VIII INTERNATIONAL MEETING ON PLANT BREEDING

"AI for Scientific Breakthrough: Applications and Innovations"

PROCEEDINGS

October - 2024

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 effort 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 their correct application within research and breeding. The International Meeting on Plant Breeding, organized by the Genetics and Plant Breeding Group “Prof. Roland Vencovsky” (GVENCK), affiliated with the Genetics Department of ESALQ/USP, is one of several events that compose the "Corteva Agriscience Plant Science Symposia Series”.

In the eighth edition, the meeting focused on advances and innovations driven by artificial intelligence. This development marked a crucial transition from Breeding 4.0, or Precision Breeding, to 5.0, with significant implications for agriculture. The event aimed to explore applications in this area to promote sustainability and food security, providing a platform for discussions on how these technologies can optimize agricultural production, increase efficiency, and help tackle future challenges. It offered participants the opportunity to stay updated on the latest research and trends in plant and animal breeding while fostering interaction and collaboration among various stakeholders in the sector.

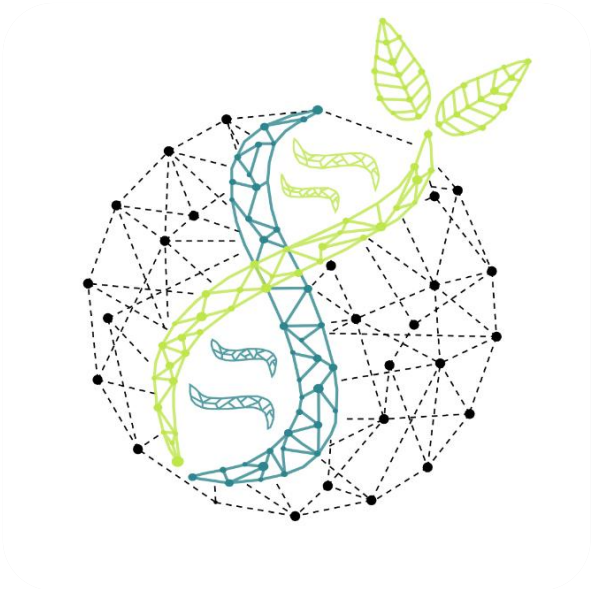
The meeting created an environment conducive to cultural and scientific exchange, bringing together speakers, students, and professionals, thereby expanding participants' networks. During the event, attendees were able to submit abstracts in poster format, promoting the dissemination of scientific knowledge and highlighting various ongoing research lines in Brazil and worldwide. This experience provided participants with the chance to enhance their communication skills and engage with the community from an analytical perspective, enriching their experiences and broadening their perspectives. Additionally, participants had the opportunity to compete for prizes. These combined activities offered excellent opportunities for establishing connections with leading companies and startups in genetic improvement and precision agriculture.

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;
- Establishing partnerships with companies and public institutions.



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SCHEDULE

October 1st, 2024

15:00 – 15:50: Registration

16:00 – 16:50: Opening Ceremony

16:50 – 17:10: Jason Rauscher (Corteva Agriscience)

17:10 – 17:50: Alexander Lipka (University of Illinois) – *"Incorporating more biology into quantitative genetics approaches"*

18:00 – 18:40: Julian Catchen (University of Illinois) – *"The comparative and population genomics of an icefish that escaped Antarctica."*

19:00: Cocktail – LEUVEN

October 2nd, 2024

7:00 – 8:00: Registration

8:00 – 8:50: Plenary 1 – Julien Garcia (UPM, Google X) – *"Modeling multiomics and their interactions in predictive models"*

8:50 – 9:00: *Roland Vencovsky Award Presentation 1*

9:00 – 9:50: Coffee – Poster and Stand Presentation

10:00 – 12:00: Mini-course 1 – Kaio Olimpio (UFV) – *"Interação genótipos x ambientes sob uma nova perspectiva: enviromics e modelos Bayesianos probabilísticos"*

10:00 – 12:00: Mini-course 2 – João Vianna (University of Illinois) – *"Empowering Scientific Research through Interactive Data Science"*

12:00 – 14:00: Lunch

14:00 – 16:00: Continuation of Mini-courses

16:00 – 16:40: Coffee – Poster and Stand Presentation

16:40 – 17:00: *Roland Vencovsky Award Presentation 2*

17:00 – 18:00: Plenary 2 – Alencar Xavier (Corteva) – *"Leveraging correlated information under multivariate settings"*

October 3rd, 2024

7:00 – 8:00: Registration

8:00 – 8:50: Plenary 3 – Rodrigo Mendes (Embrapa) – *"Rhizosphere microbiome: the second plant's genome"*

8:50 – 9:00: *Roland Vencovsky Award Presentation 3*

9:00 – 9:50: Coffee – Poster and Stand Presentation

10:00 – 12:00: Mini-course 3 – Matias Bermann (University of Georgia) – *"Genomic Selection in Animal Breeding: why, how, and future perspectives"*

10:00 – 12:00: Mini-course 4 – Thierry Pellegrinetti (CENA/USP) – *"Análise de Amplicons/Metataxonomia em Microbioma de Plantas"*

12:00 – 14:00: Lunch

14:00 – 16:00: Continuation of Mini-courses

16:00 – 16:50: Coffee – Poster and Stand Presentation

17:00 – 17:50: Plenary 4 – Matias Bermann (University of Georgia) – *"Use of AI in Animal Breeding and Genetics"*

18:00 – 18:30: Closing – *Roland Vencovsky Award Ceremony*

SPEAKERS

Alencar Xavier

Alencar Xavier holds a degree in Agronomy from the Federal University of Santa Maria (UFSM, Santa Maria/RS) and a Ph.D. in Statistical Genetics and Breeding from Purdue University (Purdue, West Lafayette/IN). He began his career at the multinational Corteva Agriscience as a Quantitative Geneticist and currently holds the position of Breeding Analyst. Additionally, he is an adjunct professor at Purdue University. Throughout his professional career, Dr. Xavier has accumulated extensive experience in agronomy, plant genetics and breeding, quantitative genetics and statistics, machine learning, phenomics, and genomics. His work in statistical genetics focuses on data-driven genetic improvement, emphasizing theoretical and computational aspects of plant breeding. He also develops quantitative analyses based on mixed models, Bayesian methods, machine learning, and high-performance computing. He is responsible for developing several statistical packages, including NAM: GWAS & breeding toolbox; bWGR: Genomic prediction; SoyNAM: SoyNAM dataset; ClickNAM: Online GWA analysis; Accuracy1: Multi-loc GS accuracy; Accuracy2: Multi-trait GS accuracy.

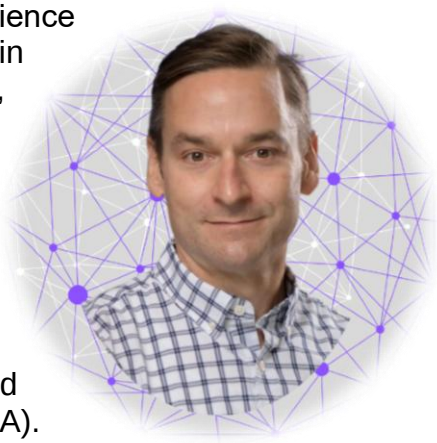
Alexander Edward Lipka

Alexander E. Lipka holds a degree in Music and Statistics from the University of Florida (Gainesville, FL), a master's in Applied Statistics, and a Ph.D. in Statistics from Purdue University (Purdue, West Lafayette, IN). He worked as a postdoctoral associate at Cornell University (Ithaca, NY) and at the United States Department of Agriculture - Agricultural Research Service (USDA - ARS, United States). He has also served as an Assistant Professor of Biometrics and currently holds the position of Associate Professor of Biometrics in the Department of Crop Sciences at the University of Illinois (Champaign, IL). Dr. Lipka applies his expertise in statistical genetics within an interdisciplinary environment to help answer questions related to crop improvement. Specifically, he is interested in the statistical approaches used for Genome-Wide Association Studies (GWAS) and genomic selection (GS). Through these statistical methods, he accelerates the development of high-performing crops by identifying specific DNA regions associated with agronomically important traits. He is the developer of the GAPIT (Genome Association and Prediction Integrated Tool) statistical package, used for GWAS and genomic prediction (or selection).

João Paulo Gomes Viana

João Paulo Gomes Viana holds a Bachelor's degree in Biological Sciences from the Federal University of Piauí (2010), a Master's in Genetics and Breeding from the same institution (2013), and a Ph.D. in Genetics and Molecular Biology from the State University of Campinas (UNICAMP, 2017). He also completed a Postdoctoral Associate position in Crop Sciences at the University of Illinois at Urbana-Champaign (2023) and currently serves as a Research Software Engineer at the National Center for Supercomputing Applications at this university. Dr. Viana has expertise in Germplasm Banks, Conservation of Native Species, Genetic Diversity, as well as Genetics and Genomics of Natural Populations.

