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Abstract Book

March 24-27, 2019
Lake Buena Vista, FL, USA

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Abstract Compilation

Abstracts are sorted alphabetical by presenting author's last name. Presenting author names appear in **bold**.

GENETIC PARAMETERS FOR AGRONOMIC TRAITS IN A REFERENCE ALFALFA POPULATION

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Alfalfa (*Medicago sativa* L.) is the most-grown cool season perennial forage legume in the world. In the U.S., alfalfa is grown on approximately 6.7 million hectares with an estimated crop value of US\$9 billion; however, alfalfa production has not been recently reported in Florida. The objectives of this study were: i) create a reference alfalfa population following a factorial mating design; ii) phenotype the population for agronomic traits at the plot/family level; iii) estimate variance components and genetic parameters using mixed models. For the mating design, a single plant per genotype was selected (plant vigor and biomass production) from a variety trial run from 2014 to 2016 in Citra, FL. Six genotypes with known adaptation in Florida (breeding lines from UF's former breeding program and 'Bulldog805') were used as male parents and crossed to 27 females following a factorial design. A total of 145 full-sib and 36 half-sib families were used for the field phenotyping experiment. The experiment was designed as a row-column (14x32) with augmented representation of three controls (Bulldog805, 'FL99' and an advanced breeding line from UF). Bulldog805 was planted as border for each experimental unit, and 20 seedlings were manually transplanted in the center of each plot for all families and controls. Borders were mowed before phenotyping for these traits: plant height, foliar disease ratings, flowering percentage and dry matter yield. Data was analyzed using linear mixed models in R and ASReml. Variance component estimates were used to calculate narrow-sense heritability (h^2). Narrow-sense heritability by harvest ranged from 0.25 to 0.50 for flowering, 0.07 to 0.27 for plant height, 0.04 to 0.33 for foliar diseases, and 0.14 to 0.33 for dry matter yield. Narrow-sense heritability across all harvests were 0.22 for flowering, 0.13 for plant height, 0.089 for foliar diseases, and 0.28 for dry matter yield. These results show great potential to develop alfalfa cultivars with improved biomass yield for subtropical environments. The phenotypic data collected in this study will be used in genomic prediction models in the future.

PRESENTER BIO: Janam Acharya is a MS student in the Agronomy Department, University of Florida. Janam is conducting research in genomic prediction in alfalfa at the Forage Breeding and Genetics Lab under Dr. Esteban Rios's supervision.

GENETIC IMPROVEMENT OF PASPALUM SPECIES IN NORTHEASTERN ARGENTINA

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The purpose of the *Paspalum* breeding program located in Corrientes, Argentina has been to develop subtropical forages with improved cool-season growth, cold tolerance, seed yield, forage quality and persistence. Three breeding methods were used within the program for polyploid species: ecotype selection, hybridization, and recurrent selection. A total of 135 ecotypes belonging to 15 different species were evaluated. The evaluation of natural diversity present in these species allowed for the identification of germplasm well adapted to the subtropics with outstanding characteristics that were unknown for some species, such as late flowering for *P. simplex*, good adaptation to acid soils for a biotype of *P. dilatatum*, and high cattle preference for an accession of *P. atratum*. Two cultivars were generated using this method, i.e. *P. atratum* cv. Cambá FCA and *P. guenoarum* cv. Chané FCA.

Hybridization within the tetraploid germplasm was possible for a few species because of the availability of induced sexual tetraploid plants. Highly apomictic hybrids were generated in a large enough number that allowed for selection in *P. notatum*, *P. simplex*, and from the interspecific cross *P. plicatulum* x *P. guenoarum*. Several hybrids for *P. notatum* exhibited a combination of high forage yield and good ground cover. Superior cool-season growth, cold tolerance, and seed set were observed for some of the interspecific hybrids. A cultivar named Boyero UNNE was developed using this technique for tetraploid *P. notatum*.

Two sexual tetraploid synthetic populations were created with the objective of using recurrent selection. One population was generated for *P. notatum* by 1) hybridizing a few sexual clones with a group of apomictic genotypes collected from different parts of the species distribution area, and 2) polycrossing the sexual hybrids. Another sexual population was created for the Plicatula group with the main difference that 8 species were crossed with an induced sexual genotype of *P. plicatulum* in the first hybridization phase. Recurrent phenotypic selection and recurrent selection based on combining ability were successfully used for these populations with the objective of accumulating additive and non-additive genetic effects in each cycle.

In conclusion, several genetic lines of *Paspalum* were selected considering a group of desirable forage traits by exploring the natural diversity, using hybridization at the tetraploid level and recurrent selection on the novel sexual germplasm.

PRESENTER BIO: Dr. Acuña is an Associate Professor whose research focuses on developing improved forage grasses and legumes. He received his M.S. and Ph.D. degrees from University of Florida. He has been doing research on genetic and physiological aspects of forage species in Argentina since 2009.

POLYPLOID ST. AUGUSTINEGRASS HYBRIDS DEVELOPED THROUGH EMBRYO RESCUE

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St. Augustinegrass [*Stenotaphrum secundatum* (Walt.) Kuntze] is uniquely adapted to the sub-tropical climate of south Florida; and the warm-humid regions of the southeastern U.S. and Gulf Coast region. It is the most commonly used turfgrass in Florida, where it accounts for 50% of the sod produced and sold. St. Augustinegrass can range in ploidy from diploids ($2n=2x=18$) to tetraploids ($2n=4x=36$) along with various aneuploids. For example, 'Floritam', the most dominant cultivar in Florida is an aneuploid with 32 chromosomes. Typically, diploids are morphologically smaller and more shade tolerant than the larger polyploid types such as Floritam. However, polyploids are typically more resistant to diseases, insects, and exhibit better drought resistance. The goal of this project was to produce variation for turfgrass characteristics and ploidy in St. Augustinegrass by using embryo rescue to facilitate crosses among ploidy levels in St. Augustinegrass. This study reports the ploidy levels of St. Augustinegrass progeny produced when making crosses between an array of ploidy levels found in parents. Flow cytometry was used to determine the ploidy levels of progeny.

Crosses were made in early morning without emasculation primarily using the aneu-polyploid lines as female parents. At approximately 10 d after pollination racemes were harvested, sterilized with 70% ethanol, then sodium hypochlorite, and followed by a water rinse. Developing embryos were dissected from florets and placed on half strength MS medium without growth regulators. When embryos germinated and developed into small plantlets they were acclimatized in peat under high humidity and then transplanted into greenhouse pots. The greenhouse grown plants were later prepped for flow cytometry using the Sysmex CyStain PI Absolute P kit. Meristems from actively growing stolons were collected, chopped, and incubated in 500 μ l of extraction buffer. The lysate containing extracted nuclei was filtered through a 30 μ m filter into a 3.5 mm tube to which 1.5 mL of propidium iodide staining solution. Samples were kept on ice, covered, and analyzed using an Accuri C6 flow cytometer.

The following crosses were made: 15 different aneu-polyploid x diploid; 6 diploid x diploid; and 2 polyploid x polyploid. From 36 racemes of aneu-polyploid x diploid crosses, 173 developing embryos were dissected and plated on MS medium. Of these, 16 germinated and developed into plant. From the diploid x diploid crosses, 60 embryos were dissected from 14 racemes and placed on MS medium. Twenty nine diploid x diploid embryos developed into plants.

Flow cytometry of all progeny from diploid x diploid crosses had values similar to the diploid parents. All hybrids recovered from aneu-polyploid x diploid crosses had flow cytometry values greater than the diploid parent, but flow cytometry values varied between hybrids of the same cross, suggesting that female aneuploid gametes may have functioned to combine with normal haploid gametes from the diploid parent. The progeny from the aneu-polyploid x diploid crosses showed marked phenotypic variability with potentially useful turfgrass attributes. Field evaluation in small turfgrass plots has been initiated.

PRESENTER BIO: Camisha Alexis is a graduate of the University of Florida with a B.Sc degree in Plant Science. She has experience in plant virology, where she was a research scholar focusing CpMMV in peanut and is now a research assistant in the UF Turfgrass and Breeding program led by Dr. Kevin Kenworthy.

PRECISION EDITING OF THE COMPLEX SUGARCANE GENOME BY HOMOLOGY DIRECTED REPAIR OF CRISPR/CAS9 INDUCED DNA BREAKS

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Genome editing tools such as CRISPR/Cas9 have been employed in several crop genomes. They enable precise targeting and introduction of double stranded DNA breaks in vivo. Subsequent cellular repair mechanisms, predominantly non-homologous end joining (NHEJ), act as critical steps to endogenous gene editing or correction. However, there is very limited control over these mechanisms, which generate an abundance of random insertions and deletions (indels). Frameshift mutations associated with these indels of unspecified size and sequence might result in loss of function phenotypes of agronomic importance. Gain of function mutations, on the other hand, generally requires precise nucleotide substitutions in the target locus. This can be accomplished with the aid of a homologous repair template and involves the cellular homology directed repair (HDR) mechanism. We are presenting data that compare HDR and NHEJ mediated genome editing efficiency in the highly polyploid sugarcane.

Presenter BIO: Dr. Altpeter is a University of Florida Research Foundation Professor. His research program integrates translational genomics, molecular physiology and metabolic engineering for crop improvement and sustainable production of value added products. The approaches include precision genome editing, synthetic biology, and molecular dissection of regulatory networks. These research activities focus on identifying, isolating and engineering limiting factors for genetic improvement of forage bioenergy and turf grasses.

GENOME-WIDE POLYMORPHISMS IN POLYPLOIDS *PASPALUM* SPECIES FROM PLICATULA GROUP

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High-throughput genotyping methods, like restriction site-associated DNA sequencing (RAD-seq), has allowed significant advances in genomic research, including non-model plant species. However, statistical methods for single nucleotide polymorphism (SNP) detection and genotype calling on polyploid species, overall without a reference genome, is a challenge in the development of SNPs markers. Recently, large-scale phylogenies are possible using RAD-seq data, so SNPs have begun to play an increasingly important role in the process of determining species boundaries and numbers in phylogeographic and phylogenetic studies. The botanical group Plicatula comprises polyploid species that presents high morphological variation and difficulties in species classification. The main species of this group is *Paspalum plicatulum* Michx., which is native to Brazil and widely distributed from the south of the U.S.A. to the south of Argentina. Due to the great importance of the species of Plicatula group as natural grasslands, this study aims to use a RAD-seq approach for the development of molecular markers to contribute with the classification of related species. With these purposes we analyzed 57 samples of ten different species of Plicatula group (*P. plicatulum*, *P. atratum*, *P. guenoarum*, *P. rhodopedum*, *P. leptum*, *P. oteroi*, *P. rojasii*, *P. lenticulare*, *P. wrightii* and *P. compressifolium*) and eight samples from the different species of Modesta, Malacophylla and Virgata groups, to be used as an external group in phylogenetic analysis. The RAD libraries were prepared according to Miller et al. (2007) using the restriction enzyme *SbfI* and each accession was barcoded with a unique 8bp sequence. Samples were multiplexed into two representative pools of DNA from each individual sample: i) pool-1 was generated across 20 libraries of *P. plicatulum* and, ii) pool-2 from 45 libraries of samples of the other species. Each pool was subjected to random mechanical fragmentation in a Covaris M220 Focused-ultrasonicator, to the size of the fragment required in the sequencing. We modified the standard protocol to target DNA fragments during shearing size selection to 600–700 bp for pool-1 and, 400 bp for pool-2. Two sequencing strategies were used: the first, pool-1 libraries were sequencing on a single flow cell lane in Illumina MiSeq System with 300 pb paired-end v3 kit; the second, with pool-2 in a single lane in the Illumina NextSeq 500 paired-end of 150 bp Medium Output. The initial processing of the data was conducted using several modules from the STACKS software. After quality filtering, more than 70 million RAD sequence reads pairs were generated across different species of *Paspalum* from the Plicatula group. Of which, 29,291,724 M of 300 bp PE was generated from the first sequencing approach of samples of *P. plicatulum*, and 44,023,836 M of 150 bp PE from the second sequencing for the *Paspalum* spp. The RAD-seq dataset will enable the estimation of allelic dosage for polyploid species of the Plicatula group and themarkers development, to infer the delimitation of their species, which will benefit the use of this valuable germplasm and the genetic breeding programs of *Paspalum*.

PRESENTER BIO: Dr. Oliveira is a Postdoctoral Researcher at Unicamp and has experience with genetic-genomic research for molecular genetic breeding of tropical forage species (*Paspalum* spp. and *Urochloa humidicola*).

GERMPLASM SCREENING FOR TOLERANCE TO BERMUDAGRASS STEM MAGGOT (BSM)

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Bermudagrass is the most important perennial forage crop grown for pasture and hay in the Southeast (~ 20 million acres). Bermudagrass Stem Maggot (BSM) (*Atherigona reversura* Villeneuve) is an invasive insect pest introduced from Asia and it significantly reduces yield of forage bermudagrass hay if it is not controlled. Over the past 5 plus years it has affected bermudagrass hayfields throughout southeastern US. A visual screening of a 300 accession core collection identified some potential lines with tolerance to BSM and have good biomass yields. Tolerant lines were then evaluated in a replicated field study for yield loss due to the insect by comparing sprayed controlled plots to unsprayed plots in Tifton starting in 2016 through the summer of 2018. For clippings in mid to late summer, BSM reduced yield of Alicia and Russell by over 40% and Tifton 85 by up to 35%. However, some of the 15 tolerant plant introductions tested exhibited less than 10% yield loss and had dry matter hay yields comparable to Tifton 85. Fiber quality and other quality traits will be evaluated for further use. The best lines will be assessed for direct release and for use in plant breeding.

PRESENTER BIO: William Anderson is a perennial forage grass geneticist on March 10, 2003. The current primary responsibilities of Dr. Anderson are to develop warm-season grass cultivars for use as forages and biomass feedstock as well as develop management systems to incorporate these species into cropping systems that include CRP lands, buffer strips, forage for livestock, and conservation programs. Dr. Anderson has published more than 70 refereed journal articles and four book chapters. He is also a co-coordinator of the USDA Southeastern Biomass Research Center to assist in development of regional testing of bioenergy feedstock crops.

STRUCTURE OF THE COVARIANCE MATRIX AND ITS IMPLICATIONS ON THE SELECTION OF *UROCHLOA RUZIZIENSIS* CLONES

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Clonal selection in *Urochloa ruziziensis* (syn. *Brachiaria ruziziensis*) is usually based on measurements taken at multiple harvests over time on the same plot. Statistical methods for the analysis of this type of data should consider the spatial variation, and the temporal correlation between repeated measurements. The methods also need to model the genetic effects over time appropriately. However, it is common analyzing these data with the split-plot-in-time model, i. e., assuming homogeneity of variances and covariances for genetic and residual over harvests. Some studies have shown that this model may not be suitable for several scenarios of repeated measurements data analysis. This work aimed to evaluate different covariance structure of the genotypic and residual matrices and verify their implications on the selection of *U. ruziziensis* clones. A total of 254 *U. ruziziensis* clones were evaluated together with the cultivars Marandu (*U. brizantha*) and Basilisk (*U. decumbens*) under nine harvests in an incomplete block design with three replications, and one-plant plot. Green biomass yield (g/plot) data were analyzed by a mixed model approach using the ASReml-R package. We tested several covariance matrices structures for residual (R) and genotypic (G) effects. Firstly, we identified the most appropriate structure for R, and secondly for G, considering R matrix previously chosen. The Bayesian Information Criterion (BIC) was used as the fit statistic of the models. We evaluated the different models by computing the Spearman's rank correlation between BLUPs, coincidence percentage, and the genetic gain with the selection of twenty-six best clones. The Antedependence model (ANTE) was the best structure for the R matrix, and the heterogeneous first-order autoregressive model (AR1H) for the G matrix according to BIC. This result demonstrated that the assumptions of homocedasticity were not appropriate. The Spearman's correlation between the model assuming homogeneous covariance (CS structure) and the model that best fit the data was 0.95 with a coincidence of 78%. These results show alterations in the ranking of clones for the fitted models. The genetic gain with CS model was 20%, and the fitted model was 25%. Therefore, the present study shows that the covariance structure might result in important impact on selection in a plant breeding program.

PRESENTER BIO: Juliana is a Ph.D. student in Plant Genetics and Breeding Graduate Program at the Federal University of Lavras, Brazil, with experience in forage breeding and data analysis.

PHENOTYPING OF *BRACHIARIA HUMIDICOLA* HYBRIDS FOR ITS BNI POTENTIAL, BIOMASS PRODUCTION, FORAGE QUALITY AND N₂O EMISSIONS

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International Center for Tropical Agriculture (CIAT), Cali, Valle, Colombia

Brachiaria humidicola (Bh) is a tropical forage grass that suppresses the activity of soil nitrifiers through biological nitrification inhibition (BNI). As a result both the nitrate (NO₃⁻) formation and nitrous oxide (N₂O) emissions are being reduced.

We aimed at investigating the link between BNI potential, biomass production, forage quality and N₂O emissions with the role of BNI on improving nitrogen (N) recovery and reducing N losses from a set of Bh hybrids.

In 2008, we started with a breeding population of approximately 2000 Bh hybrids, searching mainly for nutritional quality, spittlebug resistance and waterlogging tolerance. From this population, 118 hybrids were selected and then, based on greenhouse evaluations, twelve apomictic hybrids were selected in 2012 with different BNI capacity and planted in the field in 2013 along with seven controls Bh CIAT (26146, 26149, 679, 16888, 26159), hybrid Mulato II, *Panicum maximum* CIAT 16028 and bare soil (plots without plants) at Agrosavia 'La Libertad' Research Center located in the Piedmont region of Llanos of Colombia for field evaluation over time (4 years in total). The materials were arranged in a Randomized Complete Block Design with three replications.

We have adapted and developed phenotyping tools to characterize differences in BNI ability among Bh on the role of BNI in improving the efficiency of utilization of fertilizer nitrogen while reducing N₂O emissions from agricultural production systems. In this study, during the 4 years of evaluation, we monitored BNI potential using rhizosphere soil samples from each plot that were collected on a yearly basis during the rainy season and evaluated for a range of variation and tendency on nitrification rates using soil incubation analysis 'microcosms'. Biomass production (dry matter) and nutrition quality parameters [Crude protein (CP), In vitro dry matter digestibility (IVDMD); Neutral and Acid detergent fiber (NDF, ADF)] were determined during both the dry and rainy seasons. In the last year, N₂O emissions were determined using portable Fourier Transform Infrared Spectroscopy Gas Analyzer (GASMET DX4040) from contrasting genotypes for biomass production, BNI, CP and IVDMD.

As a result, Bh genotypes that combine high production and quality forage and high BNI activity were identified based on low soil nitrification rates (high BNI activity) and consequent low N₂O emissions compared to the control materials. These included also high forage yield and nutrition quality.

PRESENTER BIO: Dr. Arango is a Scientist with experience based on improving nutritive values of crops and forages leading projects that aim mitigation strategies from the livestock sector in Latin America. He leads the molecular environmental biology laboratory, the forage quality and animal nutrition laboratory and the [LivestockPlus](#) project.

PHENOTYPING OF *MEGATHYRSUS MAXIMUS* FOUNDER POPULATION FOR BREEDING PURPOSES FOR ITS BNI POTENTIAL, BIOMASS PRODUCTION, FORAGE QUALITY AND NITROUS OXIDE EMISSIONS

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Modern agricultural systems in the tropics are characterized by low resource use efficiency, low productivity and demand large extensions of land. To tackle this scenario, improved pastures have vast potential to contribute towards a sustainable livestock model, of high productivity and provisioning of environmental services. Guinea grass (*Megathyrsus maximus*) is a widely diverse tropical pasture, recognized for its high biomass production, nutrition quality and ease to manage under grazing. Therefore the demand for premium materials of this forage species has been increasing in Latin America, Africa and Southeastern Asia.

In view of this, the International Center for Tropical Agriculture (CIAT) has undertaken a new breeding program for this species, starting from a founder population of 130 germplasm accessions from the germplasm bank of CIAT, with the input of sexual materials from the United States Department of Agriculture (USDA). The aim of this study was to perform the first phenotyping effort to a collection of 130 germplasm accessions of *M. maximus* at greenhouse conditions for productive and environmental traits.

The Biological Nitrification Inhibition (BNI) potential (ability of plants to reduce soil nitrification, preventing nitrate leaching and emissions of nitrous oxide) of this germplasm collection was phenotyped measuring: i) nitrification potential of soil where plants were grown; ii) inhibition capacity of root tissue over the activity of nitrifying bacteria *in vitro*; iii) abundance of ammonia-oxidant microbes in soil; and iv) nitrous oxide emissions of BNI contrasting genotypes. Moreover, aerial biomass production was determined and nutrition quality parameters [Crude protein (CP), *In vitro* dry matter digestibility (IVDMD); Neutral and Acid detergent fiber (NDF, ADF)] were measured through Near Infrared Spectroscopy (NIRS). The results for these traits were integrated into a cluster analysis that suggested the splitting up of this collection into three groups, one of which clustered 51 accessions with the highest means for productivity, BNI, CP, IVDMD and low ADF. This study presents also the first evidence of BNI ability in *M. maximus*, finding ample intraspecific variability and achieving outstanding reductions in soil nitrification rates and nitrous oxide emissions (up to 65% and 30% respectively, for accessions CIAT 6299, 6901 and 688). In conclusion, this collection represents an important diversity source with desired traits for the breeding program towards the future release of climate-smart cultivars and of high quality for livestock systems in the tropics.

PRESENTER BIO: Dr. Arango is a Scientist with experience based on improving nutritive values of crops and forages leading projects that aim mitigation strategies from the livestock sector in Latin America. He leads the molecular environmental biology laboratory, the forage quality and animal nutrition laboratory and the [*LivestockPlus*](#) project.

A NOVEL KENTUCKY ADAPTED RED CLOVER LINE DISPLAYS INCREASED 2,4-D TOLERANCE

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Incorporation of a legume, such as red clover (*Trifolium pratense*), into grass-based pasture systems offers many benefits. However, available red clover lines are highly susceptible to herbicides, including 2,4-D (2,4-dichlorophenoxyacetic acid), which is used for broadleaf weed management in pastures. A novel red clover line, UK2014, was developed at the University of Kentucky through conventional breeding and it expresses higher tolerance to 2,4-D than Kenland, a common variety used by Kentucky's forage producers. Adopting this new tolerant line would broaden weed management options in a legume-grass mixed pasture. The objective of this study was to assess the field performance of UK2014, in terms of yield and 2,4-D tolerance level, and to evaluate the tolerance of UK2014 to grazing in a clover-grass mixture following 2,4-D treatments.

UK2014 and Kenland were seeded in April 2017 in Lexington, KY. Treatments consisted of 2,4-D at a low and a high rate (1.12 and 2.24 kg/ha, respectively), applied either early (July), mid (August) or late (October) season. Each plot received only one 2,4-D treatment and treated plots were compared to controls that were not treated with 2,4-D. The entire experiment was repeated in 2018. Visual herbicide injury was evaluated one week after spraying. A visual injury rating was used from 0 (no observed injury) to 100 (death of all plants in the plot). Plots were harvested one week after treatment. Visual regrowth estimates were taken 14 days after treatment, also using a visual rating scale from 0 to 100. Both individual harvest and total season yields (dry matter ton/a) were determined. In April 2018, a grazing tolerance study was established in Lexington, KY. UK2014 and Kenland were interceded in a tall fescue (*Lolium arundinaceum*) pasture. In May 2018, 2,4-D treatments were applied at 1.68 kg/ha. The plots were submitted to mob grazing in July 19, August 9, and September 11. Red clover and fescue heights, clover stand, and clover dry weight in mixture were measured at grazing dates. Clover stands were evaluated in a 0 (weak) to 5 (very strong) visual scale. Clover dry weights were determined in two subsamples of each plot and expressed as a percentage of the total subsample weight. Data was subjected to analysis of variance and means were separated by Fisher's Protected LSD at $\alpha = 0.05$.

