundo, Rio Grande do Sul. Her current work is focused on the development of corn inbreds and commercial corn.

Dr. José Crossa

Graduated at the Universidad de la República Oriental del Uruguay (BS, Agriculture, 1974) and the University of Nebraska-Lincoln (PhD, Statistics and Quantitative Genetics, 1984), Crossa came to CIMMYT as a postdoctoral fellow in the Computing Unit and Maize Research Program in 1984. He has helped define key methodologies for conserving and using the center's maize genetic resources, covering proper regeneration procedures and strategies for forming core subsets of large germplasm collections. Prof. Roland Vencosky and Jose Crossa developed theoretical and practical work on genetic resources conservation; this work made Crossa to be selected the best scientist of the CGIAR Centers in 2008. His substantive body of research and publications has addressed many other areas of breeding and agronomy research, including genotype x environment, and QTL x environment interactions, general breeding and experimental design, hybrids and heterotic patterns, and association mapping, to name a few important subjects, and enjoys international acclaim and application.



Dr. Gabriel Margarido



Gabriel Rodrigues Alves Margarido has a degree in Agriculture from the 'Luiz de Queiroz' College of Agriculture - ESALQ / USP (2006) and a PhD from the Graduate Program in Genetics and Plant Breeding from ESALQ/USP, at the Department of Genetics (2011). He worked as a postdoctoral researcher at the eScience Group at Microsoft Research in Los Angeles. He is an Assistant Professor in the Department of Genetics at ESALQ / USP since 2013. His main areas of interest are Quantitative Genetics and Bioinformatics, working mainly in the construction of linkage maps, QTL mapping with emphasis on outcrossing and polyploid species, computational biology, genome assembly and analysis of RNA and next generation sequencing data. His current work is focused on the structural and functional analysis of the sugarcane genome, with the objective of developing algorithms for the assembly and investigation of polyploid genomes. He is also interested in other species with potential importance for bioenergy production.

SUMMARY

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ADAPTABILITY AND STABILITY OF CORN PRE-COMMERCIAL HYBRIDS IN THE CENTRAL-SOUTH REGION OF BRAZIL

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The phenotypic expression of agronomic interest traits, such as grain yield, can vary significantly under different edaphoclimatic conditions. Since maize is grown in almost all Brazilian territory, different environments should be a concern to the identification and selection of superior genotypes. In view of these observations, the identification of cultivars adapted and with stability of grain yield shows an alternative to minimize the interaction genotypes x environments and assist genotype recommendation. The objective of this research was to estimate the adaptability and stability of corn pre-commercial hybrids in four locations, aiming to identify the most stable and promising hybrids for grain yield in the Center-South region of Brazil. We evaluated 31 pre-commercial hybrids of maize, being 24 simple hybrids and 7 triple hybrids, in addition to 3 commercial hybrids as testimonials. The experiments were conducted in the following locations: Dourados - MS, Maracaju - MS, Campo Mourão - PR and Paranagi - PR. The experimental design was a randomized block design with three replications. The grain yield (kg ha^{-1}) was evaluated and the adaptability and the stability parameters were estimated by Annicchiarico, Lin and Binns and AMMI methodologies. There was significant difference ($P < 0.01$) for the purposes of hybrids, environment and genotypes x environments interaction. There are pre-commercial hybrids with performance equal to or higher than the commercial hybrids tested at the four places. The hybrid HS 0262 was recommended for the four locations by the three methodologies. In Annicchiarico e Lin e Binns analysis the hybrids HS 0997 and HS 0832 were the most stables and had greater general and specific adaptability to unfavorable environments. According to AMMI analysis, the most stable, adapted and productive hybrids were HS0805, HS 0774 and HS 0727.

Keywords: genotypes x environments interaction, grain yield, hybrids, *Zea mays* L

Acknowledgment: Agriseeds, UFGD, CNPq and Capes.

AMMONIUM TRANSPORTER FAMILIES OF *Citrus sinensis* GENOME: *IN SILICO* IDENTIFICATION AND CHARACTERIZATION

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Essential for fruit quality and yield, nitrogen (N) is one of the nutrients required in larger amounts by *Citrus*. Nitrate (NO₃⁻) and ammonium (NH₄⁺) are the main inorganic sources of N for plants. Transporters of high and low affinity mediate the NH₄⁺ uptake, which are coded by two gene families, AMT1 and AMT2, respectively. There is a lack of information about the molecular mechanisms controlling NH₄⁺ uptake by *Citrus* plants. The aim of this work was to identify and characterize AMT gene families in the *C. sinensis* genome. AMT protein sequences from plants sharing high genomic identity with *C. sinensis* (*Arabidopsis thaliana*, *Carica papaya* and *Populus trichocarpa*) were used as queries to find ortholog sequences in *C. sinensis* genome. Selected sequences were classified according to the presence of "Ammonium transporter" conserved domain, number of transmembrane (TM) domains and subcellular localization. Phylogenetic trees were constructed with AMT sequences. *In silico* expression was analyzed in four tissues (callus, flower, leaf and fruit) and expressed in Reads Per Kilobase per Million (RPKM). We identified 3 AMT1 and 6 AMT2 genes in *C. sinensis* genome. AMT1 predicted proteins were predicted to be located in the Golgi apparatus, while AMT2 sequences were equally distributed between Golgi apparatus and Cytoplasm. The number of TM domains ranged from nine to thirteen. Phylogenetic analysis showed a clear separation between the AMT1 and AMT2 gene families. The transcriptional rates of AMT1 were available for only one gene (Cs5g25370.1), which showed higher expression in leaves (2.7 RPKM). For AMT2 family the expression of all copies was available, and the main genes responsible for transcriptional activity were Cs1g19190.1 and Cs6g08950.1, that had higher rates of transcription in callus (5.8 RPKM) and leaves (6.7 RPKM), respectively. The present work was the first to perform an *in silico* analysis of all genes responsible for ammonium transport throughout the *C. sinensis* genome and these genes can be used in further studies aiming to improve the nitrogen use efficiency of Citrus plants.

Keywords: AMT; Nitrogen use efficiency; Orange; Nutrient uptake; Phylogenetic analysis.

CELLULAR COMPARTMENTS TARGETED BY CANDIDATE EFFECTOR PROTEINS FROM *Sporisorium scitamineum*, THE CAUSAL AGENT OF SUGARCANE SMUT

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Sugarcane is one of the most valuable crops worldwide mostly due to the great economic value of its subproducts. The production of sugarcane can be affected by many diseases, including smut, caused by the biotrophic fungus *Sporisorium scitamineum*. Disease establishment is fully dependent on the environmental conditions and sugarcane genotype, leading to the emission of a whip like structure responsible for teliospore spreading in the late stages. A previous analysis of the pathosystem using dual RNAseq-based transcriptome uncovered the expression of genes coding for candidate effectors. Effector proteins are one of the most important molecules released by pathogens during disease establishment and development. This work aimed to determine the subcellular plant compartments targeted by seven *S. scitamineum* candidate effectors, being four the most expressed in early interaction and three exclusive from the time of whip emission. Transient expression assay in *N. benthamiana* leaves was performed. The coding sequence of mature protein was cloned to obtain candidate effector-green fluorescent protein (Citrine) fusions downstream of a 35S promoter in an *Agrobacterium tumefaciens* binary vector. The fusion proteins were transiently expressed by agroinfiltration and their accumulation in leaf cells was determined by confocal microscopy and immunoblots. All proteins accumulated at detectable levels. Results revealed that *S. scitamineum* candidate effectors encode proteins which target various plant compartments, such as membrane, cytosol, chloroplasts and nucleus, and some of them seems to undergo post-translational modifications. Multiple target observations imply that the fungus counts with powerful tools to manipulate its host along with disease. Effectors targeting nucleus and its sub compartments are known to be crucial for host transcriptional reprogramming in favor of the pathogen. Chloroplasts are major direct targets of effectors in multiple systems, since they can influence hormone as well as key defense-related molecule production. Further analysis will be performed in order to define the potential role of these proteins within host cells during disease establishment.