Julian Catchen



Julian Catchen holds a Bachelor's degree in Computer Science from Pennsylvania State University (2000) and a Ph.D. in Computational Biology from the University of Oregon (2009), where he also completed his postdoctoral training (2014). He worked as a Software Engineer at Intel from 2000 to 2002, designing and implementing a Material Testing System (MTS). Additionally, he is the creator of the software STACKS, developed to assemble loci from short-read sequences, create genetic maps, and conduct population genomics and phylogeography analyses. Currently, Dr. Catchen is an Associate Professor of Evolution, Ecology, and Behavior at the University of Illinois at Urbana-Champaign (USA).

Julian Garcia Abadillo Velasco

Julian Garcia Abadillo Velasco holds a Bachelor's degree in Biotechnology (2020) and a Master's in Computer Science/Artificial Intelligence (2021) from the Polytechnic University of Madrid. At this university, he conducted research on genomic regions in wheat, particularly those controlling root morphology. Currently, Julian is a Ph.D. student in Data Analysis at the Center for Plant Biotechnology and Genomics at the Polytechnic University of Madrid, as well as an Artificial Intelligence/Plant Biology Resident at Google X, the "moonshot factory." Dr. Velasco has expertise in quantitative genetics and focuses on leveraging artificial intelligence to address agricultural and biotechnological challenges, aiming to promote sustainable crop development.

Kaio Olimpio das Graças Dias

Kaio Olimpio das Graças Dias holds a degree in Agronomy (2009), a Master's (2013), and a Ph.D. (2019) in Genetics and Plant Breeding from the Federal University of Lavras (UFLA, Lavras/MG). He completed postdoctoral research in Statistical Genetics at the Luiz de Queiroz College of Agriculture (ESALQ/USP, Piracicaba/SP), during which he was a visiting researcher at the University of Hohenheim's Biostatistics Unit (Stuttgart/BW). He is currently an Associate Professor at the Federal University of Viçosa (UFV, Viçosa/MG). Dr. Dias focuses on quantitative genetics, statistical genetics, polyploid genetics, genotype-by-environment interaction, mixed linear models, and Bayesian inference, with an emphasis on applications in plant breeding. His research contributes significantly to the development of advanced statistical and genetic methods, enabling more efficient identification and selection of desirable traits in plants.

Matias Bermann

Matias Bermann holds a Bachelor's degree in Agronomy from the University of Buenos Aires and a Ph.D. in Animal Genetics and Breeding from the University of Georgia (USA). His academic contributions have been applied in industry by organizations such as the American Angus Association and Zoetis, and by numerous scientists utilizing the BLUPF90 software package. Recently, Dr. Bermann developed a theory and a computer program to rapidly and accurately approximate the reliabilities of genomic breeding values for up to 4 million genotyped animals.

Rodrigo Mendes

Rodrigo Mendes holds a degree in Agronomic Engineering (2002) and a Ph.D. in Genetics and Breeding (2008) from ESALQ/USP, with two postdoctoral fellowships completed in 2010 at Wageningen University (Netherlands) and in 2023 at the Lawrence Berkeley National Laboratory (USA). He also has experience as a visiting scientist at CanaVialis/Monsanto (Brazil), the University of Lausanne (Switzerland), Rothamsted Research (United Kingdom), and the University of Cambridge (United Kingdom). Dr. Mendes served as Head of Research and Development at Embrapa Meio Ambiente from 2015 to 2022 and is a member of the scientific committee for the Promise research program (Gates Foundation). Currently, he is a researcher at Embrapa Meio Ambiente and a professor in the Graduate Program at the University of São Paulo. His research focuses on soil and plant microbial communities, aiming to elucidate how the rhizosphere microbiome influences plant growth and protection.

Thierry Alexandre Pellegrinetti

Thierry Alexandre Pellegrinetti holds a Bachelor's degree in Environmental and Sanitary Engineering (2016) from the Federal University of Lavras (UFLA), with a study period at the University of Coimbra (Portugal) from 2012 to 2013, and a Ph.D. in Sciences (2022) with a focus on Agriculture and Environmental Biology from CENA-USP. He is currently a Postdoctoral Researcher at the same institution, engaged in projects involving metagenomic and genomic data, microbial ecology, molecular biology, and bioinformatics. Dr. Pellegrinetti’s research focuses on microbial communities and their interactions in promoting plant growth, as well as the use of computational tools to explore microorganisms in Brazilian biomes.

SUMMARY

DESIGNGEN: AN APP FOR STATISTICAL ANALYSIS OF EXPERIMENTAL DESIGNS WITH FACTORIAL ARRANGEMENTS

Raissa Souza Krupek [1]; Pedro Henrique Massaro Silveira [1]; Brenda Monis Moreno [1]; Cristiane Hayumi Taniguti [2]; Antônio Augusto Franco Garcia [1]

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Abstract

There is a growing interest in developing new crop varieties that are productive, resistant to biotic and abiotic stressors, and adapted to constantly changing climate conditions. This requires advancements in plant breeding and its related fields such as statistics and experimental design. Additionally, the availability of efficient computational resources to process data is essential for researchers to estimate and interpret the desired genetic parameters. This study is built on DesignGen, a software that performs multi-purpose statistical genetic analyses. Developed by members of the Statistical Genetics Laboratory of the “Luiz de Queiroz” College of Agriculture, the platform was introduced to facilitate the interaction with users and simplify the representation of analyses without the need for in-depth knowledge of programming language. The current project focuses on DesignGen app improvements, more specifically the inclusion of statistical models applied to factorial arrangements - one of the most efficient ways to analyze the effects of two or more levels of factors investigated simultaneously, in their respective designs, with treatments being all the possible combinations of levels that can be formed from the factors being investigated. This work highlights the introduction of an additional capability to the existing app for analysis of Randomized Block and Completely Randomized Designs, applied to factorial treatment arrangement. The computational interface was developed in the R environment using the Shiny package, which offers dynamic synchronization between the code and the user interface through reactive programming logic, allowing the user to load their data and manipulate it in the interface itself. Furthermore, it was structured based on the premises of the Golem package, a framework for developing Shiny applications, enabling integration with packages that accelerate code development and standardize the organization of scripts and other files. Analysis were made using demonstrative databases, indicating that the updates on DesignGen app capabilities had satisfactory performance, allowing effective statistical analysis. This represents significant advancements in plant breeding and the performance of statistical analysis that would allow the selection of superior genotypes in a more efficient way. The software will be available from November 2024, and it can be found and installed through its repository on Github - https://github.com/statgen-esalq/StatGenESALQ_App.

Keywords: R; factorial arrangements; experimental design; statistical analysis; app

Acknowledgements

GENETIC PARAMETERS FOR RGB-BASED VEGETATION INDICES APPLIED TO A SUGARCANE BREEDING POPULATION

Igor Killer Nunes[1,2]; Gustavo Candido Jacobassi[1]; Monalisa Sampaio Carneiro[1,2,3] Flavio de Souza Costa[1]; Roberto Giacomini Chapola[1]; Hermann Paulo Hoffmann[1,3]; Rodrigo Gazaffi[1,2,3]

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Abstract

Unmanned Aerial Vehicle (UAV)-based remote sensing provides a non-destructive, low-cost, and efficient approach to monitoring crop growth. It has a high potential to accelerate the breeding process and facilitate decision-making in the field. Searching for new automated variables in sugarcane breeding is highly desirable, as it can be a tool for the early selection of varieties that are more productive in terms of sugar yield than those currently in use. Images captured through remote sensing allow for the calculation of vegetative indices, capable of characterizing vegetation cover according to its reflectance; even so, information on the genetic nature of these variables is scarce. The study aimed to assess whether RGB-based vegetation indices effectively estimate genetic parameters in a sugarcane population. The experimental area was in the Federal University of São Carlos in Araras/SP; planting was in February 2023 under the randomized blocks design, with two replications comprising 141 clones and six commercial checks. A Phantom 4 Pro was used to acquire the images between July 2023 and February 2024. The orthomosaic was obtained with Agisoft Metashape, and the vegetation indices were extracted with FieldImageR software. The vegetation indices used were NGRDI, BGI, HUE, VARI, BI, SCI, GLI, and SI. These digital variables were submitted to mixed-model analysis to predict variance components and genotypic means. The coefficients of variation of heritability in the broad sense, genetic (CVg) and residual (CVr) were obtained. The highest CVg and CVr values were obtained for SCI in November, 10.67 and 8.35, respectively. The lowest CVr values for NGRDI, BGI, and HUE were found in November, VARI in October, BI and SCI in February, GLI in July, and SI in December. For all the indices studied, the highest heritability value was observed in February, with variations between 0.72 and 0.91, with the BGI index showing the highest heritability. It was observed that early measurements provide the greatest differentiation between genotypes; however, the ideal period for selection is in February, when heritability reaches its maximum value.

Keywords: UAV; heritability; genetic variance; FieldImageR

Acknowledgements

This research was supported by the FINEP (Financiadora de Estudos e Projetos) through Convênio 01.22.0197.00 – FUNAPE – Ref. 1210-21.