In the first year of the study, visual injury one week after 2,4-D treatment was less for UK2014 than Kenland, at all treatment dates. Similarly, in plots treated with 2.24 kg/ha 2,4-D, visual estimates of regrowth were higher for UK2014 than Kenland. However, there were no differences in yield between UK2014 and Kenland at individual harvests or in the season total. While this indicated that the performance of UK2014 is equal to Kenland in terms of yield, it also indicated that the 2,4-D injury to Kenland was not enough to reduce its yield. In 2018, preliminary data show that UK2014 had lower injury ratings and higher regrowth ratings than Kenland treated plots. However, unlike in 2017, the early 2,4-D early treatment reduced Kenland yield compared to the untreated Kenland. Results of the late treatment and season total yield will be presented. In the grazing study, 2,4-D treatment did not reduce either the fescue or the clover heights. The clover stand was reduced in 2,4-D treated Kenland plots compared to UK2014, and this reduction was even larger in the percentage of clover dry weight in the mixture.

PRESENTER BIO: Lucas Araújo is a Ph.D. Student at the University of Kentucky. His experience and current assignments focus mainly on weed management in forage crops.

ROOT SYSTEM OF FORAGE PEANUT GENOTYPES UNDER TWO LEVELS OF WATER AVAILABILITY

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Forage peanut (*Arachis pinto*i and *Arachis repens*) is cultivated in regions of the humid tropics, subject to four months of drought, when forage yield decreases. Field studies have shown that there is genetic variability for drought tolerance in forage peanut. However, morphophysiological responses of different genotypes are poorly understood under water deficit conditions. The objective of this study was to evaluate the root system of different genotypes of forage peanut under two levels of water availability.

Nine genotypes of forage peanut (BRA015253, BRA034100, BRA040894, BRA042170, BRA042242, V1(59) and E5 F1 hybrids, Belmonte and BRS Mandobi), with different dry matter yield in the dry season of Rio Branco, AC, Brazil, were evaluated in a greenhouse at two levels of water availability. The design was completely randomized, with treatments arranged in a 9x2 factorial, with four replications. Rooted stolons were transplanted into acetate tubes of 95 cm in length by 9.5 cm in diameter. At the irrigated level, 95% of field capacity was maintained and in the non-irrigated level, irrigation was suspended 10 days after transplanting. When 50% of the genotypes were under 50% of the field capacity (25 days after the irrigation suspension), the experiment was interrupted and shoot dry mass (SDM), root dry mass (RDM), root system length (RSL) and root system density (RSD) were evaluated. RSD was measured at depths of 0-15; 15-30; 30-45; 45-60; 60-75; and 75-90 cm, considering a split plot design. Data were submitted to analysis of variance, Scott Knott and Tukey tests, at 5% of probability.

No significant interaction ($p>0.05$) between genotypes and water availability was observed. The effect of genotypes was significant ($p<0.05$) for RSL. There was an effect of water availability for RDM and RSL ($p<0.01$). For RSD, significant interaction ($p<0.01$) between depth and water availability was observed. The mean of RSL was 47.1 cm. V1(59) and BRS Mandobi showed the largest root length (62.8 and 53.1 cm, respectively), whereas Belmonte presented the smallest one (37.2 cm). RDM and RSL showed higher mean values for the non-irrigated level (1.26 g and 60.6 cm) when compared to the irrigated one (0.91 g and 33.7 cm). RSD was higher for the irrigated level only at depth of 0-15 cm (788.1 mg dm⁻³ for irrigated versus 677.8 mg dm⁻³ for non-irrigated). At the irrigated level, 86.2% and 96.5% of the roots were concentrated in the first 15 and 30 cm, respectively, and did not reach the deeper layers. At the non-irrigated level, 53.4% and 93.6% of the roots were allocated up to 15 and 60 cm deep, respectively.

Root system of forage peanut can be adequately study using long acetate tubes. However, to identify more drought tolerant genotypes, it is necessary to impose a more severe water deficit. BRS Mandobi and V1(59), classified as less drought tolerant in the field, have higher growth of root system, regardless of water availability. Forage peanut presents plasticity of the root system, whose growth is stimulated under moderate water availability.

PRESENTER BIO: Dr. Giselle Assis is a researcher at Embrapa, where she has been since 2005. She is also a postgraduate professor at UFAC. Her area of expertise is forage breeding. She coordinates the *Arachis pinto*i Breeding Program and collaborates in *Stylosanthes*, *Brachiaria* and *Panicum* genetic evaluation for the Brazilian humid tropics.

EFFICIENCY OF EARLY SELECTION IN THE *UROCHLOA RUZIZIENSIS* BREEDING

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The number of harvests for selecting superior genotypes in *Urochloa ruziziensis* (syn. *Brachiaria ruziziensis*) is a current and important concern for breeders, because the phenotypic evaluation process is labor and cost intensive. Therefore, early selection could significantly reduce the time and costs needed to obtain new cultivars. This work aimed to evaluate the efficiency of the early selection for green biomass yield (GBY) in the *U. ruziziensis* breeding. A total of 254 *U. ruziziensis* clones were evaluated together with the cultivars Marandu (*U. brizantha*) and Basilisk (*U. decumbens*) in an incomplete block design with three replications, and one-plant plot. The GBY (g/plot) was assessed under nine harvests. A joint analysis was carried out using the mixed model approach considering the accumulated harvests up to the third, fourth, fifth, sixth, seventh, eighth and ninth cuts, in the scheme of split-plot-in-time model (compound symmetry covariance structure). We also tested other covariance structures for residuals (R) and genetics (G) matrices, and used the Bayesian Information Criterion (BIC) as the fit statistic to choose the best model for each grouping of cuts. We estimated the Spearman's rank correlation between BLUP of the clones considering the cumulative harvests and the total number of harvests evaluated. We also evaluated the genetic gain with the selection of twenty-six best clones in all analyses. The analyses were performed using the ASReml-R package. The covariance structures for the genetic and residual effects were the same for the accumulated from the fifth to the ninth cumulative harvests. The Spearman's correlation between BLUPs was 0.96 between the accumulated five and nine harvests, showing a small alteration in the ranking of genotypes with the early selection. The genetic gain, when considering the accumulated of five harvests, was 29%, and with nine was 25%, indicating that the gain selection with five harvests was similar to the gain reached with nine harvests. The results provided important information in favor of the adoption of early selection in the *U. ruziziensis* breeding, thus saving efforts and resources, and enabling the breeding program to be accelerated.

PRESENTER BIO: Dr. Flávia is a professor at the Federal University of Lavras, Brazil with experience in researching forage and perennial crops.

BROADENING THE GENEPOOL FOR FORAGE AND TURF GRASS BREEDING – NEW COLLECTIONS OF *LOLIUM* AND *FESTUCA* SPP.

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As one of the most important forage and turf grass species, *Lolium perenne* L. (ryegrass) has been subject of intensive breeding and research. Still, it suffers from frost in winter and early spring, heat and drought as well as various diseases. The identification of effective *L. perenne* genebank accessions or inter-specific crossings with *Festuca pratensis* Huds. are opportunities to improve stress tolerance of ryegrass cultivars and to broaden the narrow genetic base of ryegrass breeding. A close relative, the allopolyploid *F. pratensis* subsp. *apennina* (de Not.) Hegi, is rarely present in genebanks, but of interest due to its strong biomass development under the extreme conditions of its habitat (1,100-2,200 m asl), and its strict homologous chromosome pairing in meiosis, as opposed to frequent homoeologous pairing in amphidiploid *Festulolium*. This study evaluates recently collected *L. perenne* and *F. pratensis* subsp. *apennina* ecotypes in order to provide useful and well described germplasm for plant breeding and research.

41 ecotypic ryegrass populations collected across Europe in 2014 were cultivated for three years at the Malchow/Poel station of the IPK in northern Germany (mean annual precipitation 530 mm, mean annual air temperature 8.9 °C). Plant vigor, winter damage, disease resistance and aftermath heading were evaluated on 30 individuals per population on a scale from 1 to 9, heading in days after first of April. Plant height was measured using a herbometer. For *F. pratensis* subsp. *apennina*, 16 out of 43 populations collected in Switzerland in 2017 were characterized for plant biomass and general disease resistance (*Xanthomonas*, leaf spot, rust) during seed multiplication.

Based on the traits evaluated in 2017 and 2018, hierarchical clustering grouped the ryegrass populations into two major clusters. Cluster 1, comprising nine populations originating from northern Germany, Belgium and France, was separated from the other ecotypes by combining low winter damage with strong vigour, large plant height and tussock size as well as a high amount of ear producing tillers. Disease incidence was low and did not contribute much to the separation of the ecotypes, except for *Xanthomonas* infestation in June 2017. For *F. pratensis* subsp. *apennina*, flow cytometry indicated the presence of both tri- (3x) and tetraploid (4x) material in 11 out of 16 populations (8 to 95 % 3x plants). Triploids can be easily recognized at the flowering stage based on their indehiscent anthers. Often, they also have shorter and more irregular awns. Average plant biomass of 3x cytotypes was 1.3 score units higher and general disease resistance (*Xanthomonas*, leaf spot, rust) 2.2 score units better than those of 4x *F. pratensis* subsp. *apennina*. In conclusion, large variation was observed for many agronomic traits in *L. perenne* and *F. pratensis* subsp. *apennina* accessions recently collected by the IPK Genebank. Based on the above evaluations, they are now well described and available as high quality seed stocks.

PRESENTER BIO: Silvia Bachmann-Pfabe has done research on sustainable crop production for a decade. Initially, she studied the effects of residues from bioenergy production on plant nutrition and soil fertility at the University of Rostock. Now she is working at IPK, evaluating nutrient efficiency, disease resistance and agronomic performance of plant genetic resources.

BIG TRIALS AND BIG DATA: NEXT GENERATION FORAGE BREEDING

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With the advent of next-generation genotyping technologies, the ability to rapidly and cost-effectively genotype plants for research and commercial applications is becoming a reality. However, correlating sequence data to the variation in plant structure, performance and function requires large populations to link phenotype to genotype. Planting these large trials have been hampered by the inability to effectively capture quantitative measurements of plant growth, development and performance. Current assessment of non-destructive yield in forage breeding programs relies largely on the visual assessment by experts, who would categorize biomass to a discrete scale. Visual assessment of biomass yield has inherent pitfalls as it can generate bias between experimental repeats and between different experts. Visual assessment is also time-consuming and would be impractical on large scale field trials.

Phenomics has the potential to overcome this bottleneck through accurate, quantitative, high-resolution phenotyping based on digital analysis of plant morphology, development and function. A forage phenomics platform has been established to allow non-destructive, high-throughput evaluation of a global perennial ryegrass reference population of 270 000 individual plants from 1300 varieties/breeding lines. Sensor technologies such as multispectral cameras (Parrot Sequoia, Micasense Red-Edge M, Sланtrange 3P, Tetracam MCA-12), hyperspectral cameras (Specim FX17) and thermal cameras (FLIR Vue Pro), LiDAR (Sick LMS400, Sparkfun LiDAR-lite, Phoenix Scout) and spectrometers (ASD FieldSpec 4) are routinely deployed on aerial and ground-based vehicles enable the capture of images covering the light-spectrum from ultraviolet to far-infrared. Protocols and screening methods have been established for key forage traits, including yield, nutritive value estimates and abiotic and biotic stress responses. This screening technique has resulted in a dramatic reduction in staff hours for the non-destructive estimation of biomass yield of single plants in this perennial ryegrass field trial.

PRESENTER BIO: Dr Pieter Badenhorst is Senior Research Scientist in Molecular Plant Breeding with Agriculture Victoria Research.

AGRONOMIC AND NUTRITIONAL VALUE EVALUATION IN A POPULATION OF *BRACHIARIA* INTERSPECIFIC HYBRIDS (*B. RUZIZIENSIS* X *B. BRIZANTHA* X *B. DECUMBENS*)

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This paper reports the evaluation of interspecific hybrids of *Brachiaria* (*B. ruziziensis* x *B. brizantha* x *B. decumbens*) for agronomic and nutritional traits in order to identify those with better agronomic performance and nutritional value. Crosses between ten sexual tetraploid plants and three apomictic cultivars were performed in a crossing block and full sib progenies have been produced. The seeds were germinated and 1,380 hybrids have been produced. These progenies were evaluated in field plots at Embrapa Beef Cattle in Campo Grande/MS/Brazil, in an incomplete block design with fourteen replications and five plants per plot spaced 1.5 x 1.5 m. The commercial cultivars *B. brizantha* cv. Marandu, *Brachiaria* spp. cv. Mulatoll, *B. brizantha* BRS Paiguás, *Brachiaria* spp. cv. BRS Ipyporã and *B. decumbens* cv. Basilisk were used as check. Field green weight (FGW), total dry matter (TDM) and regrowth ability (REG) were evaluated in seven clippings; leaf dry matter (LDM); leaf dry matter percentage (L%), Leaf blade: stem ratio (LSR), crude protein (CP), in vitro organic matter digestibility (IVD), neutral detergent fiber (NDF) and lignin (LIG) in only three clippings. Data were analyzed using the restricted maximum likelihood/best linear unbiased prediction procedure (REML/BLUP). Genetic variability for all traits assessed was detected ($p < 0.01$) and heritability estimates based on progeny means ranged from 0.34 (LDM) to 0.71 (L%), indicating the possibility of gains with selection. The accuracy estimates ranged from 0.59 (LDM) to 0.84 (L%), which are considered moderate to high values. Significant genetic correlations were observed between FGW and TDM (0.95), FGW and LDM (0.83), TDM and LDM (0.90), L% and LSR (0.89) and CP and NDF (-0.84), showing that indirect selection or selection indices to consider these associations can be used in the breeding program. Considering a selection intensity of 5% the selection response relative to cultivar Marandu was -18.6% (FGW), -21.0% (TDM); -13.3% (LDM), 16.9% (L%), 136.6% (LSR), -0.8% (REG), 5.9% (CP), 1.8% (IVD), 3.4% (NDF) and 10.5% (LIG). In relation to cultivar Basilisk was 22.3% (FGW), 35.0% (TDM); 68.3% (LDM), 23% (L%), 158.9% (LSR), 38.9% (REG), 6.1% (CP), 2.8% (IVD), 2.9% (NDF) and 4.9% (LIG). Further evaluations for other traits like resistance to spittlebugs are currently been done and need to take into account before select the progenies. Superior apomictic hybrid once identified will proceed to the next phase of the breeding program for cultivar development.

PRESENTER BIO: Dr. Barrios is a forage breeding researcher at Embrapa Beef Cattle (Campo Grande, MS, Brazil). He has been working with diallel cross and recurrent selection in *Brachiaria* since 2011.

ALFALFA BREEDING IN ARGENTINA

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INTA Manfredi, Córdoba, Argentina

Alfalfa (*Medicago sativa* L.) is the most important forage crop in Argentina, where 3.2 million hectares were grown in 2018. About 60% of this area is planted as pure stands and 40% as mixtures with temperate grasses. Pure stands are primarily for hay and dairy production, while mixtures are for beef production. Direct grazing is still important, even though the number of feedlot-type operations has dramatically increased in the last 15 years. For dairy production, the utilization of varied degrees of confinement is also significantly increasing, with corn/sorghum silage and alfalfa hay as basic feeds.

At present, more than 120 varieties, belonging to fall dormancy (FD) groups 6 to 10, are in the market. To help farmers in choosing the proper variety, the National Institute of Agricultural Technology (INTA) is conducting since 1991 the National Alfalfa Cultivar Evaluation Network. Trials are under cutting conditions in 15 locations throughout the country. The network is coordinated by INTA Manfredi and results are annually published in *Avances in Alfalfa* ("Progress in Alfalfa").

INTA's alfalfa breeding program is carried out through a joint venture with Palo Verde, an Argentine private seed company. While INTA is in charge of the cultivar development (selection, evaluation and variety registration), Palo Verde is in charge of the seed increase and commercialization processes. As a whole, INTA's varieties have about 15% market share in Argentina. In the traditional program, breeding objectives are high forage yield, high persistence (4-5 years) and multiple pest resistance. There is also special research lines aimed to develop cultivars with tolerance to abiotic stresses (salinity and drought), higher forage quality (very high multifoliolate expression), and bloat tolerance (selecting for lower initial rate of dry matter ruminal disappearance). In addition, the development of a transgenic cultivar tolerant to glufosinate ammonium is under way.

The breeding methods used are phenotypic selection under field conditions and strain crosses. Cycles of phenotypic recurrent selection under controlled conditions are also utilized to increase resistance levels to blue alfalfa aphid (*Acyrtosiphon kondoi*), spotted alfalfa aphid (*Terioaphis trifolii*), Phytophthora root rot (*Phytophthora megasperma* f.sp. *medicaginis*) and anthracnose (*Colletrichum trifolii*). In all cases, resulting experimental populations are later on conducted as synthetic varieties.

So far, the program has released 15 varieties. The latest ones are Traful PV INTA and Limay PV INTA, two non-dormant (FD 9) cultivars; and Pulmarí PV INTA, an intermediate (FD 6) cultivar. Two new varieties, Kumen PV INTA (salt tolerant) and Amaya PV INTA (77% multifoliolate expression) are currently in the registration process and will be in the market by 2020. Extensive evaluation for the deregulation of the transgenic alfalfa tolerant to glufosinate ammonium will start this year.

PRESENTER BIO: Dr. Basigalup is Senior Scientist at INTA Manfredi and responsible for INTA's National Alfalfa Breeding Program. He has released nine alfalfa cultivars, edited/co-edited three alfalfa books and authored/co-authored several book chapters and scientific papers.

EVALUATING ANNUAL LEGUME FORAGE COVER CROPS FOR THE SOUTHERN GREAT PLAINS

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Winter wheat (*Triticum aestivum*) is the basis for most of U.S. southern Great Plains agriculture where it is planted as a dual-purpose crop: as a forage during winter and as a grain crop in summer. However, a forage gap is created from February until late spring when grazing in wheat is terminated to produce grain. In addition, most of the winter wheat in the southern Great Plains is grown in continuous rotations that results in degradation of natural resources (soil fertility, soil structure, soil microbial diversity, and water quality) and aid in the buildup of pests and diseases and finally, results in decline of productivity. Winter annual legumes are a suitable and sustaining alternative to continuous wheat rotations, and can be used as forage, cover crop or as an inter-crop in a silvopastoral system. Legumes interact with rhizobia and arbuscular mycorrhiza to improve soil fertility, soil physical properties, organic matter, soil tilth, control weeds, and break disease and pest cycles when used in rotations. These traits play a vital role in sustainable crop production systems and can substitute protein supplements with locally produced legumes.

Many species of annual legumes are adapted in much of southern Great Plains. Annual legumes can be used as a means of extending the length of grazing season, and reducing the amount of nitrogen fertilizer required in a forage production program. Moreover, some of the same species that are adapted to the southern Great Plains as a forage are also used as winter cover crop in the temperate regions of United States. In this research, we are evaluating 69 plant introduction accessions and 21 advanced selections of hairy vetch, (*Vicia villosa*), 49 accessions of crimson clover (*Trifolium incarnatum*) and two ecotypic selections of arrow leaf clover (*Trifolium vesiculosum*).

Given the amount of variation present for various agronomic traits related to forage production, there is a potential to develop high quality, high biomass hairy vetch, crimson clover and arrow leaf clover cultivars for the southern Great Plains. Further studies are underway at Noble Research Institute to test the potential to breed for hard seeded varieties to suit various forage/cropping systems of Southern Great Plains.

PRESENTER BIO: Dr. Suresh Bhamidimarri is an assistant professor with more than 10 years of experience in forage breeding and research. He has released many alfalfa cultivars that are currently in market place.

ENDOPHYTES AND MYCOTOXINS IN FLORIDA PASTURES

Ann Blount

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A concern about the presence of suspect mycotoxins in our predominant Florida sub-tropical forages was brought to our attention by several Florida cattlemen members. For the past two years, forage specialists and county faculty at the University of Florida-IFAS have been evaluating selected Florida pasture grasses and weedy grass species for fungal endophyte and mycotoxin presence. Working with specific ranches in Florida, we have sampled and identified in bahiagrass, bermudagrass, limpograss, brunswickgrass, broomsedge and smutgrass the presence of several ergo-alkaloids that are considered to be mildly toxic to livestock. We expanded our sampling over this current year, focusing primarily on bahiagrass, bermudagrass and limpograss at fourteen ranches around the state. Our goals for sampling over this 1-year period is to identify if mycotoxins in these sub-tropical forages 1. are present, 2. can be identified and quantified, and 3. if they occur over a wide-range of Florida environments. In an effort to better understand the presence of endophytes in sub-tropical forage grasses and if mycotoxin activity does exist we are collaborating with Oregon State University's Endophyte Service Laboratory and their veterinarian toxicologists, Dr. Morrie Craig and Dr. Jennifer Durringer. Over the past two years, on a very limited scale, we have been examining endophyte populations in our Florida pasture grasses and have been testing for the presence of mycotoxin activity in those forages. Working jointly with the Oregon State University School of Veterinarian Medicine Toxicology Department and Endophyte Service Laboratory, we have identified the presence of fungal endophytes and also various mycotoxins occurring in several of grasses. We have confirmed from this small sample set the identification of several fungal genera living endophytically in these grasses, such as *Fusarium* spp. and *Myriogenospora atromentosa*. Additionally, we have found seasonal mycotoxin activity in these forages that are of interest to us concerning animal health. To date, we have identified and quantified the presence of zearalenone, ZEAR-4-sulfate Q1, alternariol, ergine and 15-AcetylDON, as well as several other mycotoxins that are potentially deleterious to animal health. What the mycotoxin concentrations are in these forage plants and at what level these harmful to animal health are important questions that need to be addressed. As we begin our first phase of this research, we have also cultured leaf portions of each grass sample collected to determine the presence of dominant endophytes and have begun the DNA sequencing to identify those endophytes present in the grasses. Current finding are reported here.

ROOT SYSTEM ARCHITECTURE AND ROOT DEVELOPMENT OF BAHIAGRASS MUTANTS UNDER DRY DOWN AND WELL-WATER CONDITIONS

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Eight new lines of bahiagrass (*Paspalum notatum* Flugge) with improved turf quality, developed by the University of Florida, were compared with common 'Argentine' bahiagrass for their root system architecture. Three Wilmington mutants (Wil-7, Wil-10 and Wil-13), three Argentine mutants (Arg-1, Arg-16 and Arg-27) and two wild type lines (WT-6 and WT-16) composed the new grasses. Plants were grown under greenhouse conditions in 6.5 X 120 cm clear acrylic tubes. The tubes were weighed daily for 60 days, to determine the water used by evapotranspiration (ET). Two treatments were imposed; a well-watered (WW) treatment replacing a 100% of the ET and a dry down (DD) treatment where the tubes were allowed to dry no more than 7% of the daily ET. The rate of root depth development (RRDD) was recorded by measuring the single deepest root every day. Sixty days after treatment the roots were harvested from the tubes, washed, divided in 4 sections, digitally scanned and analyzed using WinRhizo, to calculate the root length density (RLD) (cm/dm³). The RRDD on the DD were higher (0.98 cm/day) compared to the WW treatment (0.8 cm/day). The new lines did not differ from Argentine for RRDD. However, Wil-10 developed roots faster than Arg-16, Arg-1, WT-16 and WT-16. The WW treatment accumulated higher RLD in the 0-30 and 30-60 cm soil profiles. No differences were found in the 60-90 and 90-120 cm profiles between irrigation treatments. In the 0-30 cm depth, WT-16, WT-6 and Wil-13 had lower RLD values than Argentine while Wil-10, Wil-13, Arg-27 and Wil-7 had higher RLD compared to Argentine. As a percentage of the RLD, Wil-10, Arg-27, Wil-13 and Wil-7 had a more even root distribution, accumulating the higher percentages in the 60-90 cm section compared to Argentine. The results for root dry weight (RDW) were similar to the RLD in the 0-30 and 30-60 cm layers. At 60-90 and 90-120 cm, the DD had higher RDW. Wil-10 and Arg-27 had higher RDW than Argentine. Conversely, WT-16 and WT-6 had less RDW. The high RLD and RDW values of Wil-10 and Arg-27 were correlated to their RRDD values. Argentine mutants and the common type had higher RLD values in the upper layers. However, those values did not differ from Wil-10 and Wil-7. Whereas the Wilmington mutants had higher RLD values on the 0-90 and 90-120cm section. The exception was Arg-27, which was different from Wil-13 and Wil-7. The fast increase in rooting depth and the accumulation of higher RLD values in lower soil layers have been described as drought responsive traits that may allow plants to extract water from greater depths. Under drought stress conditions, all the grasses tended to develop roots faster and prioritize the development of roots in the lower layers. Except for the RDW values, where WT-6 and WT-16 had lower values than Argentine, any of the new grasses showed lower values for RRDD and RLD suggesting that the new lines may have a similar or improved drought response compared to Argentine.