Keywords: Effector biology; Plant-pathogen Interaction; Subcellular Location; *Saccharum sp.*

Acknowledgements: FAPESP (Projects 2016/16376-8 and 2016/04429-0)

CHARACTERIZATION OF A PERICENTROMERIC 160bp SATDNA FAMILY IN *ARABIDOPSIS THALIANA*

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Centromeres are the primary constrictions in the chromosomes, being a heterochromatic chromatin structure, which is mainly composed by *in tandem* repetitive DNA satellite (satDNA) sequences. Kinetochore protein deposition occurs in these structures, promoting sister chromatids cohesion and the correct chromosome segregation during cell division. *Arabidopsis thaliana* genome presents the family 180bp as the most abundant centromeric satDNA sequence in all chromosomes, which makes this family the most well characterized. However, other less abundant families, such as the 160bp, were not thoroughly described. A screening in the *A. thaliana* genome was performed to retrieve pericentromeric BACs available at the EnsemblPlant database (plants.ensembl.org/) containing 160 bp satDNA arrays. BACs containing repetitive sequences were identified by DotPlot and the motifs were extracted using the Tandem Repeats Finder software. The motifs were edited, aligned and compared for identification. The study using bioinformatic analysis tools, revealed that the 1107 motifs of the 160bp family are distributed along the pericentromeric region of the chromosomes 1 and 4, while the most abundant family 180 bp satDNA is presented in all chromosomes. Both satDNA families, 160 bp and 180 bp, share the same pericentromeric chromatin context, but never appear in contact or intermingled to each other. Comparing both sequences in a DotPlot analysis, a terminal conserved region of 15bp was identified. A weblogo of the 160bp satDNA motifs show the level of nucleotide conservation, especially for the terminal 15bp that is also well conserved in the 180bp. The 160 bp satDNA family is chromosome-specific and shows just a shallow relationship to the 180 bp satDNA family. This weak relationship between both families can provide evidences for the 160 bp satDNA family origin, that could had suffered a completely turnover of the sequences after chromosome rearrangements during *A. thaliana* diversification. Further analysis is required for thorough understanding of the 160 bp satDNA origin, specially comparing complete genome sequencing in ecotypes and related species.

Keywords: Bioinformatics; satellite DNA; 180pb; 160pb.

EPIGENETIC DIVERSITY OF SUGARCANE BASIC GERMPLASM

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Sugarcane crop has a high sugar, ethanol and biomass production potential. Modern sugarcane varieties have an interspecific origin derived from crosses between a few number of accessions mainly from *S. officinarum* and *S. spontaneum*. There is a huge genetic variability in the *Saccharum* species and related genera that can be exploited by the breeding programs in the development of sugarcane varieties. Although the genetic variability of sugarcane basic germplasm has being study at the genome level, there is no information about the genetic variability at the epigenome level. DNA methylation is one of the main epigenetic modifications. The MSAP (*Methylation sensitive amplification polymorphism*) technique has being used to quantify the DNA methylation level. In the present study the MSAP technique was applied to investigate the genetic diversity within and among six groups of accessions (*S. officinarum*, *S. spontaneum*, *S. barberi*, *S. robustum*, *Erianthus* sp. and commercial varieties). Two DNA samples of the same genotype were digested with the restriction enzymes *EcoRI*-*MspI* and *EcoRI*-*HpaII*, ligated to the respective adapters followed by pre-selective and selective amplification. The amplification products were compared side by side for methylation patterns determination. Each MSAP marker was classified as Methylation-Susceptible Loci (MSL) and Non-Methylated Loci (NML). The molecular variance analysis (AMOVA) and Principal Coordinate Analysis (PCoA) were conducted by the R MSAP package (R 3.2.5. program). A total of 1341 loci (1117 MSL and 224 NML) were obtained. The majority of the MSL was found in the commercial and *S. barberi* accessions. The genetic differentiation (ϕ_{ST}) among the six groups was 0.329 and 0.170 respectively for the NML and MSL. Most of the identified polymorphisms occurred due to hemi-methylation of external cytosine. The PCoA showed different cluster patterns when performed with the NML and MSL. The cluster formed by the *S. officinarum* and commercial accessions were distant each other in the PCoA conducted with the MSL compared to that obtained by the NML. In the NML PCoA, the cluster formed by the *Erianthus* sp accessions was apart from the other species. Our results revealed that different levels of methylation exist among the investigated groups. However, within each group the level of external cytosine methylation was higher than the internal. The difference between ϕ_{ST} values obtained by the NML and MSL suggest that epigenetics probably had a lesser contribution in the differentiation of the investigated groups.

Keywords: sugarcane; epigenetic; MSAP; R program.

EVALUATION OF INTERMEDIATE HYBRIDS AND CORN S₂ LINES

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In maize breeding programs the identification of good combining ability is a key strategy for success in the development of high performance hybrids. However, the development of hybrids from Partially Inbreeding Lines (LPE) may be an alternative to the use of double or triple hybrids of lines with complete endogamy. The aim of this study was to evaluate a group of LPE hybrids obtained through prediction based on a diallel cross group between S₂ lines of the PC 0201 and PC 0202 maize populations, and in parallel with the evaluation and characterization of these lines in evaluation *per se*. The LPE trials was conducted in Londrina, Guarapuava and Santa Tereza do Oeste, in the state of Paraná, Brazil, with 117 treatments, 115 hybrids between S₂ progenies and two commercial single hybrids as controls (2B 810 from Dow AgroSciences and DKB 290 from Dekalb). Lines evaluation *per se* was conducted in Londrina and Santa Tereza do Oeste. In both trials, the following characteristics were evaluated: plant height and ear (AP and AE), lodging (CA) and plant breakage (QB), stand (ST), ear number (NE), female flowering (FF) and grain yield (REND). In both experiments the experimental design of randomized blocks with two replications per location was used, the plot was composed of a line of 5 meters in length and five plants per meter. In the evaluation of the hybrids, it was observed that in relation to (REND), the controls (mean of 14.329 Kg/ha⁻¹) were statistically similar (P <0.01) when compared to the two most productive LPE hybrids (mean of 13.062Kg/ha⁻¹), 91.2% of the mean in relation to the controls. Presenting expected data with the use of triple hybrids, and obtaining a lower seed cost than simple hybrids. In relation to the evaluation of lines *per se*, for the REND character, the average of the S₂ lines corresponded to 63% of the average of the original populations (PC 0201 and PC 0202) with average productivity of 12.210Kg/ha-1. The data obtained show that the LPE under study, demonstrate good potential for hybrid seed production.