ESTIMATING THE CANOPY COVER WITH RGB-SENSORS BOARDED IN UAV TO DISCRIMINATE THE SUGARCANE GROWTH PATTERN

Gustavo Candido Jacobassi[1], Igor Killer Nunes[1,2], Monalisa Sampaio Carneiro [1,2,3], Flavio de Souza Costa[1], Roberto Giacomini Chapola[1], Hermann Paulo Hoffmann[1,3], Rodrigo Gazaffi[1,2,3]

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Abstract

Sugarcane breeders are always looking for new technologies to maximize the efficiency of identifying new varieties. Based on remote sensing principles, using UAV boards with RGB sensors allows the continuous monitoring of experimental areas where all the genotypes can be regarded in their growth pattern. The growth pattern can be summarized for breeding purposes by the canopy cover, which results are still sparse for the sugarcane breeding context. This work aimed to study the canopy cover variable to evaluate the development of reference genotypes (checks) from the UFSCar/RIDESA sugarcane breeding program; also, the growth patterns were compared with climatic variables to comprehend the trait development over the crop cycle.The experiment was planted in February 2023 at the Araras campus, UFSCar, under the randomized complete blocks design, with two replications. Six checks were considered: CTC9001 and RB966928 as an early-maturing cycle, RB015177 as a medium-maturing cycle, RB005014 and RB975201 as a medium- or late-maturing cycle, and CTC4 as a late-maturing cycle.. A Phantom 4 Pro UAV was used to obtain the RGB images monthly from July/2023 (5 months) to February/2024 (12 months). The Orthomosaics were obtained via Agisoft Metashape, and the canopy cover was obtained from the NGRDI index (Green Red Difference Index) using the FieldImageR software. The experimental area's Evapotranspiration (ET0) data was obtained from the Zeus Agro Meteorological Station. ET0 and canopy cover from each genotype were correlated to compare the growth speed. The correlations between Canopy Cover data and accumulated ET0 were positive, i.e., vegetative growth is associated with water supply. The checks RB005014 and RB015177 achieved 60% of canopy cover in the shortest time (150 DAP), followed by RB966928 and CTC9001 (180 DAP), and CTC4 and RB975201 had a slower the slowest time (210 DAP) and uneven growth pattern. The two newest checks are the ones with the shortest canopy cover. Considering that 60% of canopy cover is a milestone for the inter-row closure limit, genotypes that reach this percentage early are highly preferable, e.g., weed control is facilitated with genotypes with the fastest canopy cover. Finally, in this study, we identified genotypes with faster growth patterns that are not necessarily related to their maturing cycle, allowing the selection for transgressive genotypes, i.e., genotypes with early closing interrow for the broad maturing cycles, which is desired for breeding.

Keywords: Remote Sensing; FieldImageR; Growth pattern; biomass accumulation; maturing cycle

Acknowledgements

This research was supported by the FINEP (Financiadora de Estudos e Projetos) through Convênio 01.22.0197.00 – FUNAPE – Ref. 1210-21.

PRIME EDITING AND KNOCKOUT OF LONG TANDEM REPEAT ARRAYS IN MAIZE KNOBS USING CRISPR-CAS9: CONSEQUENCES FOR HETEROCHROMATIN STRUCTURE, ORGANIZATION, AND BEHAVIOR

Eduardo Velloso de Oliveira[1,2]; Gabriel Lasmar dos Reis[1,3]; Eloisa Vendemiatti[1]; Vagner A. Benedito[1]; Mateus Mondin[2]

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Abstract

Knobs are heterochromatin regions in the maize genome well-visualized in pachytene chromosomes during meiosis or as conspicuous bands in prophase or metaphase mitotic chromosomes. These structures were first described more than 80 years ago and have been extensively studied since then. However, their primary function in the maize genome remains unclear. We started the development of a CRISPR-Cas9 system to knock out the K180 satellite DNA family motifs, which is the major component of the knobs, and evaluate the resulting impact on the plant's phenotypes. Additionally, we are engineering a CRISPR-Cas9 prime editing system to change the arrays nucleotide composition, aiming to induce structural changes in the heterochromatin blocks, including epigenetic modifications. Initially to these experiments, we selected the maize inbred line Fast Flowering Mini Maize A Transformable 6 [FFMM-A6], due to its short development cycle and compact size. An embryogenic tissue culture was well-stablished producing highly friable callus, making it suitable for *Agrobacterium tumefaciens* transformation and subsequent plant regeneration. The pDirect_23C binary vector plasmid, harboring the bar selection gene, which confers resistance to bialaphos was chosen, and had two gRNAs targeting the K180 regions inserted by Golden Gate cloning technique. By altering the knob region, we expect to observe changes in the heterochromatin structure, which may lead to variations in genotypic organization, chromosomal behavior, and potentially phenotypic abnormalities. These observations could help clarify the function of this region. Furthermore, our research may provide insights into the role of what has long been considered "useless DNA," potentially redefining our understanding of these genomic structures.

Keywords: Maize; Knobs; Heterochromatin; CRISPR-Cas9; K180; Prime editing.

Acknowledgements

We are thankful for the follow scholarship supports: EVO by PET-MEC, EVO by CNPq and MM is a PET-MEC fellow.

ESTIMATION OF GENETIC PARAMETERS FOR A MULTI-PARENTAL POTATO POPULATION IN MULTI-ENVIRONMENT TRIALS WITH NON-TRADITIONAL EXPERIMENTAL DESIGNS

Marco Guerra Hinostroza[1], Saulo Chaves[1], Carla Cristina da Silva[1], Mariela Aponte[2], Moctar Kante[2], Thiago Mendes[2], Guilherme da Silva Pereira[1]

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Abstract

The evaluation of many early generations genotypes requires non-traditional experimental designs, where lack of full replication can negatively affect estimates of genetic parameters such as variance, heritability, and ultimately breeding values. Frequently, high heritability values may be found in early selection cycles for highly environmentally sensitive traits. These values suggest two misconceptions: first, that selection in early cycles is as effective as in advanced cycles; and second, that the same unbalanced designs can still be used in later cycles. The goal of the present work is to increase the precision of genetic parameter estimation in unbalanced trials with correlated genotypes by decomposing genetic effects and correcting for kinship relationships established in pedigree files. The breeding team of the International Potato Centre generated a multi-parental population (5 females × 12 males) composed of 30 full-sib families (with 50 to 120 individuals per family, totaling 2992 individuals) with a genetic background resistant to the Oomycete *Phytophthora infestans* from various sources. This population was evaluated in three trials between 2022 and 2024. Two experimental designs were used: one trial employed an augmented row-column design, while the other two used partially replicated designs with different levels of replication. Variables evaluated included the area under the disease progress curve (AUDPC) and yield components such as the number and weight of total and marketable tubers. Pedigree information included two previous generations, and genotypic information consisted of a panel of 1955 DArTag-derived single nucleotide polymorphisms. The R package updog (v2.1.5) was used to perform dosage calling and the R package AGHmatrix (v2.1.4) was used to construct the numerator relationship matrix. Parental analysis implemented in Polygene software (v1.7) allowed the correction of pedigree information. Variance components were estimated by restricted maximum likelihood (REML) using Asreml-R (v4.2.0). Models considering alternate relationship matrices such as pedigree-based (A) and marker-based (G) were compared using Akaike Information Criterion (AIC). Likelihood Ratio Tests (LRT) were used to evaluate the significance level of the genetic variance components associated with additive and non-additive effects. Additive effects were significantly different from zero at the 0.001 or 0.01 probability levels, depending on the variable, while non-additive effects were significant at the 0.01 or 0.05 levels. The broad- and narrow-sense heritabilities were moderate, with values ranging between 0.38~0.56 and 0.16~0.36, respectively. After fixing pedigree errors, the standard error was reduced by ~20% when using only an A-matrix and by ~5% when using a G-matrix.. Although the procedures for estimating genetic parameters in unbalanced experimental designs and correlated individuals still present challenges, the sensitivity of the model and the incorporation of kinship information improve the quality of these estimates when properly applied.

Keywords: Variance, Heritability, Pedigree, Relationship matrix

Acknowledgements

BAYESIAN INFERENCE IN THE COMPARISON OF INDUCERS AND DONOR ENDOSPERM TEXTURES IN MAIZE PUTATIVE HAPLOID SEED PRODUCTION

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Abstract

Data generated in experiments with haploidy induction in corn (*Zea mays* L.) present a dichotomous response, with success represented by the production of putative haploid seeds. The use of models with binomial distribution, which has a fixed dispersion parameter, may not capture the variability in the data. Mixture models that use the beta-binomial distribution can present satisfactory adjustments in the presence of overdispersion. Thus, this study aimed to compare the adjustment of the binomial and beta-binomial models, through Bayesian inference, and to evaluate the influence of inducers and endosperm textures on the a posteriori means of the treatments. For the experiment were used seeds obtained from the crossing of twelve donor genotypes, classified into four endosperm textures (dent, semi-dent, semi-flint and flint), pollinated with four haploidy inducing genotypes (TAIL 7, TAIL 8, TAIL 9 and UH400 x UH401), in the 2016/17 harvest year in the municipality of Abelardo Luz - SC. The response variable analyzed was the number of putative haploid seeds (y_i) in a total of seeds (n_i), classified by means of the phenotypic marker R1-navajo. For analysis, it was first considered that yi follows a binomial distribution with probability of success π_i, y_i ~ Binomial (n_i, π_i) and logit link function. Subsequently, models with beta-binomial distribution were adjusted, where yi ~ Beta-binomial (n_i, α_i, β_i), assuming that y_i ~ Binomial (n_i, π_i) and πi ~ Beta (α_i, β_i). Non-informative priors were considered for model adjustment. Based on leave-one-out cross-validation (LOOCV), all models with beta-binomial distribution were more adequate and should be used for correct estimates of credibility intervals. The model that considers the main effects of inducers and textures without the presence of interaction presented the lowest estimate of the leave-one-out information criterion (LOOIC), and its use was justified. Therefore, the inducing factors and endosperm textures can be analyzed independently. Thus, the inducers UH400 x UH401 and TAIL 7 obtained the highest a posteriori means, 0.0773 and 0.0683, respectively, and the semi-dent and flint textures obtained the highest and lowest a posteriori means, 0.1305 and 0.0302, respectively. It was possible to establish the most suitable inducers for the groups of donor genotypes evaluated, aiming to obtain a greater number of putative haploid seeds.