PRESENTER BIO: Pablo Agustin Boeri is a graduate research assistant and Master of Science student at the University of Florida, IFAS, West Florida Research and Education Center. His project is focused on the evaluation of new turf-type bahiagrass. The objective of this project is to reduce water use and maintenance without decreasing the aesthetic value of the turf.

EVALUATING CULTIVAR AND SPECIES TRAITS WITH THE CROPGRO PERENNIAL FORAGE MODEL FOR GRASSES AND LEGUMES

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The CSM CROPGRO Perennial Forage model, now released with DSSAT V4.7 software, is capable of predicting herbage harvests, herbage protein, and re-growth of perennial forage grasses and legumes over multiple seasons. The model includes a storage organ (rhizome, taproot, crown) with carbohydrate and N storage pools that provide the ability for re-growth despite zero leaf area index caused by harvest or freeze-loss of all leaf tissue. Nevertheless, depletion of reserves from poor management and repeated damage can cause poor recovery and loss of the forage stand. The model includes seasonal dormancy, freeze thresholds, and rules for partitioning to the storage organ. Rules for dormancy, freeze thresholds, partitioning, re-growth, productivity, mobilization of carbohydrate and nitrogen from storage pools to drive re-growth, and re-fill of storage pools are included in “species” and “cultivar” files. The value of these parameters to mimic genetic improvement of forage species will be illustrated. This perennial forage version, as recently released in the DSSAT, has species/cultivar files for *Marandu brachiaria*, Tifton-85 bermudagrass (*cynodon*), and alfalfa (*Medicago sativa*). Adaptations are planned for *Paspalum notatum*, *Panicum maximum*, napiergrass, and annual ryegrass (*Lolium multiflorum*). The model requires a read-in file called “MOW” that specifies the harvest dates, the residual live stubble and associated percent leaf. The model will start from seed or vegetative cutting. It uses the DSSAT-CENTURY soil C module for soil C processes and including recycling of senesced tissue. Automated harvest routines, prediction of digestibility, and linkages to animal grazing are planned. Examples will be presented to illustrate how genetic differences (species/cultivar) affect simulated production and re-growth dynamics of two brachiaria species and eleven alfalfa cultivars.

PRESENTER BIO: Dr. Boote is a Professor Emeritus with extensive experience measuring and modeling crop and forage responses to climate, soils, genetic, and management factors. He is a co-developer of the Decision Support System for Agrotechnology Transfer (DSSAT) crop model software.

QTL MAPPING AND PROTEOMIC ANALYSIS FOR THE DEVELOPMENT OF EFFICIENT SELECTION OF FREEZE TOLERANCE IN ZOYSIAGRASS

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Zoysiagrass (*Zoysia* spp.) has increased in popularity since its introduction in the United States during the late 1880's, largely due to its reputation as a low input turfgrass. Derived from the Pacific Rim region, this primarily vegetative propagated turf was one of the first grasses well suited for the southeastern U.S., on account of its wide general tolerance to a variety of abiotic stresses. Acreage of *Z. japonica* dominates the market largely in part to the cultivar 'Meyer', which has the best freeze tolerance of any zoysia cultivar to date. However, since Meyer's introduction during the 1950s, little progress has been made in advancing the winter hardiness of zoysia, a factor that prevents further spread of the species north of the transition zone. The development of increased tolerance to extreme temperatures has become more relevant over the years, and breeding efforts stand to benefit considerably from a greater knowledge base behind the physiology of winter hardiness and freeze tolerance. The goal of our research is to investigate the physiologic and metabolic components of freeze tolerance in zoysiagrass to facilitate selection of freeze tolerant cultivars.

Prior research in our lab generated a mapping population of Meyer, the most freeze tolerant zoysiagrass cultivar x 'Victoria', a freeze susceptible cultivar with desirable turf quality. The 175 pseudo-F₂ progeny were evaluated under field conditions to determine winter survival. A SNP-based high density linkage map of *Z. japonica* was generated to identify regions of the genome associated with winter survival. Current research is focused on lab-based freezing evaluations including acclimation tests to assess true freeze response. A randomized complete block design of the mapping population was implemented for cold acclimated and non-acclimated treatments, each under two sub-zero temperature treatments (-8, -11) based off the LT₅₀ of the parent cultivars. Data was collected on regrowth and surviving green tissue for six weeks following freezing on a visual scale of 0-5. QTL analysis was performed against the high density linkage map to validate previously identified regions of interest for freeze tolerance.

Differentially regulated proteins expressed in cold vs non acclimated populations were evaluated via proteomic analysis in the mapping population. Following cold acclimation, crown tissues were harvested and proteins were extracted for peptide analysis. Preliminary results identified several proteins of interest associated with freezing tolerance. Segregation patterns of these proteins, as well as the correlation of their presence with freezing survival are being investigated in conjunction with the high density linkage map to identify candidate genes controlling freeze tolerance. The validated markers showing the strongest associations with these regions will be implemented in a marker assisted selection program to transfer freeze tolerance into germplasm of interest with the ultimate goal of creating elite zoysiagrass cultivars.

PRESENTER BIO: Jessica Brown is a Masters student in plant breeding under Dr. Susana Milla-Lewis at North Carolina State University. She graduated from the University of Georgia in 2017 with a BS in Genetics and a BS in Plant Biology.

IDENTIFYING ST AUGUSTINEGRASS OFF-TYPES IN SOD PRODUCTION FIELDS

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Cultivars of St. Augustinegrass are clonally propagated genotypes. 'Floritam' St. Augustinegrass represents approximately 80% of the St. Augustinegrass (*Stenotaphrum secundatum* Walt. Kuntze) sold annually in Florida, and 'Bitterblue' was one of the first cultivars produced as sod in Florida. The genetic purity of these two widely-grown cultivars is unknown. The presence of other genotypes within production fields of these two cultivars is possible considering the extent of their acreage and how long they have been commercially available. The objective of this study was to ascertain the level of genetic purity among sampled production fields of Floritam and Bitterblue.

Sod growers in Florida were asked to submit samples from fields of both cultivars. They were also encouraged to submit samples from known questionable or problematic areas within fields. Samples were grown in the greenhouse, and morphologically different grasses were separated. Flow cytometry (FCM) was used to determine 2C DNA content. For samples where FCM results were unclear, chromosome staining and counting was attempted. Samples also were screened using a set of 22 primer pairs that amplified simple sequence repeats within the St. Augustinegrass genome. This set of primers amplified several unique alleles between cultivars in previous research in St. Augustinegrass. Amplification patterns for each sample were compared to amplification patterns of verified samples of commercial cultivars to determine if the samples and suspected off types had patterns similar to the verified cultivars. Based on visual observations of mature stolons, 17 of 46 samples from Floritam fields and two of eight samples from Bitterblue fields were suspected to be off-types (generally differing in leaf blade or stolon morphology) or to have more than one genotype within the sample. Results of FCM supported these observations, with several genotypes having 2C DNA equivalent to diploid cultivars.

Assessments of phenotype and morphological measurements are the least expensive method of identifying genotypes and off-types; however, the use of flow cytometry can provide a quick determination that phenotypically similar genotypes differ in ploidy level. When a more informative answer is needed, molecular markers can be utilized to identify the source of an off-type as well as distinguish between genotypes that share the same ploidy level. These methods can be utilized to verify suspected off-types and assist producer efforts to maintain clean sod production fields.

PRESENTER BIO: Jamie Buhlman holds a B.S. in Plant Science with a focus in Plant Health from the University of Florida. She is currently pursuing a M.S. in Agronomy, while working as an Agricultural Assistant in the UF turfgrass breeding program under Dr. Kevin Kenworthy.

MEASURING MOWING FREQUENCY IN GENETICALLY MODIFIED ST. AUGUSTINEGRASS

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Mowing is one of the key inputs required to maintain a healthy and beautiful lawn. St. Augustinegrass (*Stenotaphrum secundatum* Walt. Kuntze) is the most popular lawn grass in Florida. The recommended mowing height of St. Augustinegrass is 3-4 inches, removing no more than one-third of the leaf tissue per mowing. Due to vigorous canopy growth of current St. Augustinegrass cultivars, this equates to a mowing frequency of once a week. For homeowners, this represents a repetitive expense that continues for life of the lawn, costs personal time, labor, fuel or a lawn maintenance service.

'Floritam' St. Augustinegrass represents 80% of the St. Augustinegrass market in Florida. The Scotts Company recently released 'ProVista' St. Augustinegrass, a derivative of Floritam which has been genetically modified to resist glyphosate damage and to have reduced expression of gibberellic acid (GA3). The goal of modifying GA3 expression was to produce a St. Augustinegrass that required less mowing than standard cultivars but to also take advantage of other potential benefits that come with the regulation of GA3, such as enhanced shade tolerance. The objective of this study was to compare the mowing requirements among genetically modified genotypes and commercial cultivars of St. Augustinegrass.

Nine genotypes of genetically modified St. Augustinegrass and three commercial cultivars, 'Floritam', 'Palmetto', and 'Seville', were planted in 4' by 7' plots in 2017 with fifteen 4" by 4" plugs in a randomized complete block design with three replications at the Plant Science Research and Education Center, Citra, FL. Beginning in 2018, after establishment, plots were visually rated for color, density and turf quality; and mowed, as needed, at 3.0" based on an individual plot's measured plant height. Three plant height samples were taken per plot and when the average height of a plot reached 4.5 inches, the entire plot was mown to 3.0 inches and the mowing event recorded. On average, seven of the eight genotypes were mown at least half as frequent as 'Floritam', with one genotype mown two-thirds as often. The difference in mowing frequency between the modified genotypes and Palmetto was less evident, and all but two of the genotypes were mown at least twice as often as Seville.

Overall, the genetically modified St. Augustinegrass required less mowing than standard cultivars. For end-users, this means a direct decrease in the maintenance cost of their St. Augustinegrass lawns. In the future, other potential areas of interest are to look at how GM St. Augustinegrass compares to standard cultivars in other aspects, such as turf quality or pesticide usage.

PRESENTER BIO: Jamie Buhlman holds a B.S. in Plant Science with a focus in Plant Health from the University of Florida. She is currently studying for a M.S. in Agronomy, while working as an Agricultural Assistant in the UF turfgrass breeding program under Dr. Kevin Kenworthy.

CHARACTERIZATION OF GENETIC OFF-TYPES IN 'CELEBRATION' BERMUDAGRASS BY INFERRED PLOIDY LEVEL THROUGH FLOW CYTOMETRY

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In 2017, 511 hectares of 'Celebration' bermudagrass (*Cynodon dactylon*) turfgrass sod production fields in Florida were inspected three times to visually detect off-type bermudagrasses, and other eligibility factors, for certification by the Southern Seed Certification Association. The purpose of this study was to genetically characterize off-types. 'Celebration' bermudagrass is a vegetatively propagated cultivar with bluish-green leaf color and medium-coarse leaf width compared with 'Tifway,' and is grown to plant golf course fairways and sports turf fields. In some inspected 'Celebration' fields, spots of an off-type bermudagrass were observed, which had taller, more yellow-green leaves compared with adjacent areas that appeared to be homogeneous 'Celebration' bermudagrass. The off-type spots were most noticeable when viewed in the direction of a low sun, in actively growing fields. The off-type bermudagrasses are referred to here as LYG, for "lanky yellow-green." LYG spots were from about 25 cm width to 3 m width. In fields regrowing from ribbons in previously cut sod, LYG had stolons that were narrower and more reddish in color than adjacent 'Celebration,' and seemed to spread faster into bare areas than 'Celebration.'

Paired samples of 'Celebration' and LYG bermudagrasses were collected from 11 locations on 10 turfgrass sod farms and grown in 3-L clay pots. Each pair consisted of a 'Celebration' plant and an LYG plant that were sampled within 3 m of each other in the same field. Once samples rooted and established, each was replanted from a single stolon. Stolons and attached leaves were harvested and sent to the University of Florida, Department of Agronomy, to be grown out again and evaluated through flow cytometry. Samples for flow cytometry were processed using a CyStain absolute P kit with extraction buffer and staining buffer and stained with propidium iodide. Samples were analyzed on an Acuri C6 flow cytometer at the University of Florida, ICBR flow cytometry core laboratory.

Among 11 'Celebration' samples, all were homogeneous phenotypically and had 3.64 to 4.10 pg DNA/2C nucleus (average 3.79) and were inferred to be tetraploids ($2n = 4x = 36$). Among 11 LYG samples, seven were similar phenotypically to other LYG samples, but different from 'Celebration,' and had 2.83 to 3.17 pg DNA/2C (average 3.01), and were inferred to be triploids ($2n = 3x = 27$). Another four LYG samples were not consistent. One sample resembled 'Common' bermudagrass and was inferred to have $2n = 4x = 36$, based on DNA content; one resembled LYG but was inferred to have $2n = 4x = 36$; one resembled a 4x phenotype but had nuclear DNA content between 3x and 4x; and one resembled 'African bermudagrass' (*Cynodon transvaalensis*) and had nuclear DNA content between 2x and 3x.

In conclusion, while there was variation among LYG off-types occurring in 'Celebration' bermudagrass fields, the majority of LYG samples were genetically similar to one another and different from 'Celebration' based on inferred ploidy level. LYG spots in 'Celebration' bermudagrass cannot be explained as effects of environmental variation.

PRESENTER BIO: Dr. Busey is University of Florida Professor Emeritus of Environmental Horticulture with more than 40 years of experience researching turfgrass. He has extensive experience with plant identification.

NUTRITIVE VALUE OF NEW *PASPALUM* ACCESSIONS FOR DIVERSIFICATION IN TROPICAL LIVESTOCK

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There is a growing demand for diversification of tropical forages adapted to climate change and that may contribute to the reduction of large monoculture areas with palisade grass in tropical livestock. The objective of this project is selecting among of 26 accessions of six groups of genus *Paspalum*, those have desirable characteristics (high nutritional value, high productive and adaptive capacity) as alternatives to animal production systems. The treatments were corresponded to cut frequencies represented by intervals of 28 and 70 days, according to the rainy (spring and summer) and winter seasons of 2016-2017. The treatments were allocated the experimental units (2.5 m² plots) according to a complete randomized blocks design, with 4 replicates. Each plot is represented by an access and is composed of 5 plants. The cuts had been made a height of 10 cm from the soil and the border plants was discarded and considered the middle (1.5 m²) as a form of standardization and each cycle was being evaluated the nutritional value. The determinations of the materials were carried out by means of analysis of the dried samples and milled of each cut. Subsequently taken to the laboratory for chemical analyzes: Crude protein (CP) was analyzed by the micro method Kjeldahl, neutral detergent fiber (NDF) by Van Soest and dry matter *in vivo* digestibility (IVDM) incubated in non-lactating fistulated cow feeding by hay. Statistical analysis was performed using the PROC MIXED of the Statistical Analysis System (SAS). The effects of cutting frequency, season of the year and the interaction between them will be considered fixed effects and the random effects are the blocks. Analysis of variance was performed based on the following causes of variation: cut frequency, season of the year and interaction between them. The means of the treatments were estimated using the "LSMEANS" and the comparison between them, when necessary, by means of the "Student" test and 5% probability of error. The results are shown in relation to dry matter and its respective Standard Error (SE). The CP in the seasons had an average of (14.20, 15.18, 14.58% with 0.63% SE) in the spring, summer and winter respectively, with emphasis on group accesses of *P. malacophyllum* and *P. regnelli* which had the highest ($P<0,05$) average between accesses. In the NDF average (68.62, 70.63, 71.95% with 1.58% SE) in the spring, summer and winter respectively with the lowest ($P<0,05$) content with the group of *P. atratum*. In the IVDM average (75.49, 78.30, 77.00% with 3.04% SE) in the spring, summer and winter respectively with the lowest ($P<0,05$) content with the group of *P. regnelli*. The nutritive value results show that *Paspalum* accessions have potential and alternative to be used in the livestock system as forage.

PRESENTER BIO: MSc. Vinicius Carreteiro Gomes is a PhD student in University of São Paulo state in Botucatu. He has Animal Science degree and Master in Sustainable Animal Production. He works with genetic variability of *Paspalum*, beef cattle production on pasture, animal supplementation and silage in the crop-livestock system.

IMPACT OF SELECTION FOR FLOWERING TIME ON BIOMASS YIELD OF SWITCHGRASS

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Switchgrass (*Panicum virgatum* L.) is undergoing development as a perennial biomass crop to support a bioenergy industry. Biomass yield is the principal limitation to sustainable and profitable production of switchgrass as a biomass crop. As such, breeding for increased biomass yield is one of the principal goals for most of the 12 breeding programs in North America. One strategy for increasing biomass yield is to select for delayed flowering, which increases the effective duration of the growing season for the biomass crop. Peak biomass generally occurs at the time of anthesis, resulting a decline in biomass yield during the last 6 to 8 weeks of the growing season at northern latitudes. The purpose of this study was to evaluate the effectiveness of recurrent phenotypic selection for later flowering time in two switchgrass populations and the impact of selection for flowering time on biomass yield.

Three cycles of selection for later flowering and two cycles of selection for earlier flowering were conducted within the WS4U and WS8U upland-ecotype germplasm pools. These 10 populations plus the two original populations were grown in replicated and randomized field studies at four locations, ranging from 42°N latitude (northern Illinois) to 46°N latitude (northern Wisconsin). Biomass yield was determined on all plots for 2 years. All 12 populations were also evaluated for anthesis date at the southern Wisconsin location (43°N).

Realized heritability for anthesis date ranged from 0.68 to 0.80. Selection responses for anthesis date were linear, accounting for 88 or 90% of the sums of squares across all six generations. On average, each generation of selection changed anthesis date by 5 days, regardless of ploidy level or direction of selection. On average, selection for later anthesis increased biomass yield and selection for earlier flowering decreased biomass yield. However, the responses in biomass yield were strongly associated with latitude of the evaluation location. At the northernmost location (46°N with the shortest growing season), each 1-day delay in anthesis date resulted in an increase of only 0.3 Mg/ha for biomass yield. However, at the southernmost location (42°N with the longest growing season), each 1-day delay in anthesis date resulted in an increase of 0.12 Mg/ha for biomass yield. The two intermediate locations had intermediate responses.

This study provides significant proof-of-concept that delays in anthesis date for upland switchgrass can be used as a mechanism to increase biomass yield, largely by taking advantage of a longer portion of the growing season. This mechanism was equally effective in two very different populations, one tetraploid and one octoploid, indicating that this mechanism is somewhat generalizable, at least within upland germplasm. The impact of later flowering on increased biomass yield diminished as the length of the growing season was reduced at more northern latitudes, creating a window of latitude at which this approach will be most effective.

PRESENTER BIO: Dr. Casler is Research Geneticist with USDA-ARS, conducting research on breeding, quantitative genetics, and genomics of forage and energy grasses. He has published extensively on approaches and methodology for breeding perennial grasses and on experimental designs for agronomic research.

DETERMINING SITES IDEAL FOR A PILOT EXPERIMENT IN COLOMBIA TO TRIAL NEW FORAGES IN EAST AFRICA

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At the International Center of Tropical Agriculture (CIAT) located in Colombia, a new breeding program is being established with the aim of sending robust forage breeds to the East African region. These forages will be selected for their tolerance to drought, flooding and soil nutrient availability, which are ever important plant traits needed to adapt to the stresses of future agriculture under climate change. Quality needs to be ensured before these forages become available to East Africa, however, which means a trial of the first selection cycles needs to be performed in a pilot experiment in Colombia. The question here presented is: How to effectively and remotely decide an experimental site in Colombia that is representative of the climate and soil conditions existing in East Africa? Using a target population of environments analysis, we characterized the different environmental zones of East Africa and calculated how similar they were to conditions in Colombia. Using R to perform a cluster analysis of East Africa and a multivariate distance time warp to calculate similarity for climate and soil data at a 10 km by 10 km scale for the period of 1980 – 2010, we produced a map of Colombia that indicates highly suitable areas for our forage pilot experiment. This method of determining experimental sites is highly valuable as it can be performed remotely with free and powerful statistical software, and is able to be easily replicated for other breeding programs for other regions. Input climate data need not only be those that are for current climate conditions, but can easily be changed for future climate conditions under climate change forecasts.

PRESENTER BIO: MSc Valheria Castiblanco is Scientist at the tropical forages program of CIAT. She leads the *Urochloa* Breeding Program.

IDENTIFICATION OF BRACHIARIA HUMIDICOLA HYBRIDS WITH WATERLOGGING TOLERANCE AND BIOLOGICAL NITRIFICATION INHIBITION CAPABILITY

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Soil waterlogging is a major limitation to pasture productivity due to the slow diffusion of gases in water that reduces plant growth, as O₂ availability in the root zone decreases. On the other hand, Biological nitrification inhibition (BNI) is a process where roots exudate organic substances that inhibit the activity of soil nitrifiers. *Brachiaria humidicola* (Bh) is an important forage grass in humid lowland tropics that has been identified and characterized for having good waterlogging tolerance and high soil nitrification inhibitory potential.

The objective of this study was to evaluate the variation in waterlogging tolerance and BNI of 27 Bh hybrids that were developed by the *Brachiaria* breeding program of CIAT. Two controls of Bh (cvv. Tully and LLanero) were included for comparison purposes. Waterlogging tolerance was tested for traits such as shoot growth, leaf chlorophyll content and biomass production in plants growing in drained or waterlogged soil for 23 days under greenhouse conditions. Soil used was a top Oxisol from Santander de Quilichao, which was mixed with river sand in a proportion of 2:1 (w/w) and fertilized to avoid nutrient deficiencies. Plants were planted into PVC pipes of 80 cm high and 7.5 cm diameter in a factorial combination of two drainage conditions: drained (field capacity) and waterlogged. The trial was established in a four-replicate randomized complete block. We also evaluated Biological nitrification inhibition (BNI) capability from these 27 Bh hybrids, through Nitrification potential from top soil samples taken in plots of 1m² using modified shaken slurry procedure with 1 g soil (air-dried) was mixed with 10mL assay solution (30mM KH₂PO₄; 0.7mM K₂HPO₄ and 0.75 mM ammonium sulfate, pH = 7.2) in a 50 mL flask, which was then covered and incubated, one by one to sampling at 0h, 24h, until 96h intervals at 120 rpm at 25 °C in dark.