Keywords: *Zea mays* L.; Diallelic crossing; Grain yield; Partially Inbreeding Lines.

GANHO DE SELEÇÃO PARA CARACTERES QUANTITATIVOS EM UM TESTE DE PROGÊNIES DE *Pinus maximinoi*

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O *Pinus maximinoi* é uma espécie que ocorre principalmente em florestas tropicais e subtropicais úmidas em elevações variando entre 700 e 2400 m de altitude, cuja área de ocorrência se encontra na América central. Os estudos referentes ao melhoramento genético são recentes, ainda em fase de domesticação. Contudo, sabe-se que a espécie apresenta potencial para a produção de celulose e as propriedades da madeira se assimilam ao *Pinus taeda*, espécie de Pinus mais plantada comercialmente no Brasil. Dessa forma, o objetivo foi avaliar o ganho de seleção, utilizando uma intensidade de seleção (IS) de 10% para o incremento médio anual (IMA), o diâmetro à altura do peito (DAP) e a altura (H) em um teste de progênie de *Pinus maximinoi*. Esse teste foi implantado em Buri, SP, no delineamento de blocos completos com informação dentro de parcela, onde os tratamentos foram constituídos de progênies obtidas das 23 melhores matrizes do Instituto Nacional de Bosques da Guatemala e adicionados 3 testemunhas, totalizando 26 tratamentos, distribuídos em 10 blocos, com 6 plantas por parcela no arranjo de 3 x 2m, contendo 4 linhas de bordadura, as características DAP, H e IMA foram mensuradas aos 5 anos de idade. Para a realização das análises, utilizou-se o modelo 1 do SELEGEN REML/BLUP, sendo estimado a correlação de Spearman para as três características sob avaliação. As herdabilidades para o IMA o DAP e a H foram 0,396; 0,278 e, 0,65 respectivamente. Essas estimativas foram consideradas moderada para a altura e baixas para o DAP e o IMA. Constatou-se que o ganho com a seleção para o IMA dos 10% de melhores indivíduos foi de 22,1% enquanto que para DAP foi de 8,34%, já para a altura o ganho foi de 16%, isso porque a herdabilidade é diretamente proporcional ao ganho e por se tratar de uma espécie nova, são esperados valores elevados de ganho, uma vez que se trata do primeiro ciclo de melhoramento dessas progênies. Com relação a correlação entre os caracteres, foi encontrado um valor médio de 0,95 para as três correlações quando se envolveu todos os indivíduos. Levando em conta apenas os selecionados (157), as correlações foram de 0,74 para altura e DAP, 0,78 para altura e IMA e 0,80 para DAP e IMA. Portanto, apesar da baixa herdabilidade encontrada para o DAP, esta pode ser a melhor característica a ser avaliada, uma vez que é a mais prática de ser obtida e apresenta elevadas correlações com a altura e com o IMA.

Palavras chave: Correlação; Herdabilidade; Pinus; Intensidade de seleção.

Agradecimentos: FAPEMIG, CAPES e CNPq e GRUPO RESINAS BRASIL

GENETIC DIVERSITY IN *Campomanesia adamantium* POPULATIONS BASED ON FRUITS MORPHOLOGICAL CHARACTERISTICS

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Guavira (*Campomanesia adamantium*), belonging to the Myrtaceae family, is a Brazilian cerrado native fruit. Its fruits are consumed in natura and used as agents in liqueurs, juices, sweets and ice creams. The *C. adamantium* leaves and fruits have medicinal properties, including anti-inflammatory, antidiarrheal and antiseptic activities in the urinary tract. Although the species presents several possibilities of economic exploitation, little is known about the levels of genetic diversity of natural populations. Understanding the genetic diversity of native species allows the identification of promising genotypes that can later be included in breeding programs. This work's objective is to evaluate the genetic diversity of guavira access through fruits physical characteristics. A total of 360 guavira accessions were collected from six natural populations in the municipalities of Bonito and Jardim, Mato Grosso do Sul. From each population, ten fruits obtained from six adult trees were evaluated and the average was used in the genetic divergence study. The fruits were analyzed for longitudinal diameter (LD), transverse diameter (TD), epicarp thickness (ET), total fruit mass (FM), pulp mass (PM), seed mass (SM), color (COR) and soluble solids content (SSC). The genetic diversity was estimated by the Tocher optimization method, using the average Euclidean distance. The relative contribution of the eight variables evaluated in guavira fruits indicated that PM was the most important feature (62.02%), which means, this characteristic was the one that most contributed to the variability. The SSC was the second characteristic that most contribute to genetic diversity (14.81%) and the variables COR (0.79%) and ET (0.006%) were the ones that contributed the least. These results suggest that the COR and ET variables can be eliminated from the next evaluations, which would reduce laborious work, time and costs spent on experimentation. The Tocher grouping method joined the 360 guavira accesses into eight groups. Group I was composed of the highest number of accesses, 341 or 94.72%. Groups II to IV were formed, respectively, by five (1.4%); three (0.83%) and seven accessions (1.94%). Groups V to VIII presented an access each, indicating that these individuals are highly divergent in relation to the others. The formation of the different groups suggests the existence of genetic variability between and within the populations studied. The genetic variability observed in this work may be a reflection of the environmental effect associated to the occurrence of sexual reproduction and / or natural mutations manifestation, and should be used in the selection of superior genotypes by breeding programs.

Keywords: grouping analysis; multivariate analysis; Brazilian cerrado species, Tocher

GENETIC DIVERSITY OF CASSAVA ACCESSIONS BY MULTIVARIATE ANALYSIS

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Genetic diversity measures of cassava accesses, by predictive means or multivariate techniques, help on selecting parents who will be used to obtain hybrids. For this, it is necessary to perform diallel crosses, which require a great experience from the breeder due to the low rate and lack of genotypes flowering synchrony. The purpose of this study was to evaluate the genetic divergence of cassava accessions through multivariate statistics. A total of 127 cassava accessions from Embrapa Cassava and Fruits Germplasm Active Bank, and 12 cassava commercial cultivars were evaluated in an experiment set up in a randomized complete block design, with three repetitions. The plots consisted of five plants spaced of 0.80m between plants and 0.90m between rows. The traits measured were: first branch height (FBH - meters), plant height (PH - meters), stem diameter (SD - cm) and fresh root weight (FRW - kg plant⁻¹) and above ground yield (AGY – kg plant⁻¹). After data analysis of variance, a multivariate analysis was performed using the generalized Mahalanobis distance (D^2) as a measure of dissimilarity. The mean values for FBH, PH, SD, FRW and AGY were, respectively, 0.39 m, 0.79 m, 0.78 cm, 0.74 kg plant⁻¹ and 2.58 kg plant⁻¹. The highest distances between the genotypes were between IAC-12 and BGM-1447 (7.26), BGM-2022 and BGM-1447 (7.0), BGM-1134 and BGM-1447 (6.95), BGM- 1338 and BGM-1447 (6.91) and BGM-1328 and BGM-1447 (6.90). The lowest distances were between BGM-2167 and BGM-1721 and BGM-1223 and Baianinha, both with estimates of 0.13. BGM-1447 access was more divergent among all the genotypes evaluated. Crosses with this access might generate superior genotypes in advanced generations. Genotypes with lower distance values have lower dissimilarity, which indicates that crossing them may generate low genetic variability, making it difficult to select transgressives individuals.