Keywords: Bayesian inference; Mixture Models; *Zea mays* L.; R1-navajo.

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FISH-BASED CHROMOSOME CHARACTERIZATION OF THE TRADITIONAL MAIZE VARIETY ZAPALOTE CHICO USING K180 AND B-SPECIFIC SATELLITE DNA FAMILIES

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Abstract

Zapalote Chico is a maize variety of significant cultural, agronomic, and scientific value, closely associated with the Zapotec indigenous culture of the Isthmus of Tehuantepec in Oaxaca, Mexico. This variety belongs to the group of maize races from the western coast of Mexico, including other races such as Zapalote Grande and Chapalote, as demonstrated by genetic studies. Understanding the genetics of Zapalote Chico requires knowledge of the chromosomal structure of maize (Zea mays L.). However, only traditional meiosis pachytene chromosomal analysis and metaphase C-banding were reported to this variety and molecular cytogenetics is a gap. This study aimed to physically map by FISH two different families of satellite DNA, K180 a major component of the heterochromatic knobs, and B-specific a repetitive DNA sequence exclusive of maize B-chromosome in Zapalote Chico. The USDA kindly supplied the seeds of Oaxaca 103 and Oaxaca 55 accessions. After germination the root tips were pre-treated in a solution of 8-hydroxyquinoline at 300 ppm with cycloheximide at 25 ppm for 2 hours and 40 minutes, followed by fixation in Carnoy's solution. For chromosome counting, the root tips were Feulgen stained, which revealed cells with 21, 22, 23, and 24 chromosomes, showing a significant variation in the number of B chromosomes between plants. FISH was performed using K180 and B-specific oligonucleotide probes. The K180 was mapped in almost all of the chromosome arms and B-specific were exclusively mapped in the B chromosome in both chromosome arms. A diminished signal of K180 is observed in the short arm of the B chromosome intermingled with B-Specific. The FISH experiment confirmed the chromosome counting and the variable number of B chromosomes in this accession. The chromosomal variability observed is crucial for understanding the genetic structure of Zapalote Chico, reinforcing its importance as a valuable genetic resource for cytogenetic studies and breeding programs.

Keywords: *Zea mays*, Zapalote Chico, heterochromatin, repetitive DNA sequences, knobs.

Acknowledgements

EXPLORING CHROMOSOMAL TRAITS IN SIX AMARYLLIS (*HIPPEASTRUM HYBRIDUM*) CULTIVARS

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Abstract

Commonly referred to as amaryllis, the large flowers of the Hippeastrum genus can display a variety of hues, including white, orange, red, pink, and green. This genus of bulbous, terrestrial, and perennial plants typically has a basic chromosome number of x=11 and is predominantly diploid (2n=2x=22), although tetraploids (2n=4x=44) naturally occur. This study aims to perform a cytogenetic characterization of six different amaryllis (*Hippeastrum hybridum*) including the cultivars Carmen, Orange Sovereign, 663, 1069, H-0639, and H-0614. Root tips were pretreated in 300 ppm 8-hydroxyquinoline and 25 ppm cycloheximide at a temperature of 20°C during 7,5 hours and fixed in Carnoy’s fixative solution (3 part of ethanol : 1 part of acetic acid). The root tips were stained using the Feulgen method, and fluorescent banding were carried out using CMA, CMA/DA and DAPI. The cultivars Carmen, Orange Sovereign, 663, and H-0639 displayed 44 chromosomes in their somatic cells, while the cultivars 1069 and H-0614 presented 45 chromosomes. Preliminary results from fluorescent banding procedures showed the presence of CMA bands at the short arms ends of five chromosomes in cultivars 1069 and 663, indicating G-C rich regions of approximately equal size, with no significant differences between the cultivars. The results did not differ with the application of CMA/DA. No DAPI bands were observed. The results suggest the existence of chromosomal variation between the cultivars, potentially resulting from polyploidy and a complex interspecific hybridization during breeding programs

Keywords: Amaryllis; *Hippeastrum*; Cytogenetics; Chromosome

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CYTOINFORMATICS AS A NOVEL APPROACH INTEGRATING GENOMICS AND CYTOGENETICS FOR KARYOTYPE ANALYSIS IN MAIZE (*ZEA MAYS*)

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Abstract

Since the beginning of cytogenetic studies, significant advances have emerged with the development of new methodologies and tools, greatly expanding the use and scope of this scientific field. Karyotype analyses, typically performed by observing chromosomes using chromosome banding techniques, have become essential tools for understanding and studying organisms and chromosome organization and structure. Recent advances in genomic sequencing techniques, such as telomere-to-telomere sequencing, have enabled a new approach to genomic studies based on the data it provides. In this work, we aimed to validate a novel approach, coined '*cytoinformatics*', which combines the use of genomics with the principles of cytogenetics and cytogenomics and their analysis to try to address and explore important questions in plant cytogenetics, particularly in maize. Specifically, we used the most recent sequencing data available to characterize the chromosome structure and identify variations among maize lines. The B73 maize genome (*Zea mays* ssp. *mays*) was used as a reference to compare the information from the maize NAM founder lines and the Mo17 sequenced genomes. Using programs developed for data manipulation and graphical presentation, we were able to compare genome sizes and the variation in chromosome arm ratios across different lines. This allowed us to quickly and efficiently identify and observe the translocation between chromosomes 9 and 10 of the Oh7B line graphically when compared to traditional cytogenetic methods that are more time-consuming and labor-intensive. These results demonstrate the potential of cytoinformatics as an initial and quick method for seeking cytogenomic information through markers in sequenced organisms with near complete assembly.

Keywords: Cytogenomics; Bioinformatics; Chromosome translocation; Karyotype Analysis.

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CRISPR-CAS9 HETEROCHROMATIN EDITING REDUCED RELATIVE COPY NUMBER OF TGR1 REPEATS IN TOMATO

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Abstract

The study of constitutive heterochromatin domains harbouring repetitive DNA persists as a significant challenge. The same silencing mechanism that enables the propagation and maintenance of heterochromatic portions makes it difficult to access them. However, even a small disturbance in these regions might provide remarkable findings. Using CRISPR-Cas9 to edit repetitive DNA in heterochromatin regions helps investigate genome organization and heterochromatin dynamics. As a model crop, the tomato exhibits a distinctive genome organization characterized by extensive heterochromatin domains, which comprise approximately 77% of the genome and are rich in repetitive DNA sequences. TGR1 repeat is the main in tandem repeat of tomato, represented as a 162 bp motif, with ~77000 copies per genome in the subtelomeric regions and as interstitial knobs. Therefore, we aimed to promote the deletion of tomato TGR1 repeat via the CRISPR-Cas9 editing system, to provide answers to open questions related to plant heterochromatin in general. We constructed a T-DNA vector pDIRECT_22C expressing a Csy4 array of 3 gRNAs targeting TGR1 repeat, delivered in *Solanum lycopersicum* L. cv Micro-Tom (MT) explants via *Agrobacterium tumefaciens*. The first shoots emerged after ~21 days in shoot induction media, and the first plantlets emerged after ~ 15 days in shoot elongation selection media. The plantlets exhibited phenotypic variation in leaf number, leaf color, and overall size. This observation suggested individuals were edited, and presenting copy number variation (CNV) in the TGR1 repeats. A PCR for Cas9 presence revealed that 6 of them were positive for editing system. We estimated the relative CN of TGR1 repeats in the 6 positive TGR1-plantlets using quantitative real-time PCR (qPCR). CN per genome was normalized on 6 WT values to express the CN in each TGR1-plantlet as a percentage of WT CN. All 6 individuals presented relative CN reduction of TGR1 repeats, ranging from 14% to 69% compared to WT. This was the first step of an investigation of plants with heterochromatin editing presenting remarkable initial results concerning the role of heterochromatic repetitive genomic portions.