Overall, all hybrids showed similar biomass production under drained conditions when compared to the controls. From 27 Bh hybrids, we identified two hybrids as promising based on similar biomass to that of commercial cultivars under drained and waterlogged soil conditions. These two genotypes showed a comparatively larger root system (based on dry mass) than most of the other hybrids and the two controls tested. It is worth mentioning that one of promising hybrid showed both good performance under waterlogging, and also highest BNI similar BNI capability as that shown by a commercial cultivar with high BNI (cv. Tully).

PRESENTER BIO: MSc Valheria Castiblanco is Scientist at the tropical forages program of CIAT. She leads the *Urochloa* Breeding Program.

MODELS FOR ESTIMATING NUTRITION QUALITY OF *B. HUMIDICOLA* USING NEAR INFRARED REFLECTANCE SPECTROSCOPY

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Breeding forage crops requires a genetic evolution that will optimize the agronomic and compositional characteristics of the new hybrids. As a dynamic process in continuous movement, it is necessary to evaluate with precision and speed the parameters that determine the nutritional quality, in particular the contents of digestibility and fibers. Near infrared reflectance spectroscopy (NIRS) offers a low cost and an efficient alternative to obtain these parameters. The main research objective of this study was to develop a chemometric model based on measurements taken in the near infrared which can predict the contents of neutral detergent fiber (NDF), acid detergent fiber (ADF), in vitro dry matter digestibility (IVDMD) and crude protein (CP) for a hybrid population of *Brachiaria humidicola* of the CIAT breeding program.

The models to determine the parameters were generated using spectral information attained at a range between 400 to 2500nm as well as the physical-chemical composition (reference method) of each sample. These models were then used to perform a multiple regression namely a modified partial least square regression, and analyse the major components and transformations such as standard normal variate and detrend (SNVD) and transformations on the first and second derivatives, where mathematical treatments were 1,4,4,1 for ADF and IVDMD models, 2,4,4,1 for NDF and CP models, the first number is the derivative, the second number the gap, and the third and fourth numbers are the smooth.

External validation was performed with a group of samples other than the calibration group obtaining good correlation coefficients such as R^2 , 1-VR with values between 0.90 and 0.95, a standard error of cross-validation of 1.18%, 0.74%, 1.59% and 0.53 %, respectively and a predictive efficiency coefficient or RPD > 3.0. Obtaining a good correlation and prediction is possible when the model equations are developed on samples of a relatively homogeneous nature or corresponding to the same type of product, The calibrations obtained in this study present an adequate adjustment and provide a better predictive tendency of the model for this type of crop that are at the basis of animal feed in many of the livestock systems worldwide.

PRESENTER BIO: MSc Valheria Castiblanco is Scientist at the tropical forages program of CIAT. She leads the *Urochloa* Breeding Program.

PHENOTYPING *UROCHLOA HUMIDICOLA* GENOTYPES AGAINST SPITTLEBUG *AENEOLAMIA VARIA* (HEMIPTERA: CERCOPIDAE): AN EXPERIMENTAL COMPARISON BETWEEN DIGITAL IMAGE AND VISUAL EVALUATION

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Productivity of *Urochloa* pastures, mainly *U. decumbens* and *U. humidicola* are limited by susceptibility to American spittlebugs complex (Hemiptera: Cercopidae). The *Urochloa* breeding program at International Center for Tropical Agriculture (CIAT), aims to improve resistance to such biotic stress. To quantify plant host resistance, a high-throughput phenotyping method was standardized by the entomology program of CIAT. Current assessments of genotype for spittlebug resistance are based on visual damage percentage. This method has been successful, as shown by the high and fast genetic gain achieved by the *Urochloa* breeding program. Image analysis, has been used to maximize the accuracy of data collection from quantitative traits like yield and resistance to biotic or abiotic stress.

The main objective of this study was to optimize our phenotyping process in *U. humidicola* to detect spittlebug resistance by comparing two evaluation methods (visual and digital image evaluation) under greenhouse conditions. Damage percentage was calculated through visual and digital image evaluation at 7, 14, 21, 28 and 35 days after infestation. These data were used to calculate respective area under the disease progress curve (AUDPC). In order to identify plant antibiosis, the number of live nymphs (in five stage) and/or adults in each pot was recorded 35 days after infestation and insect survivorship percentage (ISP) was estimated.

Lin's concordance correlation coefficient showed that digital image evaluation reproduces the visual evaluation with accuracy of 0.81 (95% CI 0.79 – 0.82). Visual damage percentage was always greater than damage calculated with digital image evaluation, suggesting an overestimation of the damage when calculated visually. ANOVA calculated from AUDPC variable revealed significant differences among genotypes ($P < 0.001$), while calculated from ISP variable revealed no statistically significant differences between genotypes ($P = 0.08$). These results suggest that the resistance mechanism against spittlebugs in *U. humidicola* hybrids is tolerance.

Digital image analysis provided the advantage to measure if progression of the damage was the outcome of senescence induced before the imposition of the stress, or whether resistance was the outcome of production of new leaves during the stress. This distinction is important because it can be related to the ability of a plant to resist spittlebug *per se*, or whether the plants use an escape mechanism to compensate increased leaf senescence. In the present study, genotypes showing greater new leaf production at early stages of the stress, where the ones that showed less damage.

PRESENTER BIO: MSc Valheria Castiblanco is Scientist at the tropical forages program of CIAT. She leads the *Urochloa* Breeding Program.

DEVELOPMENT OF NOVEL POLYMORPHIC EST-SSR MARKERS IN GUINEAGRASS (*PANICUM MAXIMUM*) FOR VARIABILITY GENETIC STUDIES

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The availability of genomic tools, such as molecular markers, to support *Panicum maximum* breeding programs is rather limited. Recently, RNA-seq technology has been applied for the identification of expressed sequence tag-derived-simple sequence repeats (EST-SSRs) loci. In this study, we report on the development of 21 novel polymorphic EST-SSR markers, obtained from a *de novo* assembly of the transcriptome from *P. maximum* leaves.

A total of 12,605 EST-SSRs loci were detected in 10,203 transcripts. EST-SSRs with three or more nucleotides motives were selected and their sequences were blasted against *Setaria italica* genome aiming to obtain a list of markers widely distributed throughout the genome. From this list, 144 EST-SSRs were chosen for validation in 15 genotypes from germplasm collection of *P. maximum* maintained at Embrapa.

Of the 144 EST-SSRs, 76 were successfully amplified, and from those, 21 polymorphic EST-SSRs were used to evaluate the genetic diversity among 190 genotypes of the *P. maximum* germplasm collection. The number of alleles observed for each locus ranged from 2 to 19 (average 9.8), with EST-SSR 1089-1090 being the lowest value and EST-SSR 1187-1188 the highest. The polymorphism information content values (PIC) ranged from 0.02 to 0.91 (average 0.67), and the discriminatory power of these loci ranged from 0.4 to 0.98 (average 0.76). Two subpopulations among set accessions of guineagrass were identified by structure analysis and were not related to the country of origin of them. This novel set of EST-SSR evidence good diversity properties and may be recommended for further studies in *P. maximum*.

PRESENTER BIO: Dr. Chiari is researcher of the Brazilian Agricultural Research Corporation (Embrapa) since 2006 with molecular biology of forages. Currently, she leads a big project about functional and structural genomic of tropical forage species at Embrapa.

BOOSTING GENETIC GAIN IN TALL FESCUE VIA SPEED BREEDING AND GENOMIC SELECTION

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Breeding schemes that utilize modern breeding methods like genomic selection (GS) and speed breeding (SB) have the potential to accelerate genetic gain for different crops. We investigated through stochastic computer simulation the advantages and disadvantages of adopting both GS and SB into commercial breeding programs for allogamous crops. In addition, we studied the effect of omitting one or two selection stages from the conventional phenotypic scheme on GS accuracy, genetic gain, and inbreeding. As an example, we simulated GS and SB for five traits with different genetic architectures and heritabilities for a tall fescue breeding program. The phenotypic selection scheme required eleven years, while the proposed GS/SB schemes required four to nine years per cycle. Our results showed that running more SB rounds resulted in higher genetic gain per cycle when compared to phenotypic or GS only schemes and this increase was more pronounced per year when cycle time was shortened by omitting cycle stages. While GS accuracy declined with additional SB rounds, the decline was less in round three than in round two, indicating that it may be stabilizing. However, more SB rounds resulted in higher inbreeding rate, which could limit long-term genetic gain. The inbreeding rate was reduced by approximately 30% when generating the initial population for each cycle through random crosses instead of generating half-sib families. Our study demonstrated a large potential for additional genetic from combining GS and SB. Nevertheless, methods to mitigate inbreeding should be considered for optimal utilization of these highly accelerated breeding programs.

PRESENTER BIO: Dr Noel Cogan is Research Leader, Molecular Genetics with Agriculture Victoria Research, and Senior Lecturer with the School of Applied Systems Biology, La Trobe University.

CULTIVAR SUB-SELECTION FOR DELAYED INFLORESCENCE AND IMPROVED WATER-SOLUBLE CARBOHYDRATE CONTENT AND YIELD IN PERENNIAL RYEGRASS

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Increasing herbage yield production and improving herbage quality are the two predominant objectives for pasture plant breeders. High levels of within cultivar variability for herbage yield and quality in perennial ryegrass have been documented. The purpose of this study is to investigate cultivar sub-selection as a method to improve certain traits efficiently while maintaining overall genetic identity. Four subsets of selections were made from cultivar 'Trojan' for delayed heading date, improved water-soluble carbohydrate (WSC) content, yield, and total WSC yield. Four synthetic populations were developed from the four subsets of selections and tested along with the original cultivar in two field trials. A simulated sward trial was conducted to assess yield and WSC of the four synthetic populations and compared to the base cultivar 'Trojan'. A spaced plant trial was used to record the heading date. Original plants and synthetic populations were genotyped using 384 single nucleotide polymorphism (SNP) markers. Yield was estimated by mowing the plots and WSC was estimated by near infrared reflectance spectroscopy (NIRS). Results showed that heading date was delayed by 5 days, high WSC content and high WSC yield selections resulted in 10.7% and 20.2% increase compare to the original cultivar respectively. SNP marker-assisted selection enabled maintenance of the genetic diversity, hence, the genetic identity of a cultivar as demonstrated in a genetic marker-based cultivar relationship dendrogram. The derived populations from the subsets of selections have the potential to be developed further and released as an advanced cultivar and commercialized to realize the genetic gain.

PRESENTER BIO: Dr Noel Cogan is Research Leader, Molecular Genetics with Agriculture Victoria Research, and Senior Lecturer with the School of Applied Systems Biology, La Trobe University.

DEVELOPING CANOPY BASED REFLECTANCE TECHNIQUES FOR THE NON-DESTRUCTIVE ASSESSMENT OF NUTRITIVE CHARACTERISTICS IN PERENNIAL RYEGRASS BREEDING PROGRAMS

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Perennial ryegrass (*Lolium perenne* L.) is the most important temperate perennial grass species sown for dairy production. Although perennial ryegrass is recognized as having a relatively high level of nutritive value, there are times of the year when the quality of perennial ryegrass is inadequate to meet the requirements of high-producing dairy cows and at these times farmers resort to the use of more expensive supplements to maintain productivity. Important characteristics include dry matter digestibility (DMD), water-soluble carbohydrates (WSC), crude protein (CP) and cell wall characteristics such as neutral detergent fibre (NDF) and lignin. The concentrations of these traits are known to be under both genetic and environmental control and the costs associated with screening these traits across seasons and environments have limited the number of active breeding programs that target these traits, despite their economic importance. We describe the results of using hyperspectral analysis to facilitate the real time assessment of perennial ryegrass genotypes for forage quality parameters. Initial results show that when incident radiation is excluded high correlations ($r^2 > 0.8$) were achieved between field and laboratory assessments of moisture content, WSC, DMD, CP, NDF and ADF. Research is ongoing to assess the seasonal specificity of these calibrations and to develop robust broad-based calibrations based on an expanded data set.

PRESENTER BIO: Dr Noel Cogan is Research Leader, Molecular Genetics with Agriculture Victoria Research, and Senior Lecturer with the School of Applied Systems Biology, La Trobe University.

DEVELOPMENT OF F₁ HYBRID BREEDING TECHNOLOGY IN RYEGRASSES

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Conventional ryegrass breeding has been limited in its ability to deliver genetic gain, partly due to the inability to effectively exploit and deliver heterosis through the formation of F₁ hybrids. Controlled crossing of geographically distant ryegrass lines demonstrated heterosis can result in c.25% increases in dry matter production. To date there have been no effective and commercially suitable methods of obtaining high proportions of F₁ hybrid seed. Advances in the understanding of the genes regulating the self-incompatibility loci in grasses (*S* and *Z*) have enabled the development of initial diagnostic markers and a methodology for the efficient generation of high proportions of F₁ hybrid ryegrass seed on a commercial scale. Initial parental populations have been developed and experimental F₁ varieties are being evaluated, that have already shown superior performance compared to reference commercial cultivars. Through the application of plant phenomics detailed trials are being undertaken to evaluate a large range of F₁ hybrid test crosses along with the parents and current market leading varieties, all of which have been comprehensively genotyped. To complement these approaches genomic selection is being developed to deliver gains directly into the parental pool development methodology, to deliver maximal advances in productivity. This technology has the ability to revolutionize ryegrass breeding, introducing a new paradigm of breeding methodologies with rapid genetic gains, supporting increased agricultural production.

PRESENTER BIO: Dr Noel Cogan is Research Leader, Molecular Genetics with Agriculture Victoria Research, and Senior Lecturer with the School of Applied Systems Biology, La Trobe University.

IMPLEMENTATION OF GENOMIC SELECTION IN COMMERCIAL BREEDING OF PERENNIAL RYEGRASS

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Genomic selection, which uses genome-wide sequence polymorphism data and quantitative genetics techniques to predict plant performance, has large potential for the improvement in pasture plants.

Global diversity of the important forage species perennial ryegrass is high and so would require a large reference population in order to achieve moderate accuracies of genomic selection. However, the diversity of germplasm within individual breeding programs is likely to be lower. Consequently, historical phenotypic records for seasonal biomass yield and heading date over an 18-year period within a commercial perennial ryegrass breeding program have been analysed and breeding lines have been characterised with a high-density transcriptome-based genotyping-by-sequencing assay. To represent the true implementation of genomic selection a forward prediction approach was assessed whereby the performance of breeding lines in subsequent years were predicted using only those breeding lines in previous years. Moderate to high prediction accuracies were achieved for biomass yield and heading date, respectively, over a period of nearly 2 decades. These results are supported by simulation studies, demonstrating the ability to predict sward based phenotypic performance early in the process of individual plant selection, so shortening the breeding cycle and dramatically increasing the rate of genetic gain. Supported by computation crossing design scripts that mitigate inbreeding depression, genomic selection has now been implemented directly into the breeding program for multiple years and the first genomically selected synthetic varieties are currently being evaluated.

PRESENTER BIO: Dr Noel Cogan is Research Leader, Molecular Genetics with Agriculture Victoria Research, and Senior Lecturer with the School of Applied Systems Biology, La Trobe University.

POLYETHYLENE GLYCOL EFFECTS ON ZOYSIAGRASS ROOT GROWTH AND LEAF HYDRATION STATUS IN A HYDROPONIC SYSTEM

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As the demand for Florida's limited water supply continues to increase, developing turfgrasses that can maintain their aesthetic value and require less supplemental irrigation during periods of drought is important to sustain urban landscapes. Field evaluations for drought responses can be difficult due to variable soil conditions, and patterns of frequent rainfall in Florida. In addition, it is difficult to differentiate between drought tolerance and drought avoidance under field conditions. These challenges can limit and slow breeding programs focused on improving drought responses. This study aimed to evaluate the use of polyethylene glycol (PEG) as a screening method to identify differences in drought tolerance between zoysiagrass genotypes in a hydroponic system.

To conduct this experiment, 13 zoysiagrass (*Zoysia spp.*) genotypes, including entries previously germinated under various PEG concentrations, were planted into 150 cc conetainers filled with calcined clay and allowed time to mature. The conetainers were then grown in hydroponic culture with either no PEG as a control, an increasing concentration of PEG over the course of four weeks to simulate increasing dry down and chronic stress (cPEG), and under 15% PEG to simulate acute drought stress (aPEG). This experiment was arranged as a randomized complete block design in a split-plot arrangement. The hydroponic cultures (no PEG, cPEG and aPEG) were split into the 13 genotypes comprised of four commercial cultivars ('Zeon', 'Icon', 'Palisades', and 'Empire') and nine experimental lines. The PEG solutions were changed weekly. Half strength Hoagland's solution (0.82 g/L) was used to provide nutrients, which was replenished as the solutions were replaced. The grasses were trimmed weekly to a height of 4 cm. The fresh leaf tissue was utilized to determine leaf hydration through measurement of relative water content as follows: the fresh leaf tissue was weighed and then soaked in deionized water for 24 hours, then blotted dry with a paper towel and weighed to establish the turgid weight. Lastly, leaves were dried at 75°C for 72 hours and weighed again to obtain a dry weight. The formula: $(\text{Fresh weight} - \text{Dry weight}) / (\text{Turgid weight} - \text{Dry weight}) \times 100\%$ was used to determine the relative water content. Other response variables evaluated in this experiment were differences among entries for turf quality, color, leaf tissue browning, and root morphological characteristics. Results from this study will indicate the potential for using PEG as an efficient screening method to select zoysiagrass genotypes that are better able to tolerate drought conditions.

PRESENTER BIO: Katherine Cox is a master's student at the University of Florida studying turfgrass science in Dr. Kevin Kenworthy's lab. Her research focuses mainly on drought tolerance in zoysiagrass and the impacts of various municipal watering restrictions on turfgrass.

EVALUATION OF MINOR FOOD AND FORAGE COOL-SEASON LEGUME GERMPLASM AS OVERWINTERING COVER CROP GREEN MANURES

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The Plant Germplasm Introduction Testing and Research Unit (PGITRU) collection of 4810 annual and perennial species is one of the most diverse and well characterized within the National Plant Germplasm System (NPGS). Characterization of minor crop species remains generally limited however. With a recurring interest in cover crops, screening underutilized accessions within minor food and forage legume germplasm species may turn up desirable germplasm with multi-purpose cover, green manure, and forage cropping potential. Descriptive data are needed to characterize germplasm accessions in support of cover crop and forage breeders. Specifically, which accessions thrive over winter when most cover crops are used. Key traits to screen for include rapid stand establishment, winterhardiness, early spring regrowth, vegetative biomass and high feed values where forage is desired. This study evaluated 597 accessions in 28 cool-season food legume taxa and 156 accessions in 32 cool-season forage legume taxa over one season for overwinter survival, C:N ratios and biomass production (dry weight and area based on canopy width and height) at the PGITRU's Central Ferry research station in southeastern Washington. Ten seeds were sown in replicate on black plastic and irrigated as necessary. Although air temperature remained above -10°C over the 2015-16 winter, 141 of the accessions did not survive. *Lathyrus ochrus*, *Vicia ervilia*, *V. benghalensis*, and *V. narbonensis* were generally less hardy than *L. sativus*, *V. sativa* and *V. villosa*, yet intraspecific variability blurred any definitive line. Simple screening of winter hardiness under field conditions and measurement of canopy height and width at flowering was enough to identify promising accessions with a large biomass production (>500 g DW). The top 30 largest biomass producing accessions were either *V. villosa*, *V. sativa*, *L. tingitanus* or *L. clymenum*. A handful of forage legume accessions had an area at flower >5000 cm², but this was accompanied by scant ground coverage over winter and rather late flowering (late May) compared to annual food legume germplasm (April-early May). All accessions that were tested for C:N at flowering were well under 20:1, suggesting that any of these accessions would be excellent green manures that quickly release inorganic nitrogen as they decompose. Further screening of both food and forage legume germplasm is necessary to accurately characterize each species trait value distribution within a given environment. Field screening for overwintering is relatively cheap and simple but inherently unpredictable. Therefore, it is advisable for interested researchers to screen these selections within their intended production environment and share their characterization data with WRPIS curators.

PRESENTER BIO: Dr. Coyne is the curator/geneticist with the USDA ARS Plant Germplasm Introduction and Testing Research unit co-located with Washington State University, Pullman, WA. Dr. Coyne's research focuses on increasing the utilization of plant genetic resources.

DOMESTICATION AND CULTIVATION OF *LIMONIUM AUREUM* (L.) HILL

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Wild golden sealavender (*Limonium aureum* (L.) Hill), widely distributed on salty gravel beach, loess slope and sand land in northern China, is of high ornamental value with long flower display period, beautiful color and the lush flowering braches. Besides, *L. aureum* is strong resistant, which only needs one tenth of water consumption of common lawn, and can maintain normal growth under 4‰ salt condition. *L. aureum* can be used as ornamental flower in city garden construction especially for cities in which lacking of water or having salinity problem. Based on these important features of *L. aureum*, to breed new cultivar with high height, splendid color and long display period, 100 copies of germplasm from Dawa Mountain in Lanzhou City and Longquan Township in Yongdeng County were screened in 2004. Wild seed germination rate was highly enhanced from 8-12% to 75% with being treated by 75% alcohol for 30s firstly and then 5% NaClO for 7 min. After a series of experiments such as plant transplanting, production test and comparison test with *Limoniumbicolor* (Bag.) Kuntze, *Limonium otolepis* (Schrenk) Ktze and *L. aureum*, the domestication variety was certified by Chinese Herbage Cultivar Registration Board in 2018, named as *Limonium aureum* (L.) Hill Longzhong. The flowers of *L. aureum* (Longzhong) are golden, bright and the display period can last for 7 months, nearly 50 days longer than the wild ones. Its height is 40-50cm, nearly 20cm higher than the wild ones, and each plant contains about 150-390 pieces of inflorescences, nearly 120 pieces more than the wild ones. Evaluated by the comprehensive score method, we found that the flower aesthetic order is *L. aureum* (Longzhong) > *L.bicolor* > *L. otolepis* > *L. aureum*, which is highly consistent with our breeding purpose. And we found that the salt concentration it can tolerate ranges from 100 to 300 mM, a large amount of Na⁺ can be accumulated in the plant, and a higher concentration of K⁺ would be maintained in the leaves. At the same time, superfluous Na⁺ can be excreted through its salt gland. So it is recommended to cultivate and prompt in the desertification, arid and salinity regions in northern China.

PRESENTER BIO: Dr. Guang-xin Cui is an assistant researcher mainly focusing on ecology and husbandry in alpine region and new forage cultivar breeding of cold and dry area.

DEVELOPMENT OF SINGLE NUCLEOTIDE POLYMORPHISMS (SNP) MARKERS FOR GENETIC MAP SATURATION OF HEXAPLOID *UROCHLOA HUMIDICOLA*

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Large areas of Brazil are destined for the cultivation of pasture, where the purpose is to feed cattle. Among these, it stands out *Urochloa humidicola* (syn. *Brachiaria humidicola*), an apomictic polyploid species that tolerates waterlogged soils. The single sexual accession (H031) of the Germplasm Bank conserved at Embrapa is hexaploid ($2n=6x=36$). In the breeding program developed by Embrapa Gado de Corte, H031 was crossed with the commercial cultivar BRS Tupi, also hexaploid, generating 279 hybrids F₁, which were used in the construction of a genetic map. However, this map was based on single-doses marks from 124 microsatellites and has low resolution. It is known that high resolution in specific regions are fundamental for the identification of loci controlling relevant quantitative traits. Furthermore, little is known about the molecular basis of apomixis, whose understanding may also benefit from a saturated genetic map. In order to increase the density of markers on map, the main objective of this work was the development of single nucleotide polymorphism (SNP) by Genotyping-By-Sequencing (GBS). For this purpose, DNA was extracted from young leaves of each hybrid and the parents. Genomic libraries were constructed from the reduction of DNA complexity with two restriction enzymes, PstI and MspI, and were sequenced as 150-bp single-end reads on Illumina NextSeq 500 platform. After quality filters, we obtained a total of 1,732,628,260 sequence reads covering 260 Gb of sequence data, with an average greater than 5M reads per sample. SNP calling was performed using Tassel-GBS pipeline modified for polyploids, which allows the use of the total amount of reads available. We have used as reference the genomes of *Urochloa ruziziensis*, *Setaria italica*, *Setaria viridis*, *Panicum halli* and *Panicum virgatum*; and transcriptomes of *U. humidicola* and *Urochloa decumbens*. The *U. ruziziensis*'s genome provided the best alignment (30,81%), and 172,325 SNPs were identified. Dosage of each bi-allelic loci was estimated using the SuperMASSA software. Ploidy was fixed as 6, and after filtrations, 17,145 markers had the dose estimated with high reliability. The OneMap 2.0.6 software will be used to build an integrated genetic map using these SNP data and the SSR previously developed. As this software does not account for allele dosage, another map will be built with SNP markers considering dosage information using the polypmapR. The proposal to construct a genetic map of high resolution and with genetic information relevant to *U. humidicola* will be of great importance for the breeding program in progress at Embrapa. From reproductive mode data previously phenotyped, apomixis can also be mapped on this population. The project will also develop a tool that in the future can be associated with other phenotypic traits of economic importance, raising the *U. humidicola* breeding program to high technology levels.