Keywords: *Manihot sculenta* Crantz; genetic dissimilarity; Mahalanobis distance; Active Germplasm Bank.

GENETICALLY MODIFIED PLANTS WITH VIA ABA GENE DEPENDENT UNDER WATER DEFICIT

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Despite the complexity of the drought tolerance characteristic in plants, the biotechnological tools have allowed advances in understanding the mechanisms involved in this interaction. Commonly, in studies related to plant tolerance to water deficit, genes encoding proteins known as transcription factors are used. These proteins are involved in the early stages of gene expression and regulation, as well as in the transduction of stress response signals, being divided into ABA (Abscisic Acid) dependent and independent routes. Studies with events of *Arabidopsis thaliana*, where the overexpression of the *AtAREB1* factor was carried out, showed that transgenic plants presented greater sensitivity to ABA and greater tolerance to drought. The objective of this study was to evaluate genetically modified (GM) plants overexpressing the *AtAREB1* dependent ABA transcription factor, under stress due to water deficit. The experiment was conducted in greenhouse pots, and two genetically modified soybean events (GM1Ea2939 and GM1Ea15) and the conventional background (BR16 cultivar) were tested. The plants were compared under control (irrigated) and under water deficit, applied at 12 and 14 days after the onset of stress. Gas exchange, chlorophyll content (mg cm⁻²) and relative water content were evaluated. Regardless of genotype, all variables were negatively affected by water deficit. During short periods of stress, GM1Ea2939 and GM1Ea15 events differed significantly from BR16, with higher relative water content. Under short stress, events GM1Ea2939 and GM1Ea15, presented higher stomatic conductance in relation to BR16, 0.2974, 0.2668 and 0.1917 mol H₂O m⁻² s, respectively. By visual observation, it was verified that the GM plants, especially the GM1Ea2939 event showed slow wilt compared to the conventional background BR16. Regardless of the water status, the GM plants presented lower transpiration than the BR16 cultivar, which may have contributed to the greater water preservation and, consequently, better performance under water deficit.

Keywords: *Glycine max*; Transcription factors; *AtAREB*; Drought.

GENOMIC DIVERSITY OF FOOD-TYPE SOYBEAN ACCESSIONS

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In order to be suitable for human consumption as a vegetable, additionally to other traits, soybean must have large seeds, usually represented by one hundred seed weight (HSW). In this context, we studied the diversity of 28 large-seeded accessions of soybean, aiming to understand the relationship among them and guide their application to produce bred lines. These genotypes are plant introductions used in crosses to develop food-type soybean and they were selected based on the threshold of $HSW \geq 20g$. A set of 42509 SNPs was obtained from the fingerprinting of the USDA Soybean Germplasm with the SoySNP50K beadchip, publicly available at Soybase. Using the software TASSEL5, we obtained distance matrices and generated a Neighbor-Joining tree and a multidimensional scaling plot (MDS). Population clusters were inferred using the software FastStructure, which was run on default settings with 10-fold cross-validation testing for subpopulations (K) ranging from 1 to 8. The script ChooseK, included with the FastStructure package, was used to choose the number of subpopulations that maximize the marginal likelihood. The MDS analysis showed the formation of two groups: one of Japanese origin and other mainly of Chinese ancestry. This result was validated by the inferences of FastStructure, where a value of $K = 2$ was considered the most suitable for the genotypes. Further observations showed that 16 genotypes were grouped in the Japanese cluster and four genotypes were in the group of Chinese origin. The other eight accessions had mixed ancestry, where three genotypes had a major proportion of Chinese genetic information and five accessions had most Japanese ancestry. The accession PI200497 which so far had no known origin and Ivai (PI628859), an accession collected in Brazil, exhibited more similarity to the Japanese cluster. The North Korean accessions PI090241 and PI091725-3 are more related to the Japanese accessions, which is also true for Araçatuba (PI285090) an accession collected in Venezuela and cultivated in Brazil many years ago; beyond, this latter accession also had a strong association with the Chinese cluster. Regarding the accessions collected in the USA, Majos (PI548697) and Hampton (PI614156) are most related to Japanese accessions and FC031665 is completely related to the Chinese group. This research provided informations about the diversity of soybean accessions and may guide the breeding of new soybean lines for human consumption.

Keywords: *Glycine max* (L.); Germplasm; Plant introduction; FastStructure.

Acknowledgements: CNPq for the financial support.

IMAGE ANALYSIS FOR MEASURING POD DIMENSIONS IN VEGETABLE SOYBEAN

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The full nutritional properties of soybean are not exploited because it is not typically used as a food in Western cultures. Food-type soybean may be used for human consumption as a vegetable, and additionally to other traits, it must have large pods and seeds. Furthermore, large seeded genotypes are associated with a better flavor for fresh consumption. In order to obtain high quality soybean and offer this food option to farmer and consumers, a breeding program needs to implement an efficient procedure to analyze pod and seed size to select the most prominent genotypes. Pod dimensions usually are measured using a caliper or in a visual evaluation, which are time consuming and prone to error. Here, we employed ImageJ [1] a software for image analysis to measure the pod length (PLR6) and width (PWR6) in the R6 stage. We also study the correlation between these two traits with one hundred seeds weight (HSW) which is an evaluation to select large seeded genotypes. An F₃ population consisting of 21 progenies was evaluated in a randomized complete block design with four replicates. We performed an analysis of variance, a Scott-Knott test and a correlation study of the traits. The ANOVA showed that genotypes had a significant difference in all the traits evaluated and the plot with the three dimensions (PLR6, PWR6, HSW) distinguished the performance of parents and crossings. The traits exhibited a high correlation, confirming that the use of image analysis in the R6 stage may optimize the process of selection of large seeded soybean.

Keywords: Glycine max; Soyfood; ImageJ; Plant phenotyping

Acknowledgments: We are thankful to CNPq for the financial support and Dr. G. Polder for developing the plugin used for our analysis.