Keywords: cytogenetics; heterochromatin; microtom; repetitive DNA

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CYTOGENETIC ANALYSIS OF FOUR SPECIES OF *CATTLEYA* SUBGENUS *CATTLEYA* SERIES *SOPHRONITIS*

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Abstract

Cattleya series "*Sophronitis*" (Orchidaceae) are epiphytic plants endemic to Brazil, primarily distributed in the southeastern and southern regions of the country. The species are small, unifoliate orchids with single flowers or clusters with multiple flowers of different color variations (red, orange to pink and yellow). Due to their significant ornamental traits, many species within this group are utilized in orchid breeding and genetic improvement programs. Cytogenetic analyses in plants are of considerable importance, as they provide extensive information that can be applied to various purposes, including phylogenetic and evolutionary studies and genetic improvement. According to the new botanical classification of *Cattleya*, the subgenus *Cattleya* series *Sophronitis* has 9 species. Molecular phylogenetic analyses characterize all species, but only one species has conventional and molecular cytogenetic characterization. Observing the great lack of cytogenetic information in this important group of orchids the main aim of this work was to perform a classical cytogenetic analysis of four species of *Cattleya* series *Sophronitis* (*C. cernua*, *C. wittigiana*, *C. brevipendiculata* and *C. rio-grandensis*) to determine chromosomes numbers and intraspecific chromosomal variations. Root tips of each species were pre-treated with a 17:3 solution of 8-hydroxyquinoline and cycloheximide and fixed with Carnoy 3:1. The root tips were stained by Feulgen method, followed by an enzymatic treatment to soften tissues for slide preparation by squash technique. For observation and chromosome counting, an optical microscope with a 100x objective and ImageJ software were used. It was determined that the somatic chromosome number is 2n=40 and no intraspecific variation was observed in the four species studied. Despite the significant morphological variability and diversity among the species, the results indicate that this group exhibits high chromosomal stability, which could be valuable for genetic improvement strategies involving interspecific crossings.

Keywords: Orchidaceae; *Sophronitis* series; cytogenetic; chromosomes

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AMPLIFICATION TEST OF SATELLITE SEQUENCES IDENTIFIED IN WILD SPECIES OF THE GENDER SOLANUM

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Abstract

The characterization of the repetitive fraction of the genome has significantly contributed to the understanding of the evolution and taxonomy of different plant groups, as well as revealing functional aspects of repetitive sequences. In *Solanum*, a genus with several species of economic importance such as potato, tomato, and eggplant, some species have already had their repetitive fraction described. This includes wild species from the section Commersoniana, such as *S. chacoense* (2n=2x=24), *S. commersonii* (2n=2x=24), and *S. calvescens* (2n=3x=36). The latter is considered a promising source of resistance and tolerance alleles to biotic and abiotic factors for potato breeding, although its taxonomic status remains controversial. In previous work, exclusive and shared satellite sequences were identified among the species, with varying levels of abundance. Mapping the chromosomal location of these sequences in these species is expected to complement the variability data and contribute to inferences about their relationships. Therefore, the aim of this study was to define the optimal conditions for amplifying satellite sequences, using primers designed for each sequence, for subsequent physical mapping via Fluorescent *in situ* Hybridization (FISH). Three sequences were selected: SOL8, identified in *S. calvescens*, SOL11 in *S. chacoense*, and SOL1 in *S. commersonii*. Amplification was performed using primers designed to amplify the consensus sequences with the Geneious Prime software, employing the Primer3 tool. DNA from *S. calve*scens, *S. chacoense*, and *S. commersonii* was extracted from young leaves of greenhouse-grown plants using the CTAB protocol. DNA quality was evaluated through 1% agarose gel, and concentration was measured with a NanoDrop spectrophotometer. PCR amplification was performed in a total volume of 25 µL containing ultrapure water, buffer, dNTPs (10 µM), forward and reverse primers (10 µM), species-specific DNA (100 ng), and Qiagen HotStart Taq (2.5 U). Tests included variables such as annealing temperature (50° to 59°C) and additives (Q-Solution, Mg+2). Ladder-like amplification patterns are expected, given the tandem nature of the sequences; however, under the proper conditions, only specific amplicons were observed for each satellite. The PCR reaction that resulted in the amplification of the satellites occurred under the following conditions: initial denaturation at 95°C for 15 minutes, followed by 30 cycles of three steps: denaturation at 94°C for 30 seconds, annealing at 54°C (SOL8 and SOL11) and 57°C (SOL1) for 45 seconds, and extension at 72°C for 1 minute, with a final extension at 72°C for 10 minutes. The amplified satellite sequences will be purified, sequenced, and labeled for localization and confirmation of their tandem pattern, abundance, and variability among related Solanum species.

Keywords: Breeding; *Solanum*; patato; DNA; PCR; Primer

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INPACTOR2: A FAST AND EFFICIENT METHOD FOR THE ANALYSIS OF LTR RETROTRANSPOSONS IN *VIGNA UNGUICULATA* L. WALP.

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Abstract

LTR retrotransposons (Long Terminal Repeats) are Class I transposable elements that flank internal coding regions responsible for proteins such as: proteases, reverse transcriptase, and integrase. These elements constitute a substantial part of plant genomes; their replication inserts new copies at various genomic sites, causing structural and functional changes that affect genetic variability. Their identification is highly relevant to plant breeding, as it can contribute to characterization, the study of morphological effects, and diversity among accessions. However, localizing and classifying transposons typically requires DNA extraction and genome sequencing. Inpactor2 was developed to identify and classify these only using silico methods, eliminating the need for prior molecular protocols. As a result, this study aimed to identify and classify LTR elements using Inpactor2 in the genome of *Vigna unguiculata* L. Walp. to utilize this repetitive portion in species characterization and plant breeding processes. The genome was obtained from the National Center for Biotechnology Information (NCBI), and bioinformatics analyses were performed on a system with the following specifications: Linux (Zorin OS), Intel i7 9700 processor, 64 GB DDR4 RAM (3200 MHz), 2 TB of NVMe storage, and an AMD RX6600 XT graphics card. Inpactor2 demonstrated high efficiency, accurately detecting transposable elements, filtering partial sequences, and classifying intact sequences into superfamilies and lineages/families in approximately 160 minutes. The analysis revealed that 9.61% of the cowpea genome is composed of LTR retrotransposons, with 25% Copia and 35% Gypsy, equivalent to 180,632,320 bp out of a total of 516,147,924 bp. The main Copia lineages/families found were Ale-Retrofit (2.32%), Bianca (0.64%), Ivana-Oryco (0.82%), SIRE (3.22%), and Tar-Tork (2.61%), while the Gypsy families included Athila (4.10%), CRM (4.56%), Galadriel (0.05%), Reina (0.73%), TAT (7.70%), and Tekay-Del (8.24%). The software identified the chromosomal location of the elements, with chromosome 3 containing the highest concentration of both Copia and Gypsy retrotransposons. These results may assist in designing specific primers, evolutionary studies, and genetic diversity analyses among germplasm accessions. The high precision and speed of Inpactor2, combined with functional genomics approaches, can facilitate the discovery of molecular markers and expand our understanding of plant genome evolution through LTRs.

Keywords: retrotransposons elements; genetic diversity; bioinformatic; cowpea;

Acknowledgements

PARENTAGE ANALYSIS IN AUTOPOLYPLOIDS: INSIGHTS FROM SIMULATED AND EMPIRICAL DATA IN TETRAPLOID POTATO (*Solanum tuberosum*)

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Abstract

Mislabeling and pedigree errors in outbreeding clonally propagated crops like potato are inevitable due to complex work operations during parental crossing and offspring propagation. There is an increasing loss of offspring-parental identity especially when big genetic crossing blocks are set up. Literature points to error rates of 5%-10% in many breeding programs, with pedigree errors having an adverse impact on genetic data analysis. This study seeks to obtain proportions of true and false offspring-parental assignments using dosage-sensitive molecular marker data, such as single nucleotide polymorphisms (SNPs) in tetraploid potato. Using MultiPolyPop R package, we simulated a partial diallel pedigree population from crosses between 14 females and 4 males (full-sib family size = 35) based on PedigreeSim software, together with sequence read depths and inferred dosages for 1,630 SNPs based on updog R package. By combining an increasing sample of markers (10, 30, 60, and 90 SNPs) with varying levels of missing data (0%, 10%, and 20%), we have obtained 1,000 files per combination. Empirical genotyping data for a potato population from CIP-Peru generated from crosses between 13 females and 4 males (N = 359 individuals) was done using DArTag technology with 2,315 SNPs (filtered at 10% missing data). Parentage analysis was conducted in Polygene v.1.7 software using a maximum likelihood-based polysomic inheritance model to obtain the best possible parental assignment combinations for each offspring (simulated or empirical data). Simulated data results have shown that the percentage of true assignments was higher for crosses when parent sex is known compared to when sex is unknown. In the former case, 100% true assignments were achieved at 90 markers with 0% and 10% missing data, while 90% true assignments were recorded for samples with 20% missing data. Empirical data results show 150 (41.8%) individuals with correct assignment for both parents, 172 (47.9%) individuals with one correct parent (either female or male), and 37 (10.3%) individuals with both parents incorrectly assigned. This study demonstrates the importance of parentage analysis to ensure higher quality control and fix pedigree issues before conducting any genetic data analysis. We recommend that potato breeding programs continuously improve their genetic and experimental designs through correct pedigree annotation, proper pollination control, and non-detachable labeling tags to avoid mix-ups.