PRESENTER BIO: A. C. L. Moraes is a PhD student and Research Technician, and has been working with forage since 2012. She has experience with molecular genetic breeding of other tropical polyploids plants, and has been working on the improvement of several methodologies for polyploid studies.

MIXING ST. AUGUSTINEGRASS (*STENOTAPHRUM SECUNDATUM*) CULTIVARS AS AN INTEGRATED PEST MANAGEMENT STRATEGY IN RESIDENTIAL LAWNS

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Urban landscapes are generally characterized by reduced arthropod diversity and increased herbivorous pest abundance, which reduces the ecosystem services provided by urban plants and wildlife. Warm season turfgrasses are ubiquitous to southern U.S. landscapes where when healthy, they provide benefits to humans and the environment. Unfortunately, insect pests frequently reduce lawn health. Due to few effective or practical integrated pest management (IPM) tactics, warm season lawn managers rely predominantly on insecticides for pest control, which has led to insecticide resistance, secondary pest outbreaks, and environmental contamination. Currently, warm season turfgrasses are produced, planted, and maintained as cultivar monocultures, which preserves the favorable traits selected for during turfgrass breeding. However, monoculture plantings may predispose them to insect pest attack. Evidence from other systems suggests that increasing plant cultivar diversity reduces pests, promotes biological control, supports more diverse communities, and improves plant quality. Therefore, we propose that planting mixtures of turfgrass cultivars may provide a tractable approach to achieving the benefits of increasing lawn diversity while conserving favorable aesthetic and maintenance traits. To determine if this holds true in warm season turfgrasses, we conducted a series of laboratory, greenhouse, and common garden field experiments over two years in Florida, USA. We compared St. Augustinegrass (*Stenotaphrum secundatum*) monocultures, mixtures of two cultivars, and mixtures of four cultivars to determine their effects on herbivorous insects, beneficial insects, and lawn quality. We predicted that increasing cultivar diversity would increase plant quality and arthropod diversity, while reducing plant pests and their associated long term maintenance costs and risks. Thus far, our results suggest that mixing cultivars of St. Augustinegrass reduces herbivore fitness and increases lawn quality. More specifically, fall armyworm (*Spodoptera frugiperda*) larvae feeding on more diverse mixtures develop into smaller individuals and preferentially feed on monocultures. In addition, monthly image analysis and turfgrass industry surveys indicate that more diverse lawn plantings are greener, more dense, and preferable over monoculture plantings. Ongoing work is investigating effects of cultivar diversity on arthropod communities and the effects of geographic location and regional climate on those effects. Our ultimate goal is to develop practical, industry-accepted IPM tactics that can reduce reliance on maintenance inputs and promote urban ecosystem health.

PRESENTER BIO: Dr. Dale is an Assistant Professor in the Entomology and Nematology Department at the University of Florida in Gainesville, FL. His research program focuses on developing more sustainable strategies to reduce damaging insect pests and promote beneficial insects, plant health, and the ecosystem services they provide in urban landscapes.

PLOIDY LEVEL EFFECT ON BIOMASS YIELD AND FOLIAR DISEASES IN ANNUAL RYEGRASS (*LOLIUM MULTIFLORUM* LAM.) ACROSS 12 YEARS IN FLORIDA

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Annual ryegrass (*Lolium multiflorum* Lam.) is a cool-season species native to southern Europe. In the southeastern USA, it is used in pasture systems because of its fast establishment, high yields and nutritive value, and its broad adaptation to a wide range of environmental conditions (Blount and Prine, 2016). Diploid ($2n=2x=14$) and tetraploid ($2n=4x=28$) annual ryegrass cultivars are commercially available. Spaced-planted tetraploid annual ryegrass outperformed diploids for several agronomic traits, including plant height, tiller size, and biomass yield (Rios et al., 2015); however, the relationship between ploidy level and agronomic performance is unclear for plot-based data across years. The UF-IFAS annual ryegrass breeding program conducts clipping trials yearly at the Agronomy Forage Research Unit, Hague, FL, including several diploid and tetraploid breeding lines and cultivars. Our research objective focused on investigating whether ploidy level has an effect on ryegrass biomass yield and disease resistance [crown rust (*Puccinia coronata* Corda) and gray leaf spot (*Pyricularia grisea*)], for data collected across 12 years (2006 to 2018). Trials were planted each fall and data were collected during the following winter and spring seasons. Biomass yield was assessed across multiple harvests within each year, and disease ratings were performed following a 1-9 scale (Prine, 1997). Data were analyzed using a linear mixed model in R, considering years and cultivars as random effects, while ploidy and blocks were considered fixed effects. Preliminary results showed a significant effect of ploidy level for crown rust and tetraploid lines exhibited lower disease ratings, while ploidy levels did not differ for biomass yield and gray leaf spot. The inclusion of weather data and more locations would aid in the development of models to predict yield and disease pressure in annual ryegrass in the southeastern U.S.

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PRESENTER BIO: Rocheteau DAREUS is a Master student at the Department of Agronomy (UF) working at Dr. E. Rios' Lab. His research is focused on identifying dual purpose cowpea lines that are resistant to Root knot nematodes (*Meloidogyne enterolobii*) and their potential use as cover crop in South Florida and Haiti.

EXPRESSION ANALYSIS OF TALL FESCUE HARBORING DIFFERENT ENDOPHYTE STRAINS IN RESPONSE TO WATER DEFICIT

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Tall fescue (*Lolium arundinaceum* = *Schedonorus arundinaceus*) is one of the most abundant cultivated pasture grasses in the United States, with exceptional stress tolerances partly attributed to effects of its symbiotic fungal endophyte, *Epichloë coenophiala*. Unfortunately, ergot alkaloids produced by common toxic endophyte (CTE) strains have negative effects on livestock. In contrast, novel non-toxic endophyte (NTE) strains lack ergot alkaloids, but may provide insect deterrence or other fitness benefits to tall fescue. Two plants (P27 and P46) with the *E. coenophiala* CTE strain, one with an *E. coenophiala* NTE19 strain (P12), and one with another *Epichloë* species, FaTG4, strain (P15), were evaluated together with their respective endophyte-free clones under water-withholding (stressed) versus watered control conditions in the greenhouse. Based on tiller production after resumed watering, P27 showed a positive effect of endophyte, P15 showed positive but non-significant trends, and P46 and P12 showed no significant endophyte effect. The whorls of vegetative leaf sheaths (pseudostems) were sampled and gene expression analyzed by Illumina RNA-seq sequencing. Differentially expressed genes (DEGs) were identified as affected by genotype (combined plant and endophyte), water treatment (stressed or control) or endophyte (presence or absence) if they had at least 2-fold expression difference at $P \leq 0.05$. Water treatment affected roughly a third of the plant transcriptome and the DEGs indicated the expected stress responses. Although approximately 1000 DEGs were identified as affected by the endophyte under stress or control conditions, most were unique to individual genotypes, and none was common to all genotypes. The unstressed P27 and P15 had the most DEGs, of which 84% had reduced expression in response to endophyte. The few DEGs affected by endophyte in stressed plants were genotype-specific. Our results did not support the model that positive endophyte effects on stress tolerance, but did suggest that endophyte sometimes reduces expression of anti-fungal host factors prime the plant genome for stress.

Presenter Bio: Dr. Dinkins is a Research Molecular Plant Geneticist at the USDA-ARS, Forage-Animal Production Unit in Lexington KY. Dr Dinkins has served in this position for the last 14 years working primarily on tall fescue and clover molecular research questions.

DOES ENDOPHYTE-ASSOCIATION IMPROVE PRODUCTIVITY IN TALL FESCUE? THE CASE OF THE NOVEL AR584 STRAIN IN A NON-LIMITING ENVIRONMENT

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In the symbiotic relationship between tall fescue (*Festuca arundinacea* SHREB.) and the endophytic fungus *Epichloë coenophialum*, the compounds produced by the latter offers survival benefits to the infected grass. Novel endophyte strains offer such advantages, with no toxicity associated to cattle. However, whether both the biotic and abiotic stress tolerance attributed to the endophyte infection result in an increase in dry matter productivity remains unclear. Thus, the objective of this study was to determine if tall fescues infected with the novel endophyte strain AR584 performed better than its endophyte-free counterpart in the temperate region of Uruguay. From 2010 to 2017 two cultivars, INIA Aurora and INIA Fortuna, with and without the AR584 endophyte (E+ and Nil respectively) were sowed in the tall fescue field trials of INIA's breeding program. These cultivars along with other breeding lines were sowed in 6.25 m² plots, with 4 reps in randomized complete block design, and were evaluated for 3 years periods. Both, seasonal and annual dry matter (DM) production of the swards were evaluated under cuts. Analysis of the data shows that Cultivar INIA Aurora E+ had higher annual DM yields than INIA Aurora Nil in swards of all 1st, 2nd and 3rd years, but differences were not significant ($P>0.05$). The seasonal DM yields were also higher in INIA Aurora E+ in all but one season, although the difference was significant only in spring of the 1st year ($P<0.05$). On the other hand, cultivar INIA Fortuna E+, had higher annual DM yields in swards of 1st and 2nd years and lower yield in 3rd year than INIA Fortuna Nil but differences were never significant ($P>0.05$). Except for the 3rd winter and spring, seasonal DM yields were higher for INIA Fortuna E+, but again the differences were not significant ($P>0.05$). For the total DM production over 3 years of evaluation, INIA Aurora E+ produced 4.9% and INIA Fortuna E+ produced 1.4% more than their respective Nil versions. Levels of infection measured by blotting test averaged 91 ± 5.5 for INIA Aurora E+, 3 ± 2.2 for INIA Aurora Nil, 93 ± 3 in INIA Fortuna E+, and 25 ± 7.9 in INIA Fortuna Nil. Although data can be further analyzed, these preliminary analysis suggests that the novel endophyte AR584 provides a small, but not significant advantage, in DM production to, infected tall fescue cultivars in mild environments.

PRESENTER BIO: Dr. Do Canto is a researcher and a plant breeder focused on developing improved varieties of forage crops. He has worked with both grass and legume species, and is the current responsible for INIA's ryegrass and tall fescue breeding programs.

EFFECTS OF NaCl TREATMENTS ON THE GROWTH AND SUCCULENCE OF *SALICORNIA EUROPAEA*

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Tissue succulence is an important strategy for xerophytic and halophytic species to adapt to the drought and salt environment. *Salicornia europaea* is a typical stem-succulent halophyte with excellent resistance to salinity. However, the influence of NaCl on its growth and the relationship between NaCl treatments and tissue succulence are limited. In this study, using *S. europaea* as material, the growth characteristics, anatomical structures of shoots, K^+ and Na^+ accumulation were comparatively analyzed under different NaCl concentrations. The results showed that, *S. europaea* could maintain the normal growth under salt treatment, especially NaCl concentration ranging from 50 to 200 mM could stimulate its growth, accompanying with the significant increase of fresh weight both in shoot and root. Compared with the control, the succulence degree of *S. europaea* was induced evidently by different NaCl concentrations, and peaked at 100 mM NaCl. The analysis of anatomical structures reflected that, the numbers of vessels increased significantly with the NaCl concentrations, and many tissue thicknesses such as cuticle thickness (T_c), epidermis thickness (T_e), palisade tissue thickness (T_p), aqueous tissue thickness (T_a) increased evidently. Besides, with the elevated NaCl concentrations, a mass Na^+ accumulated in the plants of *S. europaea*, and K^+ content in the shoot increased consistently. Moreover, the K^+ relative distribution in the shoot improved, also K^+ net absorption rate, K^+ , Na^+ selective absorption ability (SA value) and K^+ , Na^+ selective transport ability (ST value) improved definitely. In total, elevated water use efficiency through the change of the anatomical structures, also occupied unique absorption, transport and distribution mechanisms of K^+ and Na^+ in plants, thus *S. europaea* can maintain the normal growth under salt treatment. The results could provide theoretical basis of salt resistance in high quality forages and crops.

PRESENTER BIO: Dr. Huirong Duan focuses on plant abiotic stress physiology and molecular biology, and new forage variety breeding of cold and dry area.

DEVELOPING BLACK OAT CULTIVARS FOR FLORIDA DAIRIES

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Black oat (*Avena strigosa* Schreb) is a cool-season annual grass that has Mediterranean origin and has been used in Europe for centuries. Black oat is also successfully used in the southern portion of South America, in regions with similar latitude than Florida. Compared to annual ryegrass or other cool-season small grains, black oats are more heat tolerant and disease resistant, allowing an early planting. Black oats are not cold hardy, but they are recommended for the USDA Plant Hardiness Zones 8b-10a. Therefore, black oats could be a forage option in most of the Florida territory regarding winter temperatures. The objective of this study was to evaluate the performance of black oat entries contrasting with other cool-season grasses in four locations in Florida. Black oat entries included CI6858, SAI SELN, CI7280, CD3280, SAIA2, SAIA4, PI436103, PI436109, and Soil Saver. In addition, we included as cool-season controls the following forages: oats (Legend 567, Horizon 201, FL0720, and Cosaque), triticale (FL08128, FL01143, and Trical 342), cereal rye (FL401), and Ryegrass (earlyploid ryegrass). These 18 treatments were allocated in a randomized complete block design with four replications and established in four locations: UF IFAS NFREC Marianna, North Florida Holstein – Bell, UF Dairy – Gainesville, and RCREC – Ona. Response variables included herbage accumulation and nutritive value (crude protein and IVOMD). Early-planting dates were 9/7/16, 8/28/16, 8/29/16, and 8/30/16 for Marianna, Bell, Gainesville, and Ona, respectively. Black oats and other cool-season forages planted in this period were affected by hurricanes Hermine and Matthew and we could not collect data. Plots were re-planted in 9/30/16. In all trials we applied 300 lb/acre of 10-10-10 at planting. Plots measured 5 x 10 ft., with six rows. Plots were harvested twice in Bell and Gainesville, three times in Ona, and four times in Marianna, at 4-inches stubble height, with application of 50 lb N/acre after each harvest. In South Florida (RCREC-Ona), black oats were better than other small grains (rye, oat, triticale) and annual ryegrass. In Central (UF Dairy in Gainesville and North Florida Holstein in Bell) and North Florida (Marianna), black oats had similar productivity than the most productive oat (Legend 567) and other small grains/annual ryegrass, reaching up to 6,000 lb DM per acre in the multiple harvests. In Ona, the plant introduction (PI) CI7280 showed the best results, being a promising cultivar for future release. Nutritive value of black oats was high, comparable to other cool-season forages. Average IVOMD ranged from 75 to 80% and crude protein from 20 to 24%. During the fall, no major diseases were identified in black oats. During the summer planting, leaf spot (*Bipolaris* spp.) was observed not only in black oats, but also in all cool-season forages planted. Summer planting was also problematic regarding weed management and presence of leaf spot. Fall planting seems more adequate for black oat establishment. Black oats are one important forage alternative for Florida. This project will continue to select the best black oat entries adapted to distinct Florida environments aiming future cultivar release for producers.

PRESENTER BIO: Dr. José Dubeux is an Associate Professor at UF IFAS NFREC with more than 15 years of experience researching forages and grazing systems. He has extensive experience with nutrient cycling in grazing systems.

INHIBITION OF *GAEUMANNOMYCES GRAMINIS* VAR. *GRAMINIS* BY SOIL INHABITTING BACTERIA ASSOCIATED WITH ST. AUGUSTINEGRASS

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Take-all root rot (TARR) caused by the fungal pathogen *Gaeumannomyces graminis* var. *graminis* (GGG) is a difficult-to-manage and economically important disease with no consistently reliable fungicide options for St. Augustinegrass (*Stenotaphrum secundatum*) lawns. Host plant resistance and biological control are two attractive options for limiting TARR with many technical challenges. Our objectives were to develop an artificial GGG inoculation protocol to assess the relative susceptibility of 24 St. Augustinegrass lines, and to utilize a co-plating assay to detect bacteria antagonistic to GGG. Antagonistic bacteria present in the rhizosphere of wheat monocultures naturally suppress a related take-all wheat disease caused by *G. graminis* var. *tritici* in the well-documented natural suppression known as take-all decline. We surveyed the St. Augustinegrass rhizospheres of long-term (> 5 yr) sod production fields in Florida for antagonistic bacteria in the genera *Pseudomonas* and *Streptomyces*. Isolates of *Pseudomonas* spp. were identified by fluorescence on Kings medium B (KMB). Gram positive bacteria including *Streptomyces* spp. were isolated on inorganic salts starch (ISP4) medium, and sporulating colonies were transferred to Tryptic Soy Broth (TSB). Inhibition of GGG was observed for some isolates tested with co-plating assays on quarter strength PDA. Inoculation with oat seed infested with GGG under 70% shade resulted in significantly different TARR severity among the 24 lines of St. Augustinegrass. Bacterial isolates that inhibited the growth of GGG were identified to species and stored for future research that will focus on field testing isolates using a similar inoculation method.

PRESENTER BIO: Max Duncan is a Masters student at the University of Florida within the Plant Pathology Department. He is also the Rapid Turfgrass Diagnostician for the Rapid Turf program at the University.

LINKAGE MAP WITH SNP DOSAGE DATA IN TETRAPLOID UROCHLOA DECUMBENS

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The generation of the first intraspecific progeny of *Urochloa decumbens*, an important tetraploid forage grass in Brazilian beef production, represented a crucial advance for the breeding program of this specie. Allied to this, the rapidly decreasing cost of sequencing and availability of new tools such as genotyping-by-sequencing (GBS) and bioinformatics pipelines advances now allows important genomics studies will be perform in polyploids species. In this context, our objective was to build an *U. decumbens* integrated genetic map using SNPs markers with allele dosage information. At Embrapa Beef Cattle, an F₁ population containing 217 individuals was derived from intraspecific cross between *U. decumbens* D24/27 and *U. decumbens* cv. Basilisk. From DNA of this full-sib progeny and it parents, genomic libraries were constructed by the reduction of DNA complexity with restriction enzyme NsiI, and then sequenced as 150-bp single-end reads on the Illumina NextSeq 500 platform. Sequencing data analysis was performed using Tassel-GBS pipeline modified for polyploids, which allows the use of the total amount of reads available. Because *U. decumbens*'s genome has not yet been sequenced, GBS tags were aligned using the *Setaria viridis* genome. The output of Tassel-GBS pipeline was used as input to estimate the correct tetraploid allele dosage of each individual using SuperMASSA software. Subsequently, monomorphic SNPs and with a maximum of 25% missing data were removed manually using R software. Finally, an integrated genetic map was built with different SNPs dosage data using TetraploidSNPMap software. We obtained 1,183,089,925 sequence reads with high-quality bases covering 390 Gb of sequence data, with an average about 4M reads per sample. The alignment with *S. viridis* genome reference allowed the identification of 4,240 high-quality SNP markers after allele dosage estimation and filtrations. The final genetic map was composed of 1,000 SNPs markers distributed among nine homology groups (HGs) and spanned 1,335.09 cM with an average marker density of 1.33 cM. The length of each group ranged from 129.70 cM (HG7) to 166.57 cM (HG9), with an average of 148 cM. The largest distance between adjacent markers, 12.4 cM, was observed on homology group eight, one of the groups with the fewest mapped markers. The HG9 is the densest with 187 markers. The map contains SNP markers with different SNP configurations, being that Simplex markers (S) were the most frequent. This map will be useful for future evolutionary studies, genome assembly, and quantitative trait locus analyses in *Urochloa* spp. Besides this, information collected from this study will be integrated into traditional breeding strategies to accelerate and improve the development of superior *Urochloa* cultivars.

PRESENTER BIO: R. C. U. Ferreira is a postdoctoral research fellow at Unicamp and has been working with tropical forage since 2009. She has experience with molecular genetic breeding both in diploid and polyploid species.

HIGH-DENSITY LINKAGE MAP WITH ALLELE DOSAGE OF POLYPLOID *MEGATHYRSUS MAXIMUS*

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Megathyrus maximus (syn. *Panicum maximum*) has high nutritional quality and is the most productive forage grass in Brazil and Latin America countries. Breeding program of specie is very recent so there is little genetic-genomic knowledge about this important tropical forage. In this context, this study aims to construct a high-density linkage map with SNPs markers for the first progeny obtained in the breeding program of *M. maximus* at Embrapa Beef Cattle. A total of 136 hybrids were used to generate two 96-plex libraries of genotyping-by-sequencing (GBS) consisting of DNA fragments. Digestion were performed with a combination of a rare-cutting enzyme (PstI) and a second common-cutting enzyme (MspI). Libraries were sequenced as 150-bp single-end reads on the Illumina NextSeq 500 platform. SNP calling were performed using Tassel-GBS v.4 modified for polyploids, which allows the use of the total amount of reads available. GBS sequence tags were aligned with a transcriptome of *M. maximus* obtained from of parents this population and dosage allele of the markers identified was estimated with minimum overall depth of 50 reads using SuperMASSA software. After, monomorphic markers and with maximum of 15% missing data were removed using R software and an integrated linkage map was construed by TetraploidSNPMap software. A total of 719,854,748 reads with high quality bases evidencing the efficiency of the GBS library protocol and sequencing platform used. SNP calling allowed to identify 69,000 markers, of which 12,000 had allele dosage estimated. After other filters, an integrated linkage map was construed with 5,096 SNPs markers distributed among eight homology groups (HGs) with a cumulative map length of 1,009.1 cM and an average marker density of 0.19 cM. The length of each homology group ranged from 102.1 cM (HG3) to 176.9 cM (HG1), with an average of 126.1 cM. The HG7 is the densest with 941 SNPs markers, while the HG3 is the least dense, with 306 markers. This first linkage map will be used as base to insert more GBS-based markers from others *M. maximus* transcriptomes and get a high-resolution genetic map. Therefore, this final functional linkage map will be useful as for future quantitative trait loci (QTL) analysis, for genome assembly and studies of evolution in *M. maximus* and closely related species.

PRESENTER BIO: Thamiris G. Deo is a Master student in the Post Graduation Program in Genetics and Molecular Biology at Unicamp with three years of experience researching genetic of forage. She has experience with genetic diversity, genetic map molecular biology techniques and data analysis.