INTROGRESSION OF DROUGHT-TOLERANT *AtAREB1* EVENT IN DIFFERENT SOYBEAN GENETIC BACKGROUNDS

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The *AtAREB1* (ABA-Responsive-Element-Binding) transcription factor (TF) is an ABA-dependent protein, which regulates the expression of genes related to plant response to the water deficit. In soybean, several transgenic events overexpressing this gene were generated in the Laboratory of Plant Biotechnology at Embrapa Soybean. Among these, the event 1Ea2939 showed higher performance in field trials, with 35 to 44% greater productivity than the conventional background, when water deficit treatment was applied in growing and reproductive phases, respectively. On the other hand, these events presented longer cycle in comparison to the conventional background, increasing the crop total cycle in more than 30 days. Given these results, this study was conducted with the objective to obtain soybean plants overexpressing the TF *AtAREB1* with different cycles by the introgression of the transgene in different genetic backgrounds belonging to distinct maturity groups (MG). To obtain the hybrids, crosses were performed with soybean cultivars from the super-precocious (<6.0), precocious (6.1 a 6.3), semi-precocious (6.4 a 6.6) and middle cycle (≥6.7 a 7.4), maturity group, being five conventional soybean cultivars and two RR. Seven populations were obtained. From each cross, 20 to 50 F₁ seeds were generated, which were multiplied in greenhouse to obtain F₂ seeds generation. These F₂ seeds were sown in greenhouse in pots containing five kilos of soil, with a maximum of ten seeds per pot, totaling 7,007 plants (ranging from 693 to 1078 plants/population). In F₃ generation, Single Pod Descendent method was applied. In this methodology, a pod of each plant from F₂ generation is collected, in order to reduce the genetic variability losses caused by sampling. The obtained populations showed difference in the total cycle, and some populations showed a great reduction. Thus, these results indicated that, through the crosses performed between *AtAREB1* line and different soybean cultivars, it was possible to obtain genotypes with cycle duration compatible with the profile of cultivars used nowadays by soy producers.

Keywords: *Glycine max*; abiotic stress, transgenic, cycle.

OIL CONTENT AND GRAIN YIELD OF CASTOR BEAN LINES

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Castor bean (*Ricinus communis* L.) is an oilseed plant which plays an important role in commercial production of oil. The oil extracted from the castor bean has several uses ranging from medicine and cosmetics to replacing petroleum in the production of biodiesel, plastics and lubricants. Nevertheless, the number of well-adapted, high-yielding cultivars is still quite reduced. As a result, selecting improved genotypes is a key strategy to breed castor bean for its oil. The objective of this study was to evaluate oil content and grain yield of 24 lines of castor bean obtained by five self-pollination cycles. Field experiment was conducted in the 2014 growing season at São Paulo State University (UNESP) in the city of Botucatu on a Typic Dystrophic Red-Yellow Latosol with a loam texture. According to the Koppen Climate Classification system, the region has a humid temperate climate with annual mean temperature between 18 to 20 °C, and rainfall between 1000 to 1300 mm with altitude between 600 and 800 m. The experimental design was a randomized block with 24 lines and four replicates. Plots were 30 m² and were comprised of three lines of 10 m with only the middle line of each plot considered in the measurement. There was 1 meter between rows and between plots. Oil content in the grains was determined by nuclear magnetic resonance. Grain yield was determined by the total grain mass adjusted to 13% of moisture. There was statistical significant difference ($P < 0.0001$) among lines for both characteristics measured, with oil content ranging from 42 to 50% and grain yield from 949 to 2183 kg ha⁻¹. Lines 1, 4, 5, 8, 9, 11, 12, 14, 16, 17, 18, 19, 20, and 23 showed the highest oil content ranging from 45 to 50%. On the other hand, lines 3, 10, 11, 19 and 22 displayed the highest grain yield ranging from 1880 to 2183 kg ha⁻¹. Thus, the lines 11 and 19 had better agronomic performance and they are recommended for the cultivation in the region.

Keywords: *Ricinus communis* L; plant breeding; genotypes; industrial yield.

Acknowledgements: College of Agricultural Sciences (FCA) and São Paulo State University (UNESP)

PARÂMETROS GENÉTICOS DE CARACTERES EM LINHAGENS DE MAMONA

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A mamoneira (*Ricinus communis* L.) é uma espécie de elevado potencial econômico, e seu cultivo constitui em fonte de receita para inúmeros produtores. Porém, a cultura ainda apresenta baixa produtividade média para a região sudeste, em torno de 909 kg.ha⁻¹ na safra 2015/2016. Contudo, o desenvolvimento de materiais genéticos superiores, através do melhoramento, pode-se melhorar este problema. A eficiência dos métodos de melhoramento de plantas depende de informações que podem ser preditas por meio dos componentes de variância, como os parâmetros genéticos. Diante disso, o presente trabalho teve por objetivo avaliar os parâmetros genéticos de caracteres em linhagens de mamona. O presente trabalho foi desenvolvido no ano de 2014 na Fazenda Experimental do Lageado, pertencente à Faculdade de Ciências Agronômicas (FCA) da Universidade Estadual Paulista (UNESP), Campus de Botucatu-SP. O delineamento experimental foi em blocos casualizados com 24 linhagens e quatro repetições, tendo a parcela experimental a área útil de 8 m², com espaçamento entre plantas e entre linhas de 1,0 m. As características avaliadas foram altura da planta (AP), em cm, teor de óleo nos grãos (TO), em %, produtividade de grãos (PG), em kg.ha⁻¹. Os dados foram submetidos à análise da variância, e em seguida calculada a herdabilidade ao nível de media (h^2) pela fórmula

$h^2 = \frac{QML - QME}{QML}$, em que o QML é o quadrado médio de linhagens e o QME é

quadrado médio do resíduo; e o progresso genético estimado (ΔG) pela fórmula

$\Delta G = i \frac{\sigma^2 l}{\sqrt{\sigma^2 l + \frac{\sigma^2 e}{r}}}$, em que ΔG é a estimativa do progresso genético esperado com

20 % de intensidade de seleção, i é 1,4 de diferencial de seleção standardizado, correspondendo a 20% de intensidade de seleção, $\sigma^2 l$ é a variância genética entre as linhagens obtida por $\sigma^2 l = \frac{QML - QME}{r}$, r é o número de repetições e $\sigma^2 e$ é o QME.

A significância das variâncias das linhagens (genética) para as três características pelo teste F ($p < 0,05$), indica a existência de variabilidade genética entre as linhagens. Os coeficientes de herdabilidade estimados ao nível de médias de linhagens variaram de 0,81 a 0,97 para produtividade de grãos e altura da planta, respectivamente. O maior ganho genético estimado foi obtido para produtividade de grãos, em que a seleção de 20% das linhagens mais produtivas ocasionará um ganho de 387,27 kg.ha⁻¹. De acordo com os resultados, conclui-se que as linhagens apresentaram comportamento produtivo mais estáveis, devido à baixa contribuição do resíduo frente ao fenótipo, o que provocou condições favoráveis a eficiência da seleção de linhagens superiores.

Keywords: *Ricinus communis* L.; genótipos; herdabilidade; ganho genético.