Keywords: SNP; Dosage; Breeding program; Quality control

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IDENTIFICATION AND OVEREXPRESSION OF CANDIDATE GENES FOR HLB TOLERANCE IN *CITRUS*

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Abstract

Huanglongbing (HLB) is the main citrus disease worldwide and is caused by the bacterium *Candidatus* Liberibacter *asiaticus* (CLas). All sweet orange varieties (*Citrus sinensis* L. Osb.) are susceptible to HLB, making the development of tolerant varieties crucial. Therefore, in this work, we address gene overexpression strategy for the genetic transformation of commercial citrus varieties aiming to obtain HLB-tolerant plants. Transcriptomic analysis revealed differentially expressed genes across susceptible, tolerant, and resistant citrus genotypes. The genes were analyzed using NCBI, phytozome and Pan genome (http://citrus.hzau.edu.cn/) and we selected three targets of CAP and LCR69 superfamily protein based on sequence comparison among *C. sinensis*, *P. trifoliata*, *C. clementine*, *C. australasica*. For the overexpression of CsCAP2, CsCAP3, CsLRC69 genes, the commercial vector from @Epoch was optimized. A total of 1318 epicotyls of Hamlin, Valencia sweet oranges, and Citrange 'Carrizo' were incubated in suspension containing A. tumefaciens GV3101 with vectors specific for each overexpressed gene, resulting in 411 shoots. Transformants were identified using GUS histochemical staining. DNA extracted from GUS-positive shoots was subjected to PCR analysis, confirming genetic transformation in 12 samples. To promote elongation for grafting, PCR-positive shoots were cultured in a liquid medium containing benzyl aminopurine (BAP). The regenerated shoots were multiplied, acclimatized in a greenhouse, and subsequently challenged with HLB. Our findings provide valuable insights into HLB response mechanisms, laying the groundwork for the creation of HLB-tolerant citrus cultivars through targeted genetic engineering.

Keywords: Biotic stress, *Citrus sinensis*, Huanglongbing, Host response

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DEVELOPMENT OF A COMPUTATIONAL INTERFACE FOR THE ANALYSIS OF MIXED MODELS: EXPLORING GENOTYPE-BY-HARVEST INTERACTIONS (GHI) IN MULTI-HARVEST TRIALS

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Abstract

Linear mixed models have important features, allowing the evaluation of effects interaction and dealing with heterogeneity and dependency among model components. This approach enhances the estimation of genotype-by-harvest interactions (GHI) in analyzing multi-harvest trials (MHT), one source of variation frequently overlooked in conventional analyses. Modeling this source improves the efficiency of selecting superior genotypes in forage breeding programs. Therefore, the goals of this project are (i) to develop a user-friendly interface within the DesignGen app for analyzing phenotypic data using mixed models, and (ii) to demonstrate the selection of an optimal variance-covariance (VCOV) structure for analyzing GHI in MHT using the app's interface. DesignGen is an app that the Statistical Genetics Lab members have previously developed. This app and the interface were developed within the R environment, using packages such as Shiny to create an intuitive interface, Golem to build a modular structure, and Sommer to perform analyses within the app. From the selected model, its interface was used to elect the optimal VCOV structure for analyzing GHI in MHT data based on the Akaike (AIC) and Bayesian information criteria (BIC). The MHT data consisted of phenotypic measurements of dry leaf matter from 23 genotypes of Panicum maximum, evaluated in a completely randomized block design with up to fourteen harvests in Campo Grande, MS. The interface demonstrated efficiency, allowing users to perform their analyses clearly through an intuitive workflow. It presents the user with essential information for analyzing and interpreting the models, such as the AIC and BIC, variance components, best linear unbiased predictions (BLUP), and downloadable analysis results. The selected model confirmed the presence of GHI in the forage data, with an unstructured matrix (US) for the interaction effect and a diagonal matrix (DIAG) for the residual effect providing the best fit (AIC -566.16 and BIC -488.31). Therefore, the results demonstrate that this tool enables the construction and interpretation of models through a user-friendly interface. Also, non-homogeneous VCOV structures are adequate for modeling GHI.

Keywords: DesignGen app, breeding programs, variance-covariance structures, unstructured matrix

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HOST GENOTYPES AND FUNGAL ISOLATES SHAPE *SPORISORIUM SCITAMINEUM* TRANSCRIPTIONAL PROFILES AND SHED LIGHT ON BREEDING SMUT-RESISTANT SUGARCANE CULTIVARS

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Abstract

Sporisorium scitamineum, the causal agent of smut disease in sugarcane, presents significant challenges to sugarcane productivity worldwide. Understanding the molecular interactions between this pathogen and its host is crucial for effective disease management and breeding strategies. In this study, we aimed to investigate whether distinct Brazilian isolates of sugarcane smut, exhibiting varying levels of virulence, trigger the activation of different gene sets during the early stages of infection (48 hours post-inoculation) in sugarcane genotypes with contrasting smut-resistance levels (susceptible vs. resistant). RNA was extracted 48 hours post-inoculation (hpi) and sequenced using the Illumina platform. We used FastQC v.0.11.5 to assess read quality. The read-filtering step consisted of removing library adapters, low-quality reads (Q < 20), and ambiguous bases (N) using Cutadapt v.1.18. We performed transcript mapping with Hisat2 v.2.1.0 against the fungus reference genome. A read count table was obtained using featureCounts v.1.6.0 from the Subread package. We conducted differential expression analysis using likelihood ratio test in edgeR v.4.0.16 from R Bioconductor. Our results unveil some specific molecular events influenced by the host genotypes. The analysis of a low-virulent fungal genotype (LV) revealed the expression of 3,452 genes in planta, with 96% also expressed by the high-virulent genotype (HV) during sugarcane infection. The low-virulent genotype displayed a distinct set of expression regarding candidate effectors, sharing only three differentially regulated effector genes with HV, which exhibited opposite expression patterns. Notably, a chitin deacetylase gene was upregulated (20-fold) in LV-infected resistant plants, in contrast to HV. These variations in transcriptional responses highlight the importance of host-pathogen interactions in shaping the infection process and impacting the disease outcome. We provide preliminary insights that may guide the design of functional experiments and support the prediction of potential resistance proteins (resistance analog genes) that may interact with the pool of effectors to inhibit fungal colonization in resistant host genotypes. It underscores the importance of breeding programs that consider both fungal transcriptional diversity and isolate-specific resistance, paving the way for more durable and effective sugarcane cultivars.

Keywords: biotrophic fungi; RNAseq; candidate effectors; plant-pathogen interaction; virulence

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FUNCTIONAL ANALYSIS OF GENES RELATED TO THE PATHOGENICITY OF *ALTERNARIA ALTERNATA* TANGERINE PATHOTYPE

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Abstract

The disease known as Alternaria brown spot (ABS) caused by *Alternaria alternata* is a significant fungal disease affecting tangerine crops and their hybrids, with the most affected variety being the Murcott tangor. In the Alternaria-citrus interaction, the fungus modulates its gene expression upon recognizing host signals. Currently, the main challenge in phytopathology is understanding the interaction between pathogens and their hosts. Several *A. alternata* genes are expressed upon recognition of host signals and are possibly associated with the fungus's pathogenicity. Among them are the AALT_g5837 gene, which encodes a P450, AALT_g5454, which encodes a transcription factor, and the AALT_g10699 gene, which encodes a homeobox protein. Studies indicate that the microorganism's contact with Murcott tangor extract in culture medium led to the expression of these genes, induced as a response to the stimulus provided by the plant extract. Thus, the project's aim is to verify if these genes are involved in the pathogenicity of the *A. alternata* tangerine pathotype by using a gene replacement vector with a cassette, allowing for functional analysis of mutants. To obtain mutants, we used a gene replacement strategy and constructed the pALTmut01 vector for filamentous fungi. This vector comprises a bidirectional promoter, a gene encoding mCherry, a gene conferring resistance to hygromycin B, and two terminators with BbsI sites, which will serve as a donor for the recipient vector pUC57-Kan_Bbs. This setup allows for the cloning of arms of genomic sequences adjacent to or from the target gene to select transformants. Transformation was carried out using *E. coli* with the addition of X-Gal and IPTG. The results showed that blue colonies indicated the production of β-galactosidase, confirming the expression of the lacZ gene through X-gal metabolism. However, the white colonies did not metabolize X-gal, confirming that the transformed vector inactivated the lacZ gene. It is expected that, upon obtaining the mutants, there will be a reduction in mutant efficiency, indicating that the studied genes are related to the pathogenicity of the *A. alternata* tangerine pathotype.