BROMATOLOGICAL COMPOSITION OF SPONTANEOUS PLANTS, GRASSES AND LEGUME

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The objective of this study was to evaluate spontaneous plants, grasses and legumes as far as bromatological composition. The experiment was conducted in a greenhouse at the Animal Science Institute (Instituto de Zootecnia - IZ), in Nova Odessa, SP, Brazil. Five species were evaluated: two grasses (*Brachiaria decumbens* cv. Basilisk and *Macrotyloma axillare* NO 279) and three spontaneous plants (*Bidens pilosa*, *Sida rhombifolia* and *Amaranthus viridis*) during their vegetative and reproductive stages. The experiment was set following a randomized block design, in a 5 × 2 factorial scheme (five species and two cuts – C1 and C2), with four replications and a total of 40 experimental units (boxes of 35 kg of soil each). The concentration of crude protein (CP) in the leaves were different among the species ($P = 0.0012$ SEM 15.63). The highest levels were observed in the spontaneous plants that did not differ: 282.9, 281.6, 244.2 and 21.58 g kg⁻¹ respectively for *B. pilosa*, *S. rhombifolia*, *A. viridis*, and *M. axillare*. The lowest CP concentration was observed in the leaves of *B. decumbens* (139.3 g kg⁻¹). For acid detergent fiber (ADF) ($P = 0.0227$ SEM 13.85) and neutral detergent fiber (NDF) ($P = 0.0118$ SEM 24.89) there were significant species × cut interactions. Concentrations of ADF and NDF were respectively higher for *B. decumbens* (271.8 and 601.6 g kg⁻¹ during C2), ADF was lower for *A. viridis* during cuts C1 and C2 (101.9 g kg⁻¹) and NDF was lower for *A. viridis* during C1 (257.5 g kg⁻¹). For lignin concentration the significant difference was observed solely between species ($P < 0.0001$ SEM 25.34) as a result of the greater concentration of lignin in *B. pilosa* (141.0 g kg⁻¹) when compared to the other plants (29.0 g kg⁻¹). In general, the spontaneous plants evaluated presented greater nutritive value than the grasses and legume, and could potentially improve grazing animal diets.

Acknowledgments: To FAPESP for project funding (Grant number 2016/22463-0)

PRESENTER BIO: Dr. Flavia Maria de Andrade Gimenes, PhD in Animal Production in Pastures is currently a scientific researcher at

NITROGEN CONCENTRATION IN FORAGE LEGUME TISSUES SUBMITTED TO NITROGEN DOSES AT PLANTING

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Instituto de Zootecnia (IZ), Brazil

This study aimed to evaluate the use of nitrogen (N) in the deployment of new legume access *Macrotyloma axillare* NO 279 (macrotiloma) and *Neonotonia wightii* NO 2348 (perennial soybean) and peanut (*Arachis pintoi* cv. Belmonte). The experiment was conducted in a greenhouse according to a complete randomized block design with three replications in a factorial 3 (three species) x 3 (three nitrogen rates of 0.0, 40.0 and 80.0 kg.ha⁻¹) applied at planting in the form of ammonium nitrate. Were planted 54 pots to make two independent cuts. The legumes were planted on November 22, 2016 in all pots and 27 vessels were cut and destroyed in February 2017 (cut 1 - C1) and 27 vessels cut in June 2017 (cut 2 - C2). The plants were cut close to the soil and the morphological components (leaves and branches) of each species separated, dried in a forced circulation oven (65 ° C) for 72 hours, weighed and ground to be sent to the Laboratory for analysis according to the methodology of Kjeldahl. The data were submitted to analysis of variance using the procedure PROC MIXED of the statistical package SAS (Statistical Analysis System, version 9.3) using a level of significance of 5% and the comparison of means by the Tukey test. In the C1 for concentration of N in the tissues there was difference between species (P <0.001) and components (leaf or branch) (P <0.0001). Among species with N concentrations (g kg⁻¹) were: 26.65 ± 0.874; 19.31 ± 0.8187; 14.44 ± 0.732 for peanut, perennial soybean and macrotiloma, respectively. Among the evaluated components, the concentrations were 26.51 ± 0.696 and 13.76 ± 0.6258 for leaves and branches, respectively. In the C2 there were differences between species (P = 0.0283), components (leaf or branch) (P <0.0001) and interaction of species*components (P = 0.0010). The highest values in g kg⁻¹ were for perennial soybean leaf (19.19 ± 1.247) and penaut leaf (17.93 ± 1.247) that differed from peanut branch (12.01 ± 1.309) and macrotiloma leaf (10.91 ± 1.247) either were higher than perennial soybean (9.64 ± 1.247) and macrotiloma branch (6.46 ± 1.247). The concentrations of N were high, especially in C1. The peanut stolons had high N concentration, overcoming the leaves of macrotiloma in C2. N rates applied at planting did not influence N concentrations in the components at two plant growth times. There were differences between species for N concentration, especially at more advanced maturity (C2). The evaluated legumes have high concentration of N and potential of use in pastures with source of N for the system and for grazing animals.

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Presenter Bio: Dr. Flavia Maria de Andrade Gimenes, PhD in Animal Production in Pastures is currently a scientific researcher at Instituto de Zootecnia (IZ), Brazil

MODELING SEEDLING EMERGENCE OF DALLISGRASS AND BAHIAGRASS FORAGE CULTIVARS WITH AN EMPIRICAL ENVIRONMENTAL VARIABLE

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In temperate and sub-tropical regions, the adoption of adapted warm-season forage grasses has been a long standing objective. The adoption of these species has been hindered among other factors by their generally low and unpredictable seedling emergence. Temperature and moisture affect seed germination, seed dormancy and subsequent seedling establishment. Modeling seedling emergence with environmental variables may provide a better understanding of the processes involved leading to a better adjustment of sowing dates and technology. The hydrothermal time model (HTT) which has been used to predict seedling emergence in warm-season grasses, has a strong physiological basis, which associates seed germination and dormancy with mean temperature (T_m) and water potential (ψ). The HTT model can be predictive, but requires a considerable amount of information from germination in controlled environments. However, it can be applied empirically to field emergence data, where the incidence of environmental variables may be deduced without prior information.

To understand the best environment for crop emergence for two *Paspalum* genotypes (*P. dilatatum* cv. Chirú and *P. notatum* cv. INIA Sepé), five experiments were carried out for two years in three locations in Uruguay and Argentina (Montevideo, Salto and Buenos Aires). In each experiment, a split-split-plot design with three replications, seeds were sown on four dates (two Falls and two Springs), within irrigated and non-irrigated main plots. For each small plot, emergence was frequently recorded until no new seedlings were observed, to record the final proportion of emergence (pEm). In a previous analysis, pEm showed a linear positive association with T_m at temperatures lower than 20°C; however, emergence was lower at higher temperatures combined with high moisture. With this background, we proposed an Average Weighted Thermal Time (AWTT), an empirical variable which would linearly adjust with pEm . The AWTT is the average thermal time sum with a weighted coefficient, which takes different values according to daily T_m and ψ in reference to two temperature and water potential thresholds. First, temperature thresholds and corresponding coefficient values were iterated to adjust with pEm data obtained under irrigation with constantly high ψ . The best regression for Chirú ($r^2 = 0.47$) was achieved with lower temperature thresholds than for INIA Sepé ($r^2 = 0.46$), and a low coefficient in high temperatures for both cultivars. Then, the water potential thresholds and coefficients under low ψ were iterated to adjust with pEm data from the non-irrigated treatments. The best regressions for both genotypes was obtained with the same water potential thresholds (-0.15 and -3.0 MPa, respectively) and adjusted coefficient values, which reached -1 when T_m is high and ψ is between water potential thresholds. Significant adjustment ($P < 0.0001$) was obtained for non-irrigated data with this model ($r^2 = 0.29$ and 0.35 for Chirú and INIA Sepé, respectively). AWTT proved useful to empirically model seedling emergence, because it takes into account an interaction between temperature and water potential. These models will be used to predict climatically adjusted sowing dates which will be validated in the field.

PRESENTER BIO: MSc. Nicolás Glison is a full time Research Assistant and Doctoral Student at the Graduate School of the Facultad de Agronomía (Udelar, Uruguay). His line of research is seed germination and dormancy in native grasses, focusing on those with agricultural potential. He has published two papers as first author about seed dormancy in dallisgrass.

GENOTYPE-BY-ENVIRONMENT ANALYSIS FOR TURF QUALITY IN BERMUDAGRASS

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Genotype-by-Environment (GxE) analysis provides valuable information to breeding programs to evaluate genotype stability across environments, to select genotypes for specific environments, to define mega-environments and target environments to maximize genetic gains. The aim of this study was to estimate the GxE interaction for turf quality in bermudagrass (*Cynodon* spp.). Three trials with 165, 164 and 154 genotypes were evaluated in 2011-2012 (1), 2012-2013 (2) and 2013-2014 (3), respectively. These trials were conducted in seven locations [Citra (FL), College Station (TX), Dallas (TX), Griffin (GA), Tifton (GA), Stillwater (OK) and Jackson Springs (NC)] as randomized block designs with two replicates. The response variable evaluated was turf quality (TQ), obtained by averaging the repeated measurements over time within a given experimental unit. Data were analyzed by a mixed model approach in R using the ASReml package. The modeling of the variances and covariance matrix of residual (R) and genetic (G) were performed in the multi-environment analyses. Genetic correlations were estimated for genotypes between locations at different sites. Biplots were created using the GGEBiplotGUI package. The matrix structure that provided the least Bayesian Information Criterion was diagonal for R in all years, heterogeneous compound symmetry in 1 and 2, and factor analytic 2 in 3 for G. The estimates of type-B correlation were 0.72 for 1 and 0.66 for 2. Genetic correlations among locations were higher in the multi-environmental trials 1 (0.44 to 0.83) and 2 (0.47 to 0.93). However, 3 presented estimates varying from 0.69 to -0.95. For 1 and 2, all locations were grouped in the same mega-environment, except Tifton in 1 and Citra in 2. In 3, Citra and Dallas formed one mega-environment and Tifton and Jackson Springs the other one. The winning genotypes in the mega-environments were T070 in 1, DT1 in 2, UGB208 and UGB185 in the first and second mega-environment in 3, respectively. Tifton was the most discriminating environment for each year. In conclusion, this study provided significant information about GxE in bermudagrass across the Southeastern U.S., and turfgrass breeders will use this information to determine the best strategy to develop improved varieties.

PRESENTER BIO: Beatriz is a Ph.D. student at Genetics and Plant Breeding Graduate Program at the Universidade Federal de Lavras in Brazil. She is conducting her experimental work at the Embrapa National Beef Cattle Research Center in Brazil. Furthermore, she is conducting part of her dissertation at the University of Florida.

VALIDATION OF QUANTITATIVE TRAIT LOCI ASSOCIATED WITH FREEZE TOLERANCE IN ST. AUGUSTINEGRASS

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St. Augustinegrass (*Stenotaphrum secundatum* (Walt.) Kuntz) is a warm season turfgrass commonly used in home lawns. Its popularity in the southern United States is due to its aggressive growth habit and good shade tolerance. However, a lack of freeze tolerance has limited the spread of St. Augustinegrass into the transitional climatic region. 'Raleigh', released in the early 1980s is still the industry's standard in terms of cold tolerance. Despite the identification of freeze tolerant germplasm limited progress has been made in breeding for freeze tolerance in St. Augustinegrass. Breeding efforts to improve freeze tolerance would be aided by the identification of quantitative trait loci (QTL) and associated markers controlling the trait. An SSR-based complete linkage map was developed for St. Augustinegrass and used in conjunction with data on spring green-up and winterkill to identify multiple QTL on linkage groups 1, 3, 6 and 9.

An F₂ population of cultivar 'Raleigh' was developed to be used for validating the previously identified QTL. Laboratory based freeze tests were conducted for the population at both -3°C and -4°C. Additionally, a linkage map with 120 SSR markers was developed for the population using R/Onemap. QTL were identified via composite interval mapping (CIM) for the 'Raleigh' self-population and compared for overlap with those identified previously.

Marker assisted selection (MAS) provides a genomic flag at or near a gene of interest which allows for the genome to be screened for the marker instead of requiring a multi-year field evaluation. For a complex trait such as freeze tolerance a streamlined approach via MAS is particularly valuable. Thus, confirmation of QTL associated with freeze tolerance in St. Augustinegrass will aid breeders in the development of cold tolerant varieties to match demand.

PRESENTER BIO: Sydney Graham is a 1st year Masters Student at North Carolina State University where she studies turfgrass breeding under the direction of Dr. Susana Milla-Lewis. She received her Bachelors in Genetics from the University of Wisconsin-Madison in December 2017.

AN ETHYLENE RESPONSIVE FACTOR FROM *MEDICAGO FALCATA* CONFERS COLD TOLERANCE BY UPREGULATING POLYAMINE TURNOVER, ANTIOXIDANT PROTECTION, AND PROLINE ACCUMULATION

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M. falcata, being closely related to alfalfa and *M. truncatula*, has been used for cross with alfalfa to lead to heterosis for biomass yield due to its great tolerance to cold and drought. It is a good genetic germplasm for cold tolerance. Ethylene responsive factor (ERF) subfamily transcription factors play an important role in plant abiotic and biotic stress tolerance. The objective of this study was to identify the function of a cold responsive ERF, MfERF1, by using transgenics in combination with biochemical and physiological measurements. MfERF1 is most homologous to MtERF (MTR_8g022820) with 96% identity in amino acid sequence. Its expression was induced after 4 h of cold treatment at 5 °C, and reached to the maximum at 12 h. Transgenic tobacco plants overexpressing *MfERF1* were generated, and the homologous T3 lines were used for further analysis. Compared to the wild type, lower level of temperature that resulted in 50% lethal (LT₅₀) and higher survival rate after freezing treatment and higher level of net photosynthetic rate and lower level of ion leakage after chilling treatment were observed in transgenic plants. The results indicated that overexpression of *MfERF1* resulted in an increased tolerance to freezing and chilling in transgenic tobacco plants. In addition, down-regulation of *MtERF* (MTR_8g022820) in *Medicago truncatula*, the ortholog of MfERF1, resulted in reduced freezing tolerance in RNAi plants. Higher transcript levels of the genes involved in spermidine (Spd) and spermine (Spm) synthesis including *S-adenosylmethionine synthetase* (*SAMS*), *S-adenosylmethionine decarboxylase* (*SAMDC1*), *SAMDC2*, *spermidine synthase 1* (*SPDS1*), *SPDS2*, and *spermidine synthase* (*SPMS*) and catabolism (polyamine oxidase, *PAO*) were observed in transgenic plants than in the wild type. However, neither Spd nor Spm level was accumulated in transgenic plants as a result of promoted polyamine oxidase activity. Polyamine oxidase catalyzes oxidation of Spd and Spm for production of H₂O₂, an important signal regulating gene expression and abiotic stress responses. Transgenic plants had higher activities of antioxidants associated with the induced encoding genes including *Cu*, *Zn-SOD*, *CAT1*, *CAT2*, *CAT3*, and *cpAPX* and accumulated more proline associated with induced Δ^1 -pyrroline-5-carboxylate synthetase (*P5CS*) and reduced proline oxidase (*PROX2*) transcription as compared with wild type. The results suggest that MfERF1 confers cold tolerance through promoted polyamine turnover, antioxidant protection, and proline accumulation.

PRESENTER BIO: Dr. Guo is a Professor with more than 20 years of experience researching forage and turfgrass. He has extensive experience with plant physiology and molecular biology and has published 45 papers on internationally peer reviewed journals dedicated to abiotic stress adaptation mechanism and improvement of forage and turfgrass.

GENETIC MODIFICATION OF ASEXUAL *EPICHLÖE* ENDOPHYTES WITH THE *PERA* GENE FOR PERAMINE BIOSYNTHESIS

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Development of grass-endophyte associations with minimal or no detrimental effects in combination with beneficial characteristics is important for pastoral agriculture. The feasibility of enhancing production of an endophyte-derived beneficial alkaloid through introduction of an additional gene copy was assessed in a proof-of-concept study. Sexual and asexual *Epichloë* species that form symbiotic associations with cool-season grasses of the Poaceae sub-family Pooideae produce bioactive alkaloids that confer resistance to herbivory by a number of organisms. Of these, peramine is thought to be crucial for protection of perennial ryegrass (*Lolium perenne* L.) from the Argentinian stem weevil, an economically important exotic pest in New Zealand, contributing significantly to pasture persistence. A single gene (*perA*) has been identified as solely responsible for peramine biosynthesis, and is distributed widely across *Epichloë* taxa. In the present study, a functional copy of the *perA* gene was introduced into three recipient endophyte genomes by *Agrobacterium tumefaciens*-mediated transformation. The target strains included some that do not produce peramine, and others containing different *perA* gene copies. Mitotically stable transformants generated from all three endophyte strains were able to produce peramine in culture. Following inoculation into perennial ryegrass, *in planta* peramine production of transgenic endophytes was confirmed for novel host-endophyte associations. This study provides an insight into the potential for artificial combinations of alkaloid biosynthesis in a single endophyte strain through transgenesis.

PRESENTER BIO: Dr Kathryn Guthridge is Senior Research Scientist in Molecular Genetics with Agriculture Victoria Research.

METHOD OPTIMISATION FOR THE QUANTIFICATION OF ALKALOIDS IN ENDOPHYTE-INFECTED PERENNIAL RYEGRASS

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Rapid identification and quantification of alkaloids produced by endophyte-infected *Lolium* spp. (e.g. perennial ryegrass and tall fescue) is important for the pastoral agriculture industry. Endophyte-infection may trigger the production of beneficial alkaloids, such as peramine, which provides the grass with enhanced insect protection. Conversely, the production of lolitrem B and ergovaline can negatively impact livestock. Currently, no one method has been established to measure the concentration of these alkaloids. This study aimed to develop a robust and reproducible single method using LCMS to quantify peramine, ergovaline and lolitrem B in shoots of endophyte (*Epichloë*)-infected perennial ryegrass (*Lolium perenne* L.). Three extraction methods were explored using either 20mg or 50mg of freeze-dried, ground tissue. Method one, a single extract in one vial; method two, two extracts in one vial (dried and reconstituted to concentrate the sample), and method three, three extracts in three separate vials (to explore dilution factors). Separation of alkaloids were employed by UHPLC (ultra-high-performance liquid chromatography), and detection/quantification was compared across two mass spectrometry (MS) systems; an Agilent 6400 Series QQQ and a Thermo QExactive Plus. The concentration of ergovaline was also compared using a fluorescence detector (FLD) employing an established method. Extraction efficiency, matrix suppression, level of detection (LOD), level of quantitation (LOQ) and the recovery was established. Our studies revealed that 94-100% of the total alkaloid content in perennial ryegrass can be extracted by two extractions (method three). The LCMS (liquid chromatography-mass spectrometry) methods employed were robust (recovery of spiked samples was between 71-142%), and precise (coefficient of variations values determined at <10%). Alkaloid quantitation results were similar across both MS instruments. A difference of 5% was observed for peramine, 3% for ergovaline, and 11% for lolitrem B. Further, a 6% difference was determined for ergovaline when comparing quantitation results from the QExactive MS and the FLD instrument. This work provides the first highly sensitive quantitative UHPLC-MS method for the accurate and reproducible quantification of important alkaloids from endophyte-infected perennial ryegrass.

PRESENTER BIO: Dr Kathryn Guthridge is Senior Research Scientist in Molecular Genetics with Agriculture Victoria Research.

TRANSCRIPTOME ANALYSIS OF PERENNIAL RYEGRASS - ASEXUAL *EPICHLÖE* SYMBIOSES DURING SEEDLING GROWTH AND MATURATION

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Asexual *Epichloë* species are strictly seed-transmitted endophytic symbionts of cool-season grasses characterised by a life-cycle wholly confined to the host plant. Endophyte infection can impart environmental stress tolerance and protection from herbivory through the production of range of bio-protective alkaloid compounds. Conversely, the endophyte obtains nutrition and shelter from the host. A time-course RNA-Seq study using second generation sequencing was performed to gain a greater understanding of the major changes that occur in the endophyte transcriptome during early stages of seed germination. This has been achieved through generation and sequencing of RNA-Seq libraries of seeds and seedlings at 6 different time points from post imbibition to 10 days post germination. Seeds infected with three endophyte strains belonging to different representative taxa were used for this study. Filtered sequencing reads were mapped separately to the corresponding endophyte strain reference sequences. Reads that mapped to the reference genomes were used to generate count tables. Heat maps provided information on global gene expression patterns at each time point. Genes that were differentially expressed at one or more of the six points account for more than 40% of all the genes expressed for all 3 symbioses. The number of upregulated and down regulated genes observed at early time points were greater than those detected at later time points suggesting an active transcriptional reprogramming of endophytes at the onset of seed germination. Functional annotation was performed on genes that were differentially expressed at each time point to unravel what cellular, molecular and biological components play important roles in early stages of seed germination. Mfuzz clustering identified the unique expression patterns of alkaloid biosynthesis genes. Furthermore, comparison of the transcriptomes of *E. festucae* var. *lolii*, *LpTG-2* and *LpTG-3 in planta* identified expressed genes and gene clusters unique to each taxon.

PRESENTER BIO: Dr Kathryn Guthridge is Senior Research Scientist in Molecular Genetics with Agriculture Victoria Research.

GENOME-WIDE ASSOCIATION STUDY OF SYMBIOTIC NITROGEN FIXATION EFFECTIVENESS IN *MEDICAGO TRUNCATULA*

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Legumes are a crucial part of sustainable forage systems by virtue of symbiotic nitrogen fixation (SNF), which can inject over 100 kilograms of fixed-nitrogen per hectare of pasture each year [1]. Symbioses between legumes and rhizobia are built upon metabolic complementarity between the partners, involving the exchange fixed/reduced carbon from the plant for fixed/reduced nitrogen from the bacteria [2]. However, despite its importance, we know little about the plant genes that contribute to SNF effectiveness (SNFE) in nature. Genome-wide association studies (GWAS) have become a powerful tool to study complex traits in model and crop plants [3], and have the potential to identify genes/alleles that underlie SNF effectiveness.

In this study, we evaluated SNFE amongst 167 accessions of the *Medicago truncatula* HapMap population, spanning much of the natural genetic diversity within this species [4]. We define SNFE as the ratio of whole-plant biomass obtained via SNF divided by the mass of plants obtained with ample mineral-N. To avoid host preference for specific bacterial strains, a mixture of two broadly-effective rhizobia was used for SNF assays.

M. truncatula accessions were ranked based on SNFE, and GWAS were performed in TASSEL with a mixed linear model to analyze the association between phenotypes and genotypes [5]. A total of 1.65 million SNPs after quality filtering were used in the analysis. We are currently evaluating clusters of significant SNP markers at several chromosomal loci associated with SNFE to identify potentially-causative genes/alleles, which will be tested, in part, by comparing candidate gene transcript levels between best- and worst-performing genotypes. Candidate genes suggesting a positive effect on plant growth under SNF conditions will be functionally validated using reverse genetic tools.

By establishing proof-of-concept for discovery of genes contributing to SNFE in the model legume, *M. truncatula*, we hope to path the way for future genomics-based, plant-breeding approaches to enhance SNF in more important forage species.

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PRESENTER BIO: Dr. Raul Huertas is a postdoc with vast experience on areas such as plant abiotic stress, plant nutrition and plant-microbe interactions. He is currently working in the improvement of symbiotic nitrogen fixation in legumes, with special emphasis on the conditions of phosphorus limitation.