PERFORMANCE OF SOYBEAN CROSSES THROUGH DIALLEL ANALYSIS IN CONTRASTING RUST ENVIRONMENTS

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Rust (*Phakopsora pachyrhizi*) is one of the main diseases of soybean, being able to cause terrible losses in productivity. An important strategy for its control is the genetic tolerance, defined as the ability of the genotype to withstand the pathogen attack without significantly affecting its productivity. This work aimed to estimate the general (GCA) and specific (SCA) combining ability of 40 crosses (F₂ generation) derived from a partial diallel consisting in ten rust tolerant RR experimental lines (Group I) in crosses with four RR commercial cultivars (Group II). We evaluated the genotypes for seed yield in experiments conducted in two managements of the disease (with and without rust control) by spraying with the appropriate fungicides. The experiments were designed in randomized complete block with four replications. Individual and joint analysis of variance were performed assuming fungicide managements as a fixed effect and genotypes as random. The estimates of GCA and SCA were obtained for each fungicide management and for the average of these two environments using the Griffing methodology (model III) adapted for a partial diallel. Analysis of variance revealed significant effects for genotypes (G) and environments (E). The means of the experiments were 4620 kg ha⁻¹ (with rust control) and 3366 kg ha⁻¹ (without rust control). Joint diallel analysis revealed significant effects ($p < 0.10$) for GCA of groups I and II and for SCA. The progenies x environments interaction was not significant, indicating that there were some parents that contributed to increase seed yielding with constant behavior in both environments. The ratio of the estimate of quadratic components associated with GCA and SCA was 0.929, indicating that additive effects are much more important than non-additive effects for seed yield. The parents who contributed the most to the additive genetic action and presented the highest values of GCA were the lines USP 14-22.010 and USP 14-22.003 for group I and cultivar V Max RR for group II. The highest values of SCA were estimated for the crosses USP 14-22.003 x BMX Potência RR and USP 14-22.009 x V Max RR. We could conclude that: a) there were genetic variability due to the action of additive and non-additive genes; b) from the action of additive and non-additive genes, the best parents showed similar performances in both environments; c) the additive effects are more important than non-additive effects for seed yield.

Keywords: *Glycine max*; *Phakopsora pachyrhizi*; rust tolerance

REDUCTION ENZYMATIC ANTIOXIDANT SYSTEMS ACTIVITY CAUSE EPIGENETIC MODIFICATIONS IN RICE LINES

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Glutathione peroxidase (GPX) enzymes catalyze H₂O₂ reduction and hydroperoxides avoiding the toxicity of these compounds to the cells. In addition, GPX has been subjected to the functions of sensor and oxidative state signaling while maintaining redox homeostasis in plants. The imbalance between the antioxidant system and the production of reactive oxygen species causes oxidative stress leading to cell damages and death. In order to study epigenetic effects caused by oxidative stress, GPX knockout rice inbred lines were analyzed to the status of DNA methylation (5mC). Five knockout transgenic rice lines for GPX₁ and GPX₃ were evaluated. The experiment was carried out in a randomized block design with three replicates. Phenotypical variables were evaluated throughout the plant development. Catalase (CAT), glutathione reductase (GR), glutathione peroxidase (GPX), ascorbate peroxidase (APX) and superoxide dismutase (SOD) enzymes activity were quantified during the flowering time, using the middle third leaves. Global DNA methylation level was quantified by methylation-dependent restriction enzymes *McrBC*, *HpaII* and *MspI*. Decrease in the GPX enzymatic activity and in the DNA methylation (5mC) explains 50% of the phenotypical variation observed in the knockout rice lines, according to the outputs of the principal components analysis. Consequently, knockout plants showed a significant decrease ($p < 0.05$) in vigor (biomass and volume) compared to the control. Knockout lines for GPX presented a trend to hypomethylation, mainly in the cytosine sequence context for the restriction enzyme *McrBC*. On the other hand, DNA hypomethylation for cytosine sequence context for the isoschizomers restriction enzymes, *MspII* and *HpaI*, was not observed. Notwithstanding, demethylation of cytosine in the *McrBC* sequence context showed a strong correlation ($p < 0.05$) with the activity of the antioxidant enzymes CAT, GR and GPX ($R = 0.54, 0.68, 0.79$); and furthermore, with yields components such as seed weight and total number of seeds per plant ($R = 0.49, 0.51$). Thus, *McrBC* restriction sequence context is a site for the DNA methyltransferase 3 (*DNMT3*) that mediate transposable elements silencing, and has a distant relationship to GPX in the biochemical pathway. The knockout of GPX could affect *DNMT3* through a complex network. On the other hand, the oxidative stress could act direct against the methyl-cytosine promoting its reduction or as a consequence of the stress process.

Keywords: DNA methylation; epigenetics; oxidative stress; redox sensor

SELEÇÃO DE LINHAGENS DE MAMONA COM POTENCIAL PRODUTIVO

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A mamoneira (*Ricinus communis* L.) explorada comercialmente em função do óleo contido nas suas sementes e devido a sua grande versatilidade química no ramo industrial tem se destacado nos últimos anos como uma cultura alternativa de alta produção de biodiesel. Entretanto, as pesquisas ainda são escassas, necessitando de mais estudos e seleção de materiais mais adaptados às condições de cultivo visando atender a expansão da cultura. Face ao exposto, objetivou-se avaliar o potencial produtivo de linhagens de mamoneira, com vistas a seleção de genótipos superiores para utilização em programas de melhoramento. A pesquisa foi conduzida a campo no ano agrícola de 2015 na Fazenda Experimental Lageado, pertencente à Faculdade de Ciências Agronômicas – UNESP, localizada no município de Botucatu– SP. O delineamento experimental adotado foi em blocos casualizado, com 24 linhagens e quatro repetições, a área útil da parcela experimental sendo de 8 m², com espaçamento entre plantas e entre linhas de 1,0 m. As características avaliadas foram altura da planta (AP), teor de óleo nos grãos (TO) e produtividade de grãos (PG) corrigido para teor de água de 13%. Os resultados foram submetidos à análise de variância e as médias foram agrupadas pelo teste de agrupamento proposto por Scott e Knott ao nível de 5% probabilidade. A partir dos resultados obtidos pôde-se concluir que, para a produtividade de grãos as melhores linhagens foram 22, 20, 3, 19 e 11 com produção variando de 1880 á 2182 kg ha⁻¹, com um teor de óleo de 43 á 50%. É válido enfatizar que, para a variável altura de planta nestas linhagens ficou entre 58 e 189 cm. Portanto, podemos concluir que dentre as 24 linhagens avaliadas estas cinco mencionadas foram as que apresentaram elevado potencial produtivo.

Palavras-chave: *Ricinus communis* L.; Componentes de produção; Genótipos; Variabilidade genética.