Keywords: gene AALTg_5837; gene AALTg_5454; gene AALTg_10699; Cassete; *Alternaria Brown* Spot

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Victória Stern da Silva1,2; Mariana de Souza e Silva1; Paulo José Pereira Lima Teixeira3; Bárbara Aliende Pires3; Mariana Bossi Esteves1,3, Alessandra Alves de Souza1

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Abstract

Citrus orchards are affected by several bacterial diseases such as citrus variegated chlorosis (CVC) and citrus canker. With the aim of mitigating the damage caused by these diseases, two commercial varieties of sweet oranges, Pineapple and Hamlin, were developed overexpressing the *rpfF* gene from *Xylella fastidiosa*, which encodes for an enzyme involved in the synthesis of the diffusible signaling factor (DSF), a small fatty acid molecule responsible for regulating quorum sensing (QS) in specific bacteria. Thus, plants overexpressing DSF were able to interfere with bacterial colonization and decrease disease symptoms to CVC and citrus canker. Currently, the best lines of Pineapple and Hamlin transgenic plants, along with their non-transgenic controls, have been in field conditions for four years at the Citriculture Center 'Sylvio Moreira'-IAC, located in Cordeirópolis/SP, a region with the highest rates of HLB, a disease that is causing significant damage to citrus production. Thus, HLB severity in these plants were regularly assessed by three different evaluators, following a specific disease severity scale. The area under the disease progress curve was calculated using data collected from May 2022 to May 2024 and the data were subject to t-test ($P \leq 0.05$). Results demonstrated that the progression of HLB disease in transgenic plants is lower than in non-GM plants. Based on this result, the hypothesis was raised that DSF could be acting as an elicitor, priming transgenic plants, since *Candidatus* Liberibcater asiaticus, causal agent of HLB, does not have DSF-regulated QS system. To test this hypothesis, experiments to detect hypersensitivity reactions (HR) were conducted. For this, leaves of transgenic and non-transgenic plants were subjected to lipid extraction. The extracts, along with synthetic DSF from *X. fastidiosa* used as a positive control, were infiltrated into *Nicotiana benthamiana*. HR was visualized under white light and UV after 24 hours of infiltrations. HR was detected in the infiltration regions with extracts from transgenic plants, resembling the lesion found in the positive control. HR was not observed at the infiltration site with extracts from non-transgenic plants. So far, the results indicate that DSF may be acting as an elicitor, activating plant defense response. In future studies, the GM plants will undergo transcriptome analysis to verify possible alterations in gene expression as a result of DSF overexpression.

Keywords: *Citrus sinensis*; resistance; *rpfF*; elicitor

Acknowledgements

ESTIMATION OF THE GENETIC RELATIONSHIP MATRIX AMONG GENOTYPES OF *MEGATHYRSUS MAXIMUS*

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Abstract

Brazil's livestock sector is key to global food production, contributing to 14.34% of the world's beef supply in 2022. Approximately 85% of Brazil's 200 million cattle are raised on pastures, however, these areas are shrinking annually, highlighting the need for tropical forage breeding. One major challenge faced in *Megathyrsus maximus* breeding is the long evaluation period, which is approximately 8 to 10 years. In light of this, characterizing the relatedness of *Megathyrsus maximus* genotypes is essential for breeding programs, as this information can be useful for selecting crosses and also be applied to predictive models, such as genomic selection, which can bring significant genetic gains in perennial species. In this study, the goals were to: (i) estimate the additive relationship matrix (A matrix) for *Megathyrsus maximus* genotypes, and (ii) analyze the genetic diversity among these genotypes, based on the A matrix. The project was conducted in partnership with Embrapa Gado de Corte, which provided the pedigree records of the genotypes. To estimate the A matrix, the pedigree data was filtered, and the additive relationship matrix was calculated using the AGHmatrix package. Due to the large number of uninformative genotypes, a function was created to remove them from the pedigree. To better understand genetic proximity between individuals, clustering methods were applied: single linkage, complete linkage, UPGMA, WPGMA, and Ward's method, with UPGMA proving to be the most effective. As a result, the 640 informative genotypes were divided into six groups, revealing the genetic proximity between genotypes. The groups accurately represented full-sib and half-sib relationships, allowing for an initial understanding of the population's relationship. Limitations such as missing pedigree data, the possibility of relatedness in the base population, and the non-adoption of a specific model for polyploids were identified and should be considered in future research. In conclusion, the generated matrix can be used as a starting point for studying the relatedness in the population, although, the inclusion of molecular marker information could improve the determination of genetic similarity among genotypes. For the next steps, considering genomic selection, combining molecular markers with genealogy information could provide a model with greater efficiency.

Keywords: Tropical grasses, Pedigree, Relatedness, Clustering

Acknowledgements

GENOMIC SELECTION MODELS FOR INDUSTRIAL QUALITY TRAITS IN BRAZILIAN CASSAVA GERMPLASM

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Abstract

In the context of industrial starch production in Brazil, cassava (*Manihot esculenta*) stands out as one of the main raw materials. However, the cultivation of the species faces challenges that make genomic selection a promising approach compared to traditional breeding methods. Initial evaluation using univariate models for different traits is crucial in this process. The present study aimed to apply genomic selection models Bayesian ridge regression, BayesA, and BayesB, focusing on industrial quality traits of cassava. Genotypes from the germplasm bank of Embrapa Mandioca e Fruticultura, Brazil, at different stages of the selection cycle, were genotyped and phenotyped. Genotyping was performed using DArTseq technology, and the genomic data were aligned to the reference genome v6.0. After SNP Calling, filtering was applied based on an MAF of 1% and a Call Rate of 80%, resulting in the selection of 27k informative SNPs. Field trials were conducted in randomized complete blocks between 2018 and 2020 for assessments of traits such as Plant Architecture (Pl. Arq), Dry Matter Content (DMC), Starch Content by Gravimetry (Starch-grav), Resistance to Foliar Diseases (Leaf-disease), and fresh root yield (FRY). The clone’s BLUEs were used to validate the results through 10-fold cross-validation, with nIter set to 6000 and burnIn set to 1000, ensuring the consistency and efficiency of the models. Higher predictive accuracies were observed for FRY (0.70 - Bayesian ridge regression; 0.65 - BayesA; 0.65 - BayesB), followed by Starch Gravimetry, (0.57 - Bayesian ridge regression; 0.62 - BayesA; 0.61 - BayesB) and Plant Architecture (0.52 - Bayesian ridge regression; 0.55 - BayesA; 0.51 - BayesB). For DMC and Leaf Disease, accuracies ranged between 0.32 and 0.57. In terms of predictive ability, the best results were also obtained for Starch Gravimetry (0.40 - Bayesian ridge regression; 0.53 - BayesA; 0.53 - BayesB), followed by FRY (0.46 - Bayesian ridge regression; 0.43 - BayesA; 0.43 - BayesB) and Plant Architecture (0.39 - Bayesian ridge regression; 0.40 - BayesA; 0.37 - BayesB). The lowest values for DMC and Leaf Disease were observed, ranging from 0.13 to 0.40. Regarding heritability, values ranged from 0.17 to 0.74 using BayesB, from 0.22 to 0.23 with BayesA, and from 0.12 to 0.50 with Bayesian ridge regression. The prediction bias was below 0.06 for all traits and selection models evaluated.The results provided a solid foundation for a better understanding of the studied traits behavior and contributed to future multivariate models development.

Keywords: Bayesian models; Genomic Selection; Cassava; Cross-Validation

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HYPOCOTYL AND EPICOTHYL LENGTH IN THE DISTINGUISHABILITY OF SOYBEAN CULTIVARS BY ANDERSON'S DISCRIMANT ANALYSIS

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Abstract

The breeder of a new soybean cultivar may protect the property and moral rights of the new pure line developed through genetic improvement, upon obtaining the cultivar protection certificate, granted by the Ministry of Agriculture and Livestock. To this end, certain requirements must be met, such as distinguishability, homogeneity and stability of the cultivar candidate for protection. However, due to the narrow genetic base of soybeans, many new cultivars candidate for protection may not be distinguished from other protected cultivars. Thus, the use of additional descriptors, such as vegetative phase traits, has been suggested by some researchers for differentiation purposes. The objective of this study was to evaluate the discrimination capacity of six soybean cultivars using the hypocotyl-epicotyl length traits by Anderson's discriminant analysis. The study was carried out at the Capim Branco experimental farm of the Federal University of Uberlândia. In two sowing seasons (March and May 2023), 6 soybean cultivars (BRSGO 7560, TMG 801, UFUS 6901, BRS7380 RR, UFUS 6901, BRS 7380 RR, TMG 1179 RR, BMX Desafio RR) were evaluated in a randomized complete block design with 3 replicates. The plot consisted of 1 row of soybean plants, 5 m long, spaced 0.5 m apart. 5 plants in each plot were evaluated regarding the lengths of the hypocotyl and epicotyl, using a millimeter ruler. The data were subjected to univariate and multivariate analysis in the Genes Program. In both sowing seasons, genetic variability was found at a 5% significance level by the F test for hypocotyl length, while for epicotyl variability was detected at a 10% level by the F test for the first sowing season. The genotypic determination coefficients were 56.91% and 0.0% for hypocotyl length, respectively, for sowing seasons 1 and 2. For epicotyl length, the genotypic determination coefficients were 89.44% and 64.39%, respectively, for seasons 1 and 2. Considering that only season 1 showed genetic variability for both traits, it was decided to perform Anderson's discriminant analysis only for season 1, whose apparent error rate was 50%. It was noted that no cultivar was completely differentiated from the others, since each cultivar was distinguished from 3 or 4 cultivars. An alternative for a complete distinction of one cultivar from another is the inclusion of more traits of the vegetative phase in the discriminant analysis. It is concluded that the inclusion of the traits hypocotyl and epicotyl length in the Anderson discriminant analysis allowed the partial differentiation of soybean cultivars.