NATIONAL PLANT GERMPLASM SYSTEM'S COOL-SEASON GRASS AND TEMPERATE-ADAPTED LEGUME FORAGE GENETIC RESOURCES

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Forages play important roles in the livestock industries by providing sustenance in the form of quality hay, haylage, silage or pastures for grazing. Many cultivated, landrace, and unimproved crop and wild relative forage germplasm accessions are managed by the active collection sites within National Plant Germplasm System (NPGS). The Plant Germplasm Introduction and Testing Research Unit (PGITRU) is one of these sites with extensive cool-season-grown and temperate-adapted forage (and turfgrass) genetic resources. These accessions are exploited extensively by plant breeders who commonly mine germplasm collections, and their associated information, in search of useful genetic traits. Of the approximate 101,700 accessions presently held by the PGITRU, over 25,000 are cool-season grasses (many are forages) and more than 13,000 are temperate-adapted forages. Significant active collections within priority forage grass genera at the PGITRU include *Brachypodium* (2,977 acc.), *Festuca* (2,634 acc.), and *Poa* (2,251 acc.). Noteworthy forage legumes include the *Medicago* (8,618 acc.), *Trifolium* (3,730 acc.) and *Lotus* (921) genera. Important crop species/taxa are usually represented by large numbers of accessions within the collections, but many wild relatives in secondary and tertiary genepools are also targeted for conservation. For example, in the six genera mentioned above there are over 430 taxa represented. Responsibilities of the curatorial teams include the acquisition, regeneration, characterization, evaluation and free distribution of the germplasm to stakeholders worldwide. The primary tool utilized by Curators to manage genetic resources and their associated information is the Germplasm Resources Information Network (GRIN) Global database. Stakeholders can access GRIN-Global via internet for information and, after registration and providing a justification for germplasm (e.g., research/education), requests can also be made. In GRIN-Global, accessible information associated to specific accessions might include detailed passport and taxonomical information as well as digital voucher images. In addition, extensive characterization and evaluation data can be associated and queried using the crop descriptor information. Collection Curators welcome research collaborations and encourage the sharing of any valuable characterization and evaluation data generated by stakeholders in the research community.

PRESENTER BIO: Dr. Irish is currently the Geneticist/Curator for temperate-adapted forage legumes of the USDA-ARS National Plant Germplasm System. He has more than 14 years of experience leading research and service-oriented projects for diverse clonal and seed-propagated genetic resources.

PANICUM MAXIMUM HYBRID EVALUATION IN BRAZIL

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Among the main forage grasses grown in Brazil, *Panicum maximum* stands out for its great potential for dry matter production, easy adaptation and excellent forage quality. In search of new cultivars with differentials that better meet the needs of the cattle ranchers, crosses between five sexual plants (SP) and the apomictic accessions PM20, PM21 and Myiage were carried out at Embrapa Beef Cattle, Brazil. Accessions PM20 and PM21 were used to generate shorter hybrids for use under grazing and for crop-livestock integration while Myiage was used because of its ability to retain seeds. After visual evaluation in the field of 1,116 hybrids, we selected 154 that were cloned and planted in an evaluation experiment on February 2016 together with the progenitors and eight checks in a randomized complete block design with two replications. Plots consisted of two lines of five plants spaced 50 cm between plants and lines, and 1.5 m between plots. In May the plants were standardized and a dry-season harvest was done in September. Thereafter, plots were harvested every 35 days in the rainy-season. The experiment was conducted for two years in a total of nine harvests, encompassing two dry and two rainy seasons. Nutritive value (crude protein and organic matter digestibility) was evaluated by NIRS. Data was analyzed using SAS. Great variability was found among genotypes for total leaf dry matter yield (LDMY) ($p < 0.01$), which ranged from 10.4 to 76.5 t/ha in the total of nine harvests. The cross between progenitors SP5 and Myiage yielded hybrids with significantly higher mean leaf dry matter yields (mean of 49.5 t/ha). These progenitors individually also presented higher yields, 45.6 t/ha and 53.1 t/ha for SP5 and Myiage, respectively. Crude protein and digestibility did not vary among families. However, significant differences ($P < 0.01$) were found among hybrids obtained from crosses SP5 and SP4 x PM20, SP2 x Myiage and SP3 x PM21 for crude protein. For digestibility, differences among hybrids were only found for the cross between SP3 and PM21, which had the most hybrids under evaluation. No differences were found for crude protein among progenitors, values varied from 11.4% to 13.9%. Leaf digestibility varied among female progenitors, SP5 showing the significantly highest digestibility (65.6%). Mean crude protein values varied from 9.7% to 14.3% among hybrids and digestibility varied from 56.6% to 71.5%. The highest digestibility was found for cultivar Tanzânia-1 (73.7%). It is concluded that the use of progenitors SP5 and SP3 are indicated in the crosses aiming to increase productivity and nutritive value in the progenies. Considering the characteristics above and lack of damage due to diseases in the field, 28 hybrids from various crosses were selected and will be evaluated in six network experiments in Brazil in 2019.

PRESENTER BIO: Dr. Jank is a researcher at Embrapa with more than 30 years of experience in tropical forage breeding. She has extensive experience with germplasm evaluation and *Panicum maximum* genetic breeding with many cultivars in the market extensively being used in Brazil and other Latin American countries and has led several projects dedicated to the development of superior cultivars.

NAPIERGRASS (*CENCHRUS PURPUREUS* SCHUMACH.) GENOME SURVEY AND HIGH-DENSITY GENETIC MAP CONSTRUCTION

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Napiergrass (*Cenchrus purpureus* Schumach.) is a tropical forage grass and a promising lignocellulosic biofuel feedstock due to its high biomass yield, persistence, and nutritive value. However, its utilization for breeding has lagged behind other crops due to limited genetic and genomic resources. In this study, next-generation sequencing was first used to survey the genome of napiergrass. Napiergrass sequences displayed high synteny to the pearl millet genome and showed expansions in the pearl millet genome along with genomic rearrangements between the two genomes. An average repeat content of 27.5% was observed in napiergrass including 5,339 simple sequence repeats (SSRs). Furthermore, to construct a high-density genetic map of napiergrass, genotyping-by-sequencing (GBS) was employed in a bi-parental population of 185 F₁ hybrids. A total of 512 million high quality reads were generated and 287,093 SNPs were called by using multiple *de-novo* and reference-based SNP callers. Single dose SNPs were used to construct the first high-density linkage map that resulted in 1,913 SNPs mapped to 14 linkage groups, spanning a length of 1,410 cM and a density of 1 marker per 0.73 cM. This map can be used for many further genetic and genomic studies in napiergrass and related species.

PRESENTER BIO: Dr. Baskaran Kannan is a Senior Biological Scientist at the Department of Agronomy, University of Florida. He is working on the genetic improvement napiergrass and sugarcane for bioenergy. His research involving breeding and genetics, transgenic plant development and genome editing for bioenergy traits.

OILCANE: METABOLIC ENGINEERING FOR ELEVATING LIPID CONTENT IN VEGETATIVE TISSUES OF SUGARCANE

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Metabolic engineering to divert carbon flux from sucrose to oil in a high biomass crop like sugarcane has been proposed as a strategy to boost lipid yields per acre for biodiesel production. The energy content of plant oils in the form of triacylglycerols (TAGs) is two-fold greater compared to carbohydrates. However, vegetative plant tissues do not accumulate oil to a significant amount since fatty acid synthesis in these tissues serves primarily membrane construction, in addition TAGs undergo rapid turnover. Therefore, our objectives include:

- 1) Increasing fatty acid synthesis by expressing a transcription activator of fatty acid biosynthetic genes,
- 2) Increasing fatty acid synthesis by suppressing lipid reimport into the plastids from the cytoplasm,
- 3) Increasing TAG synthesis from diacyl-glycerol and acyl-CoA by over-expression of rate limiting enzymes,
- 4) Optimizing TAG storage by limiting the access of lipases to TAG storage compartments.

Constitutive single or multiple gene expression/suppression cassettes were generated and co-delivered with the selectable *nptII* expression cassette by biolistic gene transfer into sugarcane. Plants were regenerated on geneticin containing culture medium and analyzed for presence and expression of target constructs by PCR and RT-PCR, respectively. Quantitative real PCR was performed to study the level of gene expression in transgenic TAG plants in different vegetative tissues. Plants were analyzed for TAG content by Gas-Chromatography and Mass Spectrometry (GC-MS). The presented research outcome will add value to sugarcane for production of advanced biofuels.

PRESENTER BIO: Dr. Baskaran Kannan is a Senior Biological Scientist at the Department of Agronomy, University of Florida. He is working on the genetic improvement napiergrass and sugarcane for bioenergy. His research involving breeding and genetics, transgenic plant development and genome editing for bioenergy traits.

RELEASE OF 'FAES1307', 'FAES1312', 'FAES1313' AND 'FAES1319' ZOYSIAGRASS CULTIVARS FROM THE UNIVERSITY OF FLORIDA

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Zoysiagrass (*Zoysia* spp) use as a turfgrass was previously limited to the transition zone of the United States (U.S.). Improved cultivars in the last 20 years have led to an increased use of zoysiagrass in southern regions of the U.S. Pest pressures that continue to limit the use of zoysiagrass include the Hunting billbug, tropical sod webworm and large patch (*Rhizoctonia solani* Kuhn). Additionally, winter dormancy limits the use of zoysiagrass in central and south Florida where St. Augustinegrass stays green through winter months. The breeding objectives for zoysiagrass cultivars were improved adaptation to Florida along with increased drought resistance, increased tolerance to large patch disease and retention of color through the winter months.

'FAES1312', 'FAES1313' and 'FAES1319' were released in 2018 and 'FAES1307' was released in 2019 from the University of Florida. FAES1312 and FAES1319 were both collected from cultivated stands of zoysiagrass in south Florida, while FAES1307 and FAES1313 are both F1 hybrids from 'TAES 5309-12 × BA 123'. These grasses were evaluated as part of a USDA-SCRI funded project for their drought responses in five states across seven locations in 2011-2012. Each location was arranged as randomized complete block design with two replications. FAES1312, FAES1313 and FAES1319 were further evaluated at five locations in Florida, with each study planted in 2012 as a randomized complete block design with three replications. All four grasses were included in the 2013 NTEP (National Turfgrass Evaluation Program) Zoysiagrass Trial planted at 16 locations. Data from the USDA-SCRI trial and the Florida trial support that FAES1307, FAES1313 and FAES1319 exhibited favorable drought responses compared to commercial standards such as 'Palisades', 'Empire', 'JaMur' and 'Zeon' zoysiagrasses. These same three grasses also have better winter performance compared to Empire as indicated by the Florida trial and southern NTEP locations. These grasses exhibit delayed fall dormancy and quicker greenup in the spring. FAES1312 goes more dormant than the other three grasses; however, NTEP data supports its use further north as evidenced by its survival and performance in several transition zone and mid-western states. FAES1319 also did well in several northern NTEP locations and was among the most consistent entries across locations for each year of the study. Limited large patch data indicate that FAES1319 and FAES1307 exhibited fewer large patch symptoms compared to 'Empire', 'Zeon' and 'Meyer'

All four grasses have been grown in small production blocks and tested in landscapes through cooperation with a consortium of grower members of the Turfgrass Producers of Florida who provided financial support of the trials conducted in Florida. Foundation increase blocks have been planted with several producers through Florida with a goal of having commercially available sod in late 2019/early 2020.

PRESENTER BIO: Dr. Kenworthy is a Professor of turfgrass and forage breeding in the Agronomy Department at the University of Florida. He has extensive experience with genetics and breeding of various turf and forage crops, and has released numerous recognized cultivars of annual ryegrass.

RELEASE OF 'FSA1602' ST. AUGUSTINEGRASS FROM THE UNIVERSITY OF FLORIDA

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St. Augustinegrass [*Stenotapharum secundatum* (Walt.) Kuntze] is well adapted for use as a turfgrass throughout the Gulf Coast states and Carolinas. It is the dominant species used for residential landscapes in Florida.

'Floritam' St. Augustinegrass was jointly released from the University of Florida and Texas A&M University in 1973 and remains the primary cultivar in use today. Several other cultivars with better shade tolerance than Floritam are also utilized in Florida, but with significantly fewer acres in production. Major limiting factors to production of St. Augustinegrass include chinch bugs (*Blissus insularis* Barber), grey leaf spot (*Magnaporthe oryzae* Crouch), large patch (*Rhizoctonia solani* Kuhn), and take-all root rot [*Gaeumannomyces graminis* (Sacc.) Arx & D. Oliver var. *graminis*]. The objective of this research was to evaluate the turfgrass performance of a number of St. Augustinegrass hybrids and identify one or more lines with superior turfgrass quality and improved drought and disease resistance.

'FSA1602' is a hybrid between a maternal polyploid line named NUF216 and a diploid ($2n=2x=18$) line named 1997-6. FSA1602 is a polyploid ($2n=2x=30$) and was first planted in Gainesville, FL in 2008, where it was recognized as having a unique bluegreen color and prostrate growth habit. FSA1602 was evaluated for its drought response in five states across seven locations in 2012-2013. Each location was arranged as randomized complete block design with two replications. FSA1602 was further evaluated at five locations in Florida and one location in Raleigh, NC with each study planted in 2012 as a randomized complete block design with three replications. These experiments included Floritam, 'Palmetto' and 'Raleigh' (only in Raleigh, NC) as commercial standards. Data indicate that FSA1602 can survive colder temperatures than Floritam and it has shown an improved drought response compared to Floritam. It has proven to be well adapted throughout Florida with adequate or good resistance to shade, gray leaf spot, large patch and take-all root rot. FSA1602 is recommended for use in landscapes with moderate shade throughout Florida. A portion of the research that identified FSA1602 as a superior turfgrass was supported by a consortium of grower members of the Turfgrass Producers of Florida.

FSA1602 was officially released by the Florida Agricultural Experiment Station in January 2018 and a provisional plant patent has been filed. It was evaluated for commercial turf production by various sod growers during the selection process and was found to have desirable turf lifting and shipping attributes. It is currently being increased by sod producers who were members of the St. Augustinegrass consortium in Florida. A registered trademark of CitraBlue™ has been filed for FSA1602 and it is expected that limited commercial quantities of CitraBlue™ will be available during the 2019 growing season.

PRESENTER BIO: Dr. Kenworthy is a Professor of turfgrass and forage breeding in the Agronomy Department at the University of Florida. He has extensive experience with genetics and breeding of various turf and forage crops, and has released numerous recognized cultivars of annual ryegrass.

INVIVO GENERATION OF DIHAPLOIDS IN TALL FESCUE

B. K. Kindiger

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Within the *Lolium-Festuca* genome complex there is a need for novel breeding approaches that can facilitate the rapid development of improved germplasm, cultivars or material for molecular studies. A novel diploid, *Lolium multiflorum* (*Lolium perenne* L. subsp. *multiflorum* (Lam.) Husnot (syn. *Lolium multiflorum* Lam.) line has been identified that induces genome loss and dihaploid generation following hybridization with hexaploid, *L. arundinaceum* (Schreb.) Darbysh.) (syn. *Festuca arundinacea* Schreb.). *Lolium* x *Festuca* hybridizations are not a new area of breeding research; however, the generation of dihaploid products from *Lolium* x *Festuca* hybridizations is novel. The generation of homozygous, dihaploid lines of tall fescue creates numerous opportunities for enhanced breeding methods and molecular research in this species. This research describes the utilization of novel *L. multiflorum* inducer lines to produce dihaploid *L. arundinaceum*. The generation of dihaploids is structured around a mitotic, genome loss character that is conferred by the novel inducer lines.

PRESENTER BIO: Dr. Kindiger is a research plant geneticist with the USDA-ARS, having more than 25 years experience in forage grass research. He has extensive experience with cytogenetic and novel genetic approaches for the development of various grasses, several of which have resulted in patents and patents pending.

GENOMIC PREDICTION MODELS FOR FORAGE QUALITY IN TIMOTHY (*PHLEUM PRATENSE* L.) IN DIVERSE ENVIRONMENTS

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Timothy (*Phleum pratense* L.) is a perennial, cross-pollinating hexaploid ($2n=6x=42$) grass species distributed naturally throughout Europe and parts of North Africa and Asia. It is one of the most important input factors in the Norwegian agriculture. New improved cultivars with high yield capacity and forage quality, which are well adapted to the future climate, are very important for an economically sustainable Norwegian milk and meat production. In view of the recent developments in sequencing, molecular marker technologies and theoretical foundations, it is feasible to start the development of genomic selection (GS) based breeding schemes in timothy. The main objective is to develop GS methods for breeding of the forage grass timothy by implementing the following strategies: 1) Develop single nucleotide polymorphism (SNP) marker allele frequencies (GWAF) for 918 full-sib families of timothy using genotyping-by-sequencing technologies. 2) Assess the relative efficiency of GS for forage quality and trait stability across environments using different GS models. 3) Apply genome-wide association studies in conjunction with GS to unravel the genetic architecture of forage quality. 4) Validate the GS predictions for forage quality in 242 unrelated full-sib families. A total of 29,000 SNPs markers were identified in all the full sib families. These markers were used to test different genomic selection models for forage quality traits. Genomic predictions results will be discussed in this presentation.

PRESENTER BIO: Dr. Kovi is a Research Scientist at NMBU, Norway. His research focus on forage grass genetics at NMBU, Norway. Previously, he worked on rice quantitative genetics in China, as part of his PhD. Currently; he is working on implementing genomic selection models in timothy (*Phelum pratense* L.)

DEVELOPMENT OF SIMPLE SEQUENCE REPEAT (SSR) MARKERS IN *MEDICAGO RUTHENICA* AND THEIR APPLICATION FOR EVALUATING OUTCROSSING FERTILITY UNDER OPEN-POLLINATION CONDITIONS

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Medicago ruthenica is a perennial leguminous plant with great economic value for forage production and soil conservation in northern China. Simple sequence repeat (SSR) markers are a major molecular tool for genetic and genomic research that have been extensively developed and used in major crops. Recurrent selection procedures have been widely employed in the genetic improvement of populations and development of cultivars in *M. ruthenica*. However, genomic and sexual reproductive information is very limited, hindering its use in genetic and traditional breeding research. Thus, the present study was undertaken with the following two objectives: (1) to develop and characterize a large set of SSR markers for *M. ruthenica* and (2) to examine outcrossing fertility in *M. ruthenica* under open-pollination conditions.

Using transcriptome data, we developed 213 polymorphic SSR markers. Additionally, we used these markers to quantify selfing and outcrossing rates of *M. ruthenica* plants under field conditions. Progeny-array approach (PAA) is the powerful approach used for estimating selfing rates. The PAA is based on the comparison between offspring and maternal genotypes. The outcrossing rate was calculated to be 75.9%. These developed SSR markers will provide a useful tool for marker-trait association, QTL mapping, and genetic diversity analysis in *M. ruthenica*. In addition, the accurate fertilization mode under open pollination in the field has increased our knowledge of the sexual reproductive biology of *M. ruthenica*. The results should be highly valuable for molecular and traditional biological research.

PRESENTER BIO: Dr. Hongyan Li is a Professor with more than 10 years of experience researching forage germplasm. She has extensive experience with forage genebank working. And has led more than several projects of Investigation on collection of forage germplasm resources. Dr. Jun Li is a Research Assistant.

RESEARCH ON MOLECULAR MECHANISM OF SEEDS GERMINATION IN SHEEPGRASS

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Sheepgrass (*Leymus chinensis* (Trin.) Tzvel)) is an important perennial forage grass that is widely distributed in the Eurasia steppe. The relatively weak seed industry has been a bottleneck since long time. Our previous studies showed that germination rate of sheepseed germplasm vary significantly among genotypes. However, the underlying molecular mechanisms of seed germination are still mostly unknown.

In this study, we performed comparative transcriptomic analyses of high seed germination rate (H) and low seed germination rate (L) genotype at 14, 28, and 42 days after pollination. After comparing 3 consecutive development stages, 9255, 5366, and 4306 genes were found to be significantly differently expressed between H and L. We identified 81 transcription factor (TF) families, and some TFs genes were differently expressed between the two genotypes.

Among the identified genes, a transcription factor *LcbHLH92* which is negatively correlated with those of anthocyanin/proanthocyanidin-specific pathway genes, *anthocyanidin synthase* (*ANS*) and *anthocyanidin reductase* (*ANR*). The *LcbHLH92* gene had two transcripts, *LcbHLH92a* and *LcbHLH92b*, and their expression could be induced by ABA, cold and NaCl. Overexpression of *LcbHLH92a* or *LcbHLH92b* in *Arabidopsis* significantly inhibited the transcript levels of *dihydroflavonol reductase* (*DFR*) and *ANS* genes in leaves and seeds, which resulted in a decrease in anthocyanins and proanthocyanidins, respectively. Importantly, transgenic *Arabidopsis* seeds with a yellow color showed a higher germination rate than did wild-type controls with a brown seed color. In addition, *LcbHLH92a* and *LcbHLH92b* repressed the transcription of *Transparent Testa8* (*TT8*), *ANS*, *DFR*, and *ANR* possibly by elevating the transcript levels of Jasmonate-ZIM-domain proteins (*JAZs*) through binding to their promoters. In summary, this is the first investigation of *LcbHLH92* and its relationship to anthocyanin/proanthocyanidin biosynthesis and seed germination.

PRESENTER BIO: Dr. Liu is a Professor with more than 20 years of experience researching germplasm and gene resources of sheepgrass. His research areas include forage genetic resources and molecular breeding, sexual reproduction mechanism and the molecular basis of resistance of sheepgrass.

TRANSGENIC CENTIPEDEGRASS OVEREXPRESSING S-ADENOSYLMETHIONE DECARBOXYLASE (SAMDC) GENE FOR IMPROVED COLD TOLERANCE

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Polyamine regulates plant growth, development and stress responses as an important plant growth substance. S-adenosylmethionine decarboxylase (SAMDC) is a key enzyme for polyamine biosynthesis, while polyamine oxidase (PAO) catalyzes oxidation of polyamines to produce H₂O₂. Centipedegrass (*Eremochloa ophiuroides* [Munro] Hack.) is an important warm-season turfgrass species with low cold tolerance. The objective of this study was to improve the cold tolerance in centipedegrass by transgenics. Transgenic centipedegrass plants overexpressing a cold responsive SAMDC gene from bermudagrass (*CdSAMDC1*) were generated in this study. Transgenic plants showing *CdSAMDC1* transcript had higher levels of spermidine (Spd) and spermin (Spm) and enhanced freezing and chilling tolerance as compared with the wild type (WT). In addition, transgenic plants had higher levels of PAO activity and H₂O₂ than WT, which were blocked by pretreatment with methylglyoxal bis (guanyldrazone) or MGBG, inhibitor of SAMDC, indicating that the increased PAO and H₂O₂ were a result of expression of *CdSAMDC1*. Transgenic plants had higher levels of nitrate reductase (NR) activity and nitric oxide (NO) concentration. The increased NR activity were blocked by pretreatment with MGBG and ascorbic acid (AsA), scavenger of H₂O₂, while the increased NO level was blocked by MGBG, AsA, and tungstate (inhibitors of NR), indicating that the enhanced NR-derived NO was dependent upon H₂O₂, as a result of expression *CdSAMDC1*. Elevated superoxide dismutase (SOD) and catalase (CAT) activities were observed in transgenic plants than in WT, which were blocked by pretreatment with MGBG, AsA, tungstate and PTIO (scavenger of NO), indicating that the increased activities of SOD and CAT depends on expression of *CdSAMDC1*, H₂O₂, and NR-derived NO. Our results suggest that the elevated cold tolerance was associated with PAO catalyzed production of H₂O₂, which in turn led to NR-derived NO production and induced antioxidant enzyme activities in transgenic plants.

PRESENTER BIO: Dr. Lu is a Professor with more than 20 years of experience researching turfgrass and forage. She has extensive experience with turfgrass stress physiology and molecular breeding and has published 28 papers on internationally peer reviewed journals dedicated to abiotic stress adaptation mechanism and improvement of turfgrass and forage.