SEQUÊNCIAS CENTROMÉRICAS DE ESPÉCIES DIPLÓIDES DE *Solanum* NOS GENOMAS “A”, “C” E “D” DE ESPÉCIES ALOPOLIPLÓIDES

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No gênero *Solanum* são reconhecidos os genomas A, P, B, C, D e E. Esses genomas são encontrados em espécies diploides, com exceção dos genomas C e D, encontrados apenas em espécies alopoliplóides em associação com o genoma A. A caracterização do DNA centromérico em espécies de *Solanum* com genomas AA e PP, tem relevado uma dinâmica peculiar, com sequências cromossomo específicas e diferentes dinâmicas evolutivas nos genomas A, P, B e E. Como essas repetições ainda não foram avaliadas nos genomas C e D, o objetivo deste estudo foi verificar o padrão de distribuição e organização de sequências centroméricas dos genomas A e P de *Solanum* nos cromossomos de espécies alopoliplóides (portadoras dos genomas AC e AD), utilizando hibridização *in situ* fluorescente (FISH). As sequências centroméricas utilizadas como sondas foram identificadas em *S. tuberosum* (AA - St49 e St18), *S. verrucosum* (AA - Sv54, Sv123, Sv161.5) e *S. chomatophilum* (PP - Sc83, Sc111, Sc9/108) e a sequência de rDNA 45S foi usada como controle. O padrão de distribuição das sequências foi bastante distinto para os genomas AC e AD. No genoma AC, as sondas St18 e St49 foram ausentes, enquanto no genoma AD, St18 foi ausente e a sequência St49 presente na região centromérica de múltiplos cromossomos. A sequência Sv54 gerou 4 sinais centoméricos no genoma AD e foi ausente no genoma AC. No genoma AD, Sv161.5 foi localizada em dois pares de cromossomos e em um par no genoma AC. Sv123, tanto no genoma AC quanto no genoma AD, foi localizada em um par cromossômico. A sonda Sc83 gerou sinais dispersos, não centroméricos, ao longo dos cromossomos no genoma AC e dois sinais centroméricos no genoma AD. No genoma AD, Sc92/108 revelou quatro sinais intensos nas regiões centroméricas e nenhum sinal no genoma AC. Tanto em AC como em AD, a sequência Sc111 revelou sinais dispersos em todos os cromossomos com sinais mais intensos em alguns centrômeros. No caso do genoma AD, quatro sinais centroméricos eram co-localizados com Sc92/108, que não gerou sinal em AC. As sequências identificadas nos alopoliplóides pertencem, em sua maioria, aos cromossomos do genoma A, com padrão semelhante ora o genoma A de *S. tuberosum*, ora ao de *S. verrucosum*. Nenhuma das sequências analisadas é candidata a pertencer ao genoma C, enquanto que para o genoma D a sequência Sc83 pode pertencer a esse genoma, com padrão similar ao genoma P.

Palavras-chave: DNA repetitivo; Centrômero; DNA satélite; FISH; Evolução.

Apoio Financeiro: FAPEMIG, Capes e CNPq

STABILIZATION OF HETEROCHROMATIC KNOB SEGREGATION IN TROPICAL MAIZE INBRED LINES

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The maize genome is hypervariable at DNA content level and karyotypically, being part of this karyotypic variability explained by the presence or absence of heterochromatic knobs. S5 and S4 progenies of inbred lines were established from commercial variety Jac-Duro (JD - Flint type endosperm) (Sementes Agroceres, Brasil). The inbred lines were designated as JD 1-3, JD 2-1, for those derived from S3 and as JD 4-4 and JD 4-1 from S2 generation. The inbred-lines are homozygous for heterochromatic knobs in the chromosomal position K6L2, K6L3, K7L, K8L1 and K8L2 and segregate for presence and absence in the K3L, K5L, K7S and K9S. In order to investigate the stabilization of knobs segregation, inbred-lines 442242 and 442612, corresponding to JD 4-4 family, were analysed using the C-banding technique (described in Bertão and Aguiar-Perecin, 2002). Derived inbred-lines confirm that homozygous knobs were located in the chromosome 5 long arm (K5L) and in the chromosome 9 (K9S) short arm. On the other hand, no C-band was identified at the short and long arm in the chromosomes 3 (K3L) and 7 (K7S), respectively. Stabilization of knobs segregation and the derivation of isogenic inbred lines open a singular opportunity to the understanding of the effect of heterochromatic regions in the genome. C-banding technique allows a rapid identification of specific heterochromatic composition in inbred-lines, which can generate subsidies for the genetic mapping of the knobs, and this information can be used subsequently in crop breeding programs.

Keywords: banding-C; chromosomes; genome; heterochromatin; knobs; *Zea mays*.

Acknowledgements: CAPES, CNPq.

SUGARCANE CELL WALL DYNAMICS IN RESPONSE TO SMUT FUNGUS (*Sporisorium Scitamineum*)

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Sugarcane is the main feedstock used for sugar and ethanol production in Brazil. The crop yield can be reduced due to diseases like smut, caused by the fungus *Sporisorium scitamineum*. Smut compromises the stems formation, which implies in significant losses in cane tonnage and juice quality. In host plants, sugarcane smut initiates its colonization by the most exterior plant barrier: the cell wall. On the other hand, the infection can be modulated by changes on the plant cell wall composition after pathogen recognition. These assumptions make plant cell wall proteins as putative components of plant-pathogen interactions. Therefore, the main goal of this work is the identification of sugarcane cell wall proteins in response to smut infection. For that, two methods were used to isolate proteins from cell wall of lateral buds of the susceptible sugarcane variety (RB925345) 30 days post-inoculation with *S. scitamineum*. Mock-inoculated plants were used as control. A total of 1,249 proteins were identified by mass spectrometry, wherein 355 were found only in inoculated plants. Now these proteins started to be annotated and will be distributed into specific classes of cell wall proteins (glicosyde-hydrolases, peroxidases, signaling proteins). Results can indicate different plant cell wall protein patterns in response to *S. scitamineum* infection and may allow a better comprehension of cellular pathways related to the sugarcane defense responses against *S. scitamineum*.

Keywords: subcellular proteomics; defense; plant barrier; smut infection.

Acknowledgements: CNPq (proc. nº 155384/2016-3)

SUGARCANE SECONDARY METABOLISM DYNAMICS THROUGHOUT SMUT DISEASE: OPPORTUNITIES TO IMPROVE PLANT RESISTANCE SCREENING AND PATHOGEN DETECTION

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Sugarcane has long been recognized as one of the world’s most efficient crop in converting solar into harvestable chemical energy. However, the carbon partitioning through the well-established concept of source-sink tissues can be affected in response to pathogen attack. Sugarcane smut, caused by the biotrophic fungus *Sporisorium scitamineum*, leads to the development of whip-like structure from plant meristems in susceptible genotypes, which is composed by host and fungal tissues. The disease causes important losses in cane tonnage and juice quality. Considering that metabolomics approaches are recognized as one of the highest levels of post-genomic analysis, the present study aimed to determine changes in sugarcane secondary metabolism in response to *S. scitamineum* colonization throughout disease progression within the shoot apical meristem. We used LC-ESI-MS/MS approach in a time-course experiment from early (5 DAI), middle (65 and 100 DAI) to late infection (after whip development, 120 DAI). Statistical analysis to determine differential accumulation of metabolites between infected and control samples of the same age was performed in MetaboAnalyst 3.0 software (FDR < 0.05). Top 10 VIPs (Variable’s Importance in the PLS-DA) of each time-point comparison were selected for fragmentation. The number of metabolites quantitatively altered during the interaction (FDR < 0.05) increased with disease progression. A total of 79 metabolites were fragmented, leading to the identification of 30 compounds. Most interesting results were related to maysin biosynthesis, such as 3'-O-methyl-derhamnosylmaysin and derhamnosylmaysin metabolites, which were detected only in control plants since 65 DAI, indicating that this pathway is suppressed in susceptible plants. It may be worthwhile to evaluate if this same pattern is observed in other sugarcane genotypes, and correlate it with plant resistance. Additionally, apigenin synthesis was suppressed in the infected plants during disease progression, and increased after whip development, becoming an important marker metabolite related to healthy sugarcane plants. Among the metabolites detected only in infected plants, we identified one with a fragmentation pattern similar to Fusarin C, a metabolite of fungal origin accumulated since 65 DAI. This compound may be further investigated in applications to early detection of smut-infected plants in field previously to whip emission.