Keywords: *Glycine max*; vegetative traits; cultivar differentiation; additional descriptors

Acknowledgements

FAPEMIG

INDICATION OF BIPARENTAL CROSSINGS OF ELITE SOYBEAN LINES BASED ON GENETIC DIVERSITY STUDY

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Abstract

The selection of parents and indication of hybrid combinations is one of the fundamental steps for the success of a plant breeding program. In this regard, elite lines are potential parents in a breeding program, which can be crossed with other cultivars that add some trait of interest for breeding, such as disease resistance, cycle, and grain yield. The objective of this study was to test whether there is significant genetic diversity among 10 soybean genotypes, with the goal of indicating suitable hybrid combinations for soybean breeding focused on precocity and disease resistance. The study was carried out at the Capim Branco experimental farm, of the Federal University of Uberlândia, under greenhouse conditions. Ten soybean genotypes were evaluated, including three early-cycle elite lines (UFU J010, UFU J101, and UFU J201) and seven soybean cultivars (UFUS 7910, BRS 511, BRS 7380 RR, BRS 751 RR, TMG 803, IAC Foscarin-31, and MSoy 9350), in a randomized complete block design with 8 replicates. Each replicate consisted of a 5 dm^3 pot previously filled with substrate and grown with two plants. Sowing was carried out in November 2022. The plants were irrigated daily, and cultural treatments were carried out according to the technical recommendations of the crop. In each plant, of each replicate, the following parameters were evaluated: number of days to maturity (stage R8 - Fehr and Caviness), plant height at R8, measured with a millimeter ruler, the total number of pods per plant, determined after harvest, and grain production per plant, measured by grain mass on a 0.01 g precision scale. The data were subjected to univariate and multivariate analyses, with the aid of the Genes Program. Genetic variability was detected at the 1% significance level by the F test. Through generalized Mahalanobis analysis, it was found that the genetic distance ranged from 0.068 to 37.38, respectively between the elite lines (UFU J010 and UFU J101) and the cultivars (UFUS 7910 and BRS 7380 RR). The dendrogram generated by the intragroup average linkage method (UPGMA) and Tocher clustering revealed the formation of 4 groups, which were concordant in the clusters. The cultivars UFUS 7910 and MSoy 9350 constituted unitary groups; the cultivars BRS 511 and BRS 7380 RR formed a single group; and the other cultivars were grouped in a single group. The number of pods per plant contributed with 73.64% to the genetic diversity. From the clusters, it was possible to suggest hybrid combinations aimed at precocity and disease resistance. It is concluded that the favorable combinations with the elite lines are: UFU J010 x BRS 511, UFU J101 x BRS 511, UFU J102 x BRS 511, UFU J010 x BRS 7380 RR, UFU J101 x BRS 7380 RR, and UFU J102 x BRS 7380 RR.

Keywords: *Glycine max*; genetic dissimilarity; vegetative trais; soybean breeding

Acknowledgements

CNPq; CAPES; FAPEMIG

PERFORMANCE OF MAIZE HYBRIDS IN SOUTHERN MINAS GERAIS UNDER DIFFERENT NITROGEN DOSES

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Abstract

Since nitrogen (N) is the nutrient required in the largest volume by plants, nitrogen fertilization is essential to reach the productive potential of hybrid maize (*Zea mays* L.) cultivars. Fertilization with adjusted doses, considering the cultivars' responsiveness to increased doses, allows for yield maximization in an economically viable way. In this study, the objective was to evaluate the performance of nine single-cross maize hybrids developed by the maize genetics and breeding program at the Federal University of Lavras, under doses of 100, 200, 300, 400, and 500 kilograms per hectare (kg/ha) of urea (46% N). The experiment was conducted at the Scientific and Technological Development Center of the Federal University of Lavras (UFLA), located in the municipality of Lavras - MG, during the 2022/23 growing season. A randomized complete block design was used in a 10x5 factorial scheme (nine experimental hybrids and one control, and five doses of urea), with three replicates. Each plot consisted of four rows of five meters, with a spacing of 0.6 meters between rows and 0.25 meters between plants, maintaining an ideal population of 66,666 plants per hectare. Fertilization was split into two applications, with 50% of the dose applied at planting and the rest 30 days after plant emergence. Grain yield in tons per hectare (t/ha) was taken as the parameter and analyzed individually and jointly using the mixed linear model approach (REML/BLUP). The hybrids showed significant differences ($p<0.05$) for grain yield in both individual and joint analyses, as well as for the different fertilizer doses. However, the interaction between hybrids and fertilizer doses was not significant, according to the likelihood ratio test (LRT). The 400 kg/ha urea dose had the highest average yield among the doses, with 9.6 t/ha. Hybrid four showed the lowest yields across all doses, with 4.53, 3.98, 4.55, 6.61, and 5.80 t/ha, respectively. Five hybrids had higher yields than the control, with hybrids three and eight performing the best, yielding above 12 t/ha at all doses, standing out as promising for the market. The results allowed the selection of the best-performing hybrids and provided valuable insights into the responsiveness of these hybrids to increased nitrogen doses.

Keywords: *Zea mays* L.; Nitrogen; REML/BLUP; Yield

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UFLA, FAPEMIG, CAPES, CNPQ

CORRELATIONS BETWEEN AGRONOMIC CHARACTERS AND TECHNOLOGIES IN COMMON BEAN GENOTYPES AS GENETIC GAIN INDICATOR

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Abstract

The bean is a plant native to the American continent, domesticated in Mesoamerica and the Andes. Brazil is currently the third largest producer of the crop in the world, but productivity is low in some states, such as Mato Grosso do Sul. Genetic improvement can helpincrease production and technologics characters, but it is interesting to take into account the genetic correlation. The present study aimed to investigate genetic variability, evaluate morphoagronomic and technological characteristics, correlate them, and predict gains in the main characteristics of the crop using aselection index. Nineteen genotypes were evaluated, four of them cultivars, during the dry season, in an area located in the Federal University of Grande Dourados, MS, Brazil. The experimental design adopted was a completely randomized block design with three replicates, in which the following were evaluated: days to flowering (DF), days to maturation (DM), plantheight (ALT), height of insertion of the first pod (AIV), number of pods per plot (NVPC), number of pods per plant (NVP), number of grains per plot (NGPC), number of grains per pod(NGV), mass of one hundred grains (M100), productivity (PROD), luminosity color parameters(L), red-green component (a) and yellow-blue component (b*), percentage of water absorptionbefore cooking (PEANC) and after cooking (PEAPC),soluble solids (BRIX) and total (SOLT).It was found that most of the characteristics showed a statistically significant difference between the genotypes, and tha t seven (DF, M100, L, A, B, PEAPC and PGI) showed genetic variance higher than experimental. The production components NGPC, NVP and NVPC showed positive environmental and genotypic correlations with productivity. A total genetic gain of 23.15% and 17.85% was predicted respectively using the Mulamba e Mock (1978) index with weights assigned arbitrarily and with the heritability of each characteristic, and in productivity the estimated gain will be approximately 7 and 3% respectively, considering thesesame weights.

Keywords: *Phaseolus vulgaris* L; index selection; genetic gain; heritability.

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Universidade Federal da Grande Dourados - UFGD

Grupo de Melhoramento e Biotecnologia Vegetal – GMBV

CLASSIFICATION OF *COLLETOTRICHUM LINDEMUTHIANUM* PHYSIOLOGICAL RACES IN COMMON BEAN AIMING TO OBTAIN RESISTANT GENOTYPES

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Abstract

Anthracnose (*Colletotrichum lindemuthianum*) can cause up to 100% yield losses in common bean (*Phaseolus vulgaris*), requiring that resistant cultivars be employed in agricultural practices. However, genetic recombination of the pathogen can induce susceptibility in these cultivars, highlighting the need to develop new resistant cultivars. Backcrossing is essential to accelerate the introduction of resistance genes into susceptible cultivars. Differential cultivars assist in identifying the physiological race associated with the fungal isolate, thereby demonstrating variability in the pathogen. The study aimed to collect, map, and identify new physiological races of the fungus to support the selection of resistant genotypes and combine desirable traits with parental genotypes that have resistance breakdown. For this, indirect isolations were conducted from plants infected with anthracnose, and pure colonies of new fungal isolates were obtained. The inoculum of the fungal isolate was produced on bean pods for later inoculation on both sides of the leaves and stems of the plants (V2 growth stage), which were subsequently maintained in a controlled environment (3 days with 80% relative humidity and 20°C temperature). Disease evaluation on inoculated plants was performed using a scoring scale from 1 (resistant) to 9 (susceptible). To identify the fungal race, the isolate was inoculated on 12 differential cultivars, and for race 65, 11 specific differential cultivars were inoculated. For cultivar screening regarding resistance, 17 cultivars were inoculated with each isolate obtained and identified by race. Seven fungal isolates were identified within the following physiological races: 65.805, 81, 87, 465 (BRM 28876), 467, and 479. The six fungal races were individually inoculated on 17 cultivars to select resistant varieties. Cultivars with black seed coat, Gen 20-39-11, IAC Diplomata, and IAC Una were selected as donors of resistant genes to the fungus for crossing with IAC Veloz. For carioca seed coat, IAC 2560 Nelore, Linhagem 10, and IAC Sintonia played the same role for IAC 2051. IAC Veloz and IAC 2051 are recurrent parents which underwent resistance breakdown.

Keywords: Anthracnose; *Phaseolus vulgaris*; Plant Breeding; Backcrossing

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