Keywords: sugarcane, smut, metabolomics, meristem, maysin, mycotoxin.

Acknowledgements: Fapesp (2013/25599-2) and CNPq.

USE OF GENETIC VALUES RELATIVE PERFORMANCE FOR GENOTYPES X ENVIRONMENTS INTERACTION ANALYSIS IN CORN HYBRIDS

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The interaction of genotypes x environments (GE) evaluation is one of the main stages of a breeding program. The GE interaction presence can influence the gain selection and hinder the cultivars recommendation with wide adaptability and stability. The harmonic mean of the relative performance of genetic values (MHPRVG) allows simultaneous selection for yield, stability and adaptability, and is very useful for breeding programs. The aim of the present study was to evaluate and select corn hybrids with high crop yield, adaptability and stability, using the MHPRVG method, in environments with and without fungicide application. On 2015/16 double-crop, 16 hybrids of corn were evaluated in four environments, as follows: Amambai - MS, without/with fungicide application (environments 1 and 2) and Maracaju - MS, without/with fungicide application (3 and 4). The products used in environments 2 and 4 were Piori Xtra + Nimbus, at 300 + 600 mL ha⁻¹ doses, applied in the V8 stage and in corn pre-tasseling. The experimental design was a randomized block design, with three replications in Amambai and four replications in Maracaju. The evaluated characteristic was grain yield, corrected to 13% of humidity, in kg ha⁻¹. Selection for adaptability and stability was based on predicted genotypic value and MHPRVG. The genotype x environment interaction was significant for the grain yield characteristic. This result shows that the hybrids evaluated in the work presented variation in the productive performance according to the environments. This is problematic for breeders because of the inconsistency of hybrids superiority in the face of environments variations with and without fungicide application. When analyzing the free genotypic values of the GE interaction, it is observed that the CD384PW hybrid was the one with the best performance in environments 1, 2 and 3 and the hybrid RB9110PRO in the environment 4. According to the Genetic Values Stability, Genetic Values Adaptability, MHPRVG and with average crop yield, the best hybrids were CD384PW, RB9110PRO and DKB177PRO. These hybrids can be considered with high adaptability, stability and yield in environments with and without fungicide application.

Keywords: mixed model; genetic breeding; genotypic stability and adaptability; *Zea mays*.

VARIABILIDADE E RELAÇÕES GENÉTICAS EM MILHO TROPICAL PARA SIMBIOSE COM *Azospirillum brasiliense*

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Bactérias Promotoras de Crescimento de Plantas (BPCP) vêm sendo isoladas e comercializadas nos últimos anos como inoculantes devido à produção de fito-hormônios e fixação biológica de nitrogênio (N). No entanto, a inoculação é uma prática pouco adotada na cultura do milho pela inconsistência de resultados, o que em parte é atribuído à incompatibilidade entre genótipos e estirpes. O objetivo deste trabalho foi avaliar a variabilidade genética em um painel de 19 linhagens de milho e seus 118 híbridos em resposta à inoculação com a BPCP *Azospirillum brasiliense*. O experimento foi realizado em casa de vegetação em duas épocas de cultivo, utilizando-se o delineamento de blocos casualizados com três repetições. Os tratamentos foram sementes não-inoculadas (controle) e inoculadas com *A. brasiliense*. As plantas foram conduzidas em vasos preenchidos com solo arenoso, sem adição de N. No estágio V7, foram avaliadas massa de parte aérea seca, massa de raiz seca (MRS) e diversas características radiculares usando o software WinRHIZO™. As análises estatísticas foram realizadas por meio de equações de modelos mistos do tipo REML/BLUP. O efeito de genótipo foi significativo ($p \leq 0,01$) para todas as características avaliadas, tanto em linhagens quanto híbridos. O efeito da inoculação com *A. brasiliense* em híbridos foi significativo ($p \leq 0,01$) somente para MRS, volume radicular (VR) e diâmetro médio (DM), enquanto não apresentou significância para nenhuma característica nas linhagens. Isso indica que as combinações alélicas geradas pelos intercruzamentos geram variabilidade genética para a simbiose milho-*A. brasiliense* e que a presença de efeitos genéticos não aditivos pode estar envolvida no controle dessa característica. Nos híbridos, verificou-se incremento na média de MRS, VR e DM em relação ao controle, indicando que a bactéria possui habilidade de modificar o sistema radicular promovendo uma maior exploração do perfil do solo e, assim, maior captação de água e nutrientes. Os resultados das correlações revelaram associações positivas entre MRS, VR e DM, variando de 0,49 a 0,89 no tratamento controle. Porém, a presença de *A. brasiliense* na planta pouco afetou a magnitude dessas correlações, tornando favorável a seleção dos genótipos com maiores médias para essas características. Portanto, foram verificadas diferenças na responsividade entre híbridos e suas linhagens genitoras em resposta à inoculação, assim como uma base genética para a interação milho-*A. brasiliense* potencialmente explorável em programas de melhoramento genético que visem incrementar a frequência de alelos favoráveis em relação a essa simbiose.

VIRULENCE EVALUATION OF TWO HAPLOTYPES FOR A CANDIDATE EFFECTOR GENE OF *SPORISORIUM SCITAMINEUM* IN SUGARCANE

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Considering the central role of effectors during plant-pathogen interaction, these molecules are under constant selective pressure. Polymorphisms found in these candidate effector genes may cause changes related to the virulence level of the isolates, indicating the presence of potential races. This work aims to conduct a pilot study in order to analyze the level of aggressiveness of different isolates representing two haplotypes of the fungus *Sporisorium scitamineum* while interacting with sugarcane, under greenhouse conditions. New polymorphic sites were also investigated in four other candidate effector genes in Brazilian and Argentine isolates of the fungus *S. scitamineum*. Yeast cells from three isolates (SSC04; SSC18; SSC39) from *S. scitamineum* were used. Isolates SSC04 and SSC18 belong to the same haplotype, while SSC39 belongs to the second haplotype. Single sugarcane buds were inoculated with the isolates resulting in four treatments (SSC04, SSC18, SSC39 and Control), totalizing 15 plants per treatment. Throughout the period, observations related to the whip emission (main symptom of the disease), stalk diameter and number of lateral shoots were made. For the polymorphism identification, conventional PCR reactions were conducted using total DNA from 54 haploid yeasts derived from *S. scitamineum* isolates collected in different Brazilian and Argentinian locations. Only plants infected with isolates SSC18 and SSC04 emitted whip, resulting in 60% and 93.33% of symptomatic plants, respectively. All the treatments presented little variation in relation to the number of lateral shoots and stalk diameter. Three polymorphisms were found in the same codon for one of the candidate effector genes analyzed. Polymorphisms affected the second and third bases of the codon, causing a non-synonymous amino acid substitution. These new polymorphisms resulted a new haplotype distribution among the *S.scitamineum* isolates. The experiment demonstrated a difference in aggressiveness among the studied haplotypes, however additional experiments are needed to confirm. It was possible to identify the presence of new polymorphic sites in one of the candidate genes, potentially involved in the interaction between *S. scitamineum* and sugarcane.

Keywords: *Sporisorium scitamineum*; Sugarcane smut; Effector biology; Genetic diversity

Organization



Support