GENOTYPING BY SEQUENCING APPROACH FOR AUTOTETRAPLOIDY *UROCHLOA RUZIZIENSIS* GENETIC BREEDING

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Although Brazil is the major producer and exporter of beef in the world, the rangelands are cultivated with varieties obtained by selection on germplasm and conventional breeding. Only lately genomic information started to be used in the tropical forage breeding programs mostly because of the genome complexity and costs. *Urochloa ruziziensis* is a sexual autotetraploid forage that has a significant role in integrated systems that are currently important in the agribusiness in certain regions of Brazil and is essential as female parent in the other *Urochloa* spp. breeding programs. The objective of this work is to develop genetic information for genomic selection in the *U. ruziziensis* breeding program. A hundred and thirty individuals of a half-sib progeny and 50 progenies with bulked DNA of the first cycle of intrapopulation selection were sequenced using genotyping by sequencing (GBS). The reduction of the DNA complexity was made with two restriction enzymes, EcoT22I and MspI, and libraries were sequenced as 150-bp single-end reads on Illumina NextSeq 500 platform. A total of 727,616,969 sequence reads were obtained and processed by Tassel software, the tags created by the software were aligned using Bowtie2 to a draft genome of the specie, 15,97% of the tags aligned 0 times, 56,24% aligned more than 1 time and 27,78% aligned 1 time, only the tags that aligned 1 time were used in the genotyping, 154.467 SNPs were discovered by Tassel. SuperMASSA and Updog softwares estimated each tetraploid individual allele dosage by considering the general population model. This information enabled the construction of the first integrated genetic map for the species using tetraploid allele dosage. In addition, the SNPs markers and the phenotypic data of the 50 progenies of the first cycle of intrapopulation breeding will allow the prediction of the genomic breeding values using individual simulated best linear unbiased prediction (BLUPs) and estimate the markers genetic effects.

PRESENTER BIO: Mr. Martins is a PhD student that worked with sugarcane single copy genes in graduation and is currently researching on tropical forages for genetic breeding.

GENOMIC SELECTION OF AGRONOMIC TRAITS OF WINTER WHEAT: A CASE STUDY IN A FORAGE WHEAT BREEDING PROGRAM

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Genomic selection (GS) is an alternative form of marker-assisted selection method that can improve genetic gain in plant breeding. Phenotyping forage agronomic traits of winter wheat is expensive and time consuming because forage yield, for example, is measured multiple times during a growing season. In this study, we compared the prediction accuracies of four GS models (RRBLUP, GBLUP, GAUSS and BL) for forage agronomic traits of winter wheat. Furthermore, we assessed the impact of training population (TP) size and marker density on prediction accuracy of three agronomic traits. The Hard Winter Wheat Association Mapping (AM) panel ($n=298$) from the Triticeae Coordinated Agricultural Project (TCAP) was used in this study. The panel was genotyped with the wheat iSelect 90K SNP array and phenotyped for forage yield (FY), plant height (PH) and heading date (HD) in two field environments. We evaluated the effect of TP size and marker-density on prediction accuracy by varying the TP size ($n_{TP} = 60, 120, 180$ and 238) and the marker density ($p = 500, 1,000, 1,500, 3,000, 3,484$). The GS models evaluated in this study gave moderate to high prediction accuracies ranging from 0.46 to 0.49 for PH, 0.66 to 0.69 for FY, and from 0.73 to 0.74 for HD. The RRBLUP, GBLUP and GAUSS models produced higher prediction accuracies for most traits than BL. Prediction accuracy for all traits increased with increase in TP size and marker density in all the GS models tested. In general, prediction accuracy increase started to plateau at $n_{TP} = 180$ and $p = 1500$ SNPs suggesting that for the panel, the optimal TP size and marker density were 180 lines and 1500 SNPs, respectively. Among the GS models, the impact of TP size on prediction accuracy was more effective for RRBLUP compared to the other models. In general, the RRBLUP and GBLUP models had the highest prediction accuracy than the other models with the same marker density, and increasing TP size was more effective than increasing marker-density. This case study suggests that GS can facilitate the selection of agronomic traits during forage wheat breeding.

PRESENTER BIO: Dr. Frank Maulana is a Postdoctoral Research Fellow at Noble Research Institute, LLC, working on genome-wide association mapping (GWAS) of wheat seedling drought and heat tolerance, QTL mapping of dual-purpose traits and genomic selection (GS) of forage agronomic and quality traits of winter wheat for forage and grain production.

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RAPID DETECTION OF DROUGHT STRESS USING FIELD RADIOMETRY FOR HIGH THROUGHPUT SCREENING

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The time associated with assessing breeding lines through traditional methods serve as a bottleneck in the selection process. Improved objective measurement of spectral properties can accelerate the screening process and lead to faster selection of improved varieties. Our research objective was to compare vegetation indices (VI) associated with drought stress across soil types and turfgrass species. Treatments were arranged in a 3 x 2 factorial design across six replications under greenhouse conditions. '007' creeping bentgrass (CBG) and 'Latitude 36' hybrid bermudagrass (HB) were grown on 90:10 USGA-specified sand, sandy loam, and clay. All treatments were watered beyond field capacity and allowed to drain for four hours. Volumetric water content, spectral reflectance with a field radiometer, visual assessment of turf quality, and visual estimation of wilt were collected hourly between 0700 and 1900 from initiation until two days after the last plot reached complete wilt. Data were modeled using non-linear regression with parameters compared across soil type and grass species. Effects tests indicated differences in both grass species and soil type. Parameter shifts indicative of drought stress in CBG occurred earlier than HB, regardless of VI or soil type. Turf grown in sand maintained vigor longer than in sandy loam or clay soils. However, all VI examined were negatively correlated ($r \geq -0.67$) with visual wilt regardless of soil type or grass species, with the Water Band Index ($WBI = R_{900}/R_{970}$) being most closely related ($r = -0.85$). Index values were plotted over time and fitted using the 4-parameter logistic model. Inverse prediction of VI inflection points indicates that the WBI and the green/red ratio (GRI) were more sensitive to drought stress than the commonly used NDVI. However, the practical application of screening for drought stress using GRI is limited because of phenotypic variability across breeding lines. Unlike GRI and NDVI, WBI is unrelated to visual characteristics. Our data suggest that the WBI could be a useful tool to fast-track turf and forage breeding across grass species and soil variability.

PRESENTER BIO: Dr. McCall is an Assistant Professor of Turfgrass Pathology. His primary research focus is on early detection of biotic and abiotic stresses across turfgrass systems using field radiometry, mobile ground-based sensors, unmanned aerial vehicles. He has presented this research to professional and scientific audiences at regional, national, and international meetings.

UROCHLOA BRIZANTHA MOCK REFERENCE GENOME FROM GBS-SNP-CROP APPROACH

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Urochloa brizantha is the most important forage used as a pasture in Brazil and in the tropical world. There are several studies published about forage physiology, cytogenetic, pasture management, classic breeding and animal harvesting. However, low genomic information is available to understanding the complexity of traits and the genetic bases that control the forage grass growth. Breeding panels and polyploid species as *U. brizantha* can be evaluated genomically using genotyping-by-sequencing methods (GBS), but estimating polyploids genotypes require higher read depth compared to diploids. One of the most important step in the single nucleotide polymorphisms (SNPs) discovery pipeline is the reads alignment in a reference genome. However, the full reference genome of *U. brizantha* is not available yet, then it is necessary to use the closest related species as a reference genome. Another alternative to perform the alignment in species without reference to build a mock reference using their own GBS reads. We developed the *Urochloa mock* reference genome by using the reads of the most important cultivar in tropical regions the *U. brizantha* cv. Marandu. The GBS-SNP-CROP pipeline was used to align and to organize the nucleotides sequences in centroids. All centroids were annotated by homology using BLAST tool comparing with the Uniref90 data bank to determined candidate positions functions. Approximately 16 thousand of centroids sequences were annotated by homology and around of 20 thousand of possible functions were identified. About 1.5 thousand sequences were greater than 200 bp and their Uniref90 annotations were compared with the UniProt bank. This analysis generated around 2.1 thousand of different genes from around 200 different organisms. The use of this mock reference can improve the depth of reads during the alignment step for SNP discovery of *Urochloa* species and it can help on the genotype calling for tetraploid level.

PRESENTER BIO: Dra. Karem Meireles is a researcher at the Embrapa Beef Cattle for 12 years, and works with molecular genetics of tropical forages *Brachiaria* spp. and *Panicum maximum*.

GENETIC IMPROVEMENT OF CENTIPEDEGRASS THROUGH CHEMICAL MUTAGENESIS

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Centipedegrass [*Eremochloa ophiuroides* (Munro) Hack] is a warm-season perennial grass well adapted for turf across the southern United States. Centipede requires few inputs and is more tolerant to pests than most turfgrasses. As most U.S. centipedegrass germplasm originates from a single accession introduced from China in 1912, the genetic base for this species is narrow. A lack of morphological variation in the species has hampered breeding efforts and, as a result, few commercial cultivars are available in the market. The objective of this study was to use chemical mutagen ethyl methane-sulfonate (EMS) to generate genotypic and phenotypic variation for centipedegrass improvement.

Seeds of cultivar 'Common' were treated with each 0, 0.125, 0.25, 0.375 and 0.5% (w/v) of EMS for 16 hours. Following mutagenic treatment of seeds, 50 regenerated plantlets from each of the three highest treatments were planted in the field and allowed to self-pollinate. A total of 3,000 M₁ progenies were recovered and subsequently established at the Sandhills Research Station (Jackson Springs, NC) for initial evaluation of establishment rate, leaf texture, genetic color, drought tolerance, winter survival and seed head presence. In June of 2017, 85 lines were selected for their superior performance across traits and established in replicated trials at the Upper Mountain Research Station (Laurel Springs, NC), the Lake Wheeler Turfgrass Field Laboratory (Raleigh, NC) and the Sandhills Research Station. Commercial cultivars 'Covington', 'Santee', 'Tifblair' and 'Common' were included as checks.

Preliminary data analysis from 2017 and 2018 has identified seven lines (CEN 15116, CEN 151306, CEN 15453, CEN 15522, CEN 15773, GC 12844, and GC 12861) with superior turf quality, rate of establishment, and winter survival over the commercial checks evaluated in the trial. Additionally, these lines have been identified as having significantly higher seed yield. Evaluation of these lines for drought tolerance and establishment from seed will be conducted in 2019. Superior genotypes will be advanced to regional trials to evaluate their potential for commercial release.

PRESENTER BIO: Dr. Susana Milla-Lewis is an associate professor with 10 years of experience in turfgrass breeding and genetics. Her background is in germplasm characterization, development of breeding populations, and the utilization of new technologies in association with conventional breeding methods for plant improvement.

IS SIMULTANEOUS IMPROVEMENT OF FALL DORMANCY (FD) AND WINTER HARDINESS (WH) POSSIBLE IN ALFALFA? QTL MAPPING AND INHERITANCE OF FD AND WH

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Alfalfa (*Medicago sativa* L.), the queen of forages, possesses an important characteristic known as fall dormancy (FD) which reduces growth and yield of certain alfalfa genotypes in short day length and low temperature. Alfalfa winter-hardiness (WH) also impacts in yield and persistence. Therefore, understating the genetic basis of FD and WH is important. The objectives of this study was to identify QTLs associated with FD and WH to understand the genetic relationships between the two traits. An F1 mapping population was developed by crossing two cultivars with contrasting FD; 3010 (FD = 2) and CW 1010 (FD = 10). The F1 progenies, parents, and checks were planted using RCBD design with three replications at two locations in GA. Genotyping by sequencing (GBS) was used for discovering SNP markers. The FD of F1 and parents were estimated using a standard equation derived from the regression of FD rating and regrowth height after autumn clipping of standard checks. Dormancy assessment was also carried out in early winter. Similarly, WH was scored using visual rating of the plants. We observed a moderate positive correlation between FD and WH, especially when dormancy was assessed in winter. We filtered 5,348 single dose allele (SDA) SNPs for maternal parent (3010) and 2327 SDA for paternal parent (CW 1010). The maternal linkage map retained 1837 SNPs in 32 LG and paternal parent retained 1377 SNPs for the same number of LG with average density of 1.5 cM/SNP. 32 LG for each parent were assigned to four corresponding homologs of the eight chromosomes of model legume *Medicago truncatula*. We identified 45 significant ($P \leq 0.05$) QTLs for dormancy and 35 QTLs for winterhardiness using composite interval mapping method in WinQTL Cartographer (2.5). This is the first QTL mapping work in alfalfa using such dense genetic linkage maps. Few numbers of the QTLs detected for FD and WH for both parents colocalized completely or partially. However, more than 70% of the dormancy QTLs detected in both parents did not share genomic regions with respective winter-hardiness QTLs, suggesting that the two traits are genetically independent and therefore can be improved simultaneously in the same germplasm.

PRESENTER BIO: Dr. Missaoui is leading research in forage and bioenergy breeding and genetics at the University of Georgia and teaches advanced plant breeding at the Institute of Plant Breeding Genetics and Genomics. Prior to this role, he worked as Discovery Breeding scientist with Monsanto for 7 years focusing on developing molecular approaches for capturing genetic diversity and maximizing the efficiency of germplasm enhancement. His research interests are focused primarily on applied genetic research of perennial cool season grasses and legumes in addition to perennial grasses as feedstocks with improved suitability as biofuel. He works toward integrating molecular technologies into crop improvement.

TARGETED MUTAGENESIS OF THE COMPLEX SUGARCANE GENOME WITH CRISPR/CAS9

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Genome editing of sugarcane is challenging due to large and complex genome, high ploidy and high content of repetitive DNA. To test the efficiency and specificity of CRISPR/Cas9-mediated genome editing in sugarcane, we focus on the establishment of an easily scorable knockout target for rapid validation of edited events. Therefore, initial target genes include those that are well known to create albino or chlorotic plantlets following loss of function mutations. In this study, two target genes including 4-hydroxy-3-methylbut-2-enyl diphosphate reductase (IspH) and phytoene desaturase (PDS) were selected for gene knockout. IspH catalyzes the terminal step of the methyl-D-erythritol-4-phosphate (MEP) pathway for isoprenoid precursor biosynthesis. PDS, another essential plant carotenoid biosynthetic enzyme, is involved in the biosynthesis pathway of β -carotene which provides photoprotection of chlorophyll. So far we have amplified multiple alleles of the target genes from sugarcane, designed gRNA's *in silico*, tested the associated RNP's *in vitro* for cleavage of the target gene amplicons and constructed vectors for transformation.

PRESENTER BIO: Dr. Mohan is a visiting scientist in Prof. Fredy Altpeter's research program at the University of Florida supported by São Paulo Research Foundation. He earned his PhD in Plant Biotechnology in 2015 from the Bharathiar University, India and has gained extensive experience with genetic engineering of sugarcane as Senior Research Fellow at the Sugarcane Breeding Institute, Coimbatore, India.

INTEGRATING GENOMICS, TRANSCRIPTOMICS AND PHENOMICS TO ADVANCE PLANT BREEDING STRATEGIES

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Increased demands for agricultural products developed using environmentally friendly practices are driving forces to develop agricultural systems that are more productive, adaptable to erratic or extreme weather patterns and require fewer on-farm inputs. Current factors limiting crop production on a global scale include abiotic and biotic stresses. Integration of new technologies including genomics, transcriptomics and phenomics can be utilized to develop efficient solutions to these agricultural challenges. Alfalfa (*Medicago sativa* L.) is a perennial forage legume with global adaptability and agricultural value. Our approach includes evaluating genetic variation for stress adaptation and field performance of alfalfa germplasm, understanding the underlying mechanisms associated with these traits and identifying functional sequence variants for use during selection to increase the frequency of favorable alleles. High-throughput phenotyping strategies that utilize drones and sensors mounted on pheno-mobiles were used to identify variation for key traits in alfalfa germplasm evaluated in the field. Additionally, specific root traits or phenes that enhance the capacity for efficient nutrient and water uptake have been associated with productivity under abiotic stress conditions. The alfalfa genome sequence (V1.0), the gene expression atlas generated from plants grown under different stress conditions and multiple tissues, and genome-wide single nucleotide polymorphisms (SNPs) associated with phenotypic traits were integrated into the Alfalfa Breeder's Toolbox. The implementation and use of this user-friendly Toolbox enables basic and applied research and can facilitate integration of approaches to track the accumulation of favorable alleles during selection. Further, the Alfalfa Breeder's Toolbox will enable the use of genomics, transcriptomics and phenomics datasets to accelerate genetic gains in plant breeding programs and effectively address current and future challenges to crop productivity.

PRESENTER BIO: Dr. Monteros is an Associate Professor with over 10 years of experience developing resources for molecular breeding of forage legumes. Her research focuses on alfalfa genomics, abiotic stress responses and molecular breeding strategies.

GENOTYPING BY SEQUENCING PROVIDES NEW INSIGHTS INTO THE MOLECULAR GENETIC DIVERSITY OF NAPIER GRASS COLLECTIONS AND IDENTIFIED CANDIDATE GENES ASSOCIATED WITH IMPORTANT FORAGE TRAITS

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Napier grass (*Cenchrus purpureus* syn. *Pennisetum purpureum* L.), is an important tropical forage grass used mainly as a cut and carry feed. Napier grass has important attributes like high yielding/unit area, year-round availability, resistant to most diseases and pests, easy to establish and propagate, fast regrowth capacity, and potential as an energy crop. However, Napier grass is one of the underutilized and less researched forage crops. The ILRI genebank holds a diverse set of Napier grass accessions collected across a range of environments and origins and has a collection contributed by the Brazilian Agricultural Research Corporation (EMBRAPA). The objectives of our research are to: (i) assess the genetic diversity existing in the collections; (ii) determine genome wide linkage disequilibrium (LD) patterns; (iii) identify markers and candidate genes associated with important traits, and; (iv) subset representative genotypes that represent the diversity existing in the collections. One hundred and five accessions were genotyped by the DArTseq platform generating high-density and polymorphic SilicoDArT and SNP markers together with short sequence reads corresponding to each marker. The short sequence reads were mapped to the pearl millet reference genome, which is the closest related species to Napier grass and, based on the map position, the closest genes aligned with the markers and the corresponding annotation information extracted. In turn, these data were used to select candidate genes which could be associated with important forage traits. Population structure and diversity were assessed using selected highly polymorphic SNPs distributed across the genome. The presence of two to seven subpopulations was detected by the admixture model in STRUCTURE, phylogenetic, and principal component analysis (PCA). A few representative Napier grass accessions were subsetting from the diversity with the objective to distribute a few representative accessions for adaptation in different agroecological conditions. Genome-wide LD analyses detected a fast LD-decay, on average at about 2.54 kbp, in the overall population and showed variation in LD-decay patterns between collections. In an association analysis using agronomic and morphological traits collected in three successive harvests, 43 markers (14 SNPs and 29 SilicoDArTs) associated with plant height, and 41 markers (17 SNPs and 24 SilicoDArTs) associated with tiller number per plant were identified. Both the plant height and tiller number traits are highly correlated with total fresh and dry weights. In conclusion, the DArTseq genotyping generated high-density markers with a reasonable distribution across the genome which were suitable for genetic and molecular diversity analysis. The diversity analysis revealed the existence of a substantial amount of variation in the ILRI collection and identified some unique materials from the EMBRAPA collection, showing the suitability of the population for further genetic and marker-trait association studies. The associated markers need to be verified on successive harvests before they can be used for the selection of improved Napier grass genotypes.

PRESENTER BIO: Dr. Muktar is a Postdoctoral scientist in the Feed & Forage Development program at ILRI, Ethiopia. He holds a PhD on molecular genetic from the Max Planck institute for plant breeding research, Germany, and worked at Obihiro University (Japan) as a postdoctoral researcher in the potato germplasm enhancement laboratory.

REVIEW: BREEDING AND MOLECULAR RESEARCH OF SOME TROPICAL GRASSES IN JAPAN

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The content provided below is intended to be a review of germplasm, breeding and molecular research of some tropical grasses in Japan. The breeding and research of tropical grasses for animal feeds in Southern part of Japan was initiated in 1960's, as the consumption of beef was increasing in Japan.

Introduction, collection and evaluation: Early in Japan, *Paspalum* spp. caught attention of researchers because this genus were tolerant to frost and winter killing in the Southern Kyushu. Germplasm of dallisgrass (*P. dilatatum*) and bahiagrass (*P. notatum*) were introduced from the U.S.A. Rhodesgrass (*Chloris gayana*) and guineagrass (*Panicum maximum*) then caught the attention as potentially useful summer annuals in converted rice fields. This germplasm was mainly introduced from Australia and yield trials were made. From 1971 to 1973, two Japanese scientists made collection excursions to East Africa and collected ca. 2,300 accessions of tropical grasses. This collection is named the "Africa collection". Within the collection, 140 guineagrass accessions were evaluated for the establishment of an apomixis breeding program, trials to identify and isolate gene(s) for apomixis and the development of new apomictic varieties. Of special note is a diploid sexual accession, GR297. The importance of the Genebank activities and mutation breeding towards various utilization are discussed below.

Research: Formerly, research was focused on identifying ploidy level and reproductive methods related to the domestic seed production in Japan. Tropical grasses includes self- and/or out-crossing, and apomictic ones. First national project was focused on improving seed production of forage grasses through cytological approach, field trials and identification of key characters of seed production. Recently, genomic research on rhodesgrass, zoysiagrass, *Panicum* and *Brachiaria* has been made.

Breeding: In *Paspalum*, 1 dallisgrass, and 2 bahiagrass cultivars were developed and registered. In *Panicum*, 5 guineagrass cultivars and 1 sexual maternal line, 2 colored guineagrass (*P. coloratum*) cultivars have been registered. Three rhodesgrass cultivars were developed through maternal line selection and line selection. One of the most famous guineagrass cultivar is apomictic cv. Natsukaze, a hybrid between an isolated sexual line and an apomictic line. During the progeny testing of the open-pollinated sexual GR297 guineagrass, a vigorous apomictic 4x hybrid plant was identified from the progeny and registered as cv. Natsukaze, after the yield trials in various parts of Japan. It also utilized as a cleaning crop for the control of root-knot nematodes. Award of Japanese Society of Breeding was presented to our group for the development of this and cv. Natsuyutaka guineagrass.

Molecular Research: In guineagrass and *Brachiaria*, genomic studies of apomixis gene(s) have engaged Japanese researchers. In guineagrass, chromosome doubling of the GR297 was attempted and the 4x sexual line, Nekken No. 1 was developed. This line has been utilized as a maternal parent for the development of new varieties, mapping and linkage analysis of apomixis. Even though the locus for apospory was identified, markers linked to the gene(s) exhibited no recombination. This apospory-specific genomic region was physically mapped by FISH to a single chromosome.

PRESENTER BIO: Nakagawa is one of founders of MBFT. He was Director of Biomass Research & Development Center and Institute of Radiation Breeding with experience researching tropical forage and turfgrass, and a leader of biomass research and mutation breeding in MAFF. He is Distinguished Professor of Jiangsu Academy of Agricultural Sciences.

THE FIRST IN-DEPTH INSIGHT INTO THE GENETIC DIVERSITY OF A BUFFELGRASS (*CENCHRUS CILIARIS* L.) COLLECTION USING GENOTYPING-BY-SEQUENCING ANALYSIS

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Buffelgrass [*Cenchrus ciliaris* (L.) Link (syn. *Pennisetum ciliaris* L.)] is an important drought tolerant, warm season, perennial forage grass widely grown in the tropics and subtropics. It is native to different parts of Africa, the Middle East and Asia. A large collection of buffelgrass obtained from different parts of Africa is maintained in the genebank at the International Livestock Research Institute (ILRI) in Ethiopia. So far, most of the diversity information on the collection is based on agro-morphological characterization, where plenty of variation has been identified. Thus, there is an opportunity to complement this with molecular data to advance and accelerate selection of climate resilient superior genotypes. In this study, we report on the genetic diversity of a buffelgrass collection based on a genotyping-by-sequencing (GBS) approach using the DArTseq platform. Genotyping data of 111,917 Silico DArT and 93,501 SNP markers were obtained for 185 accessions with average polymorphism information content values between 0.0 to 0.5 per marker. Of the generated markers, 8,053 (7 %) Silico DArT and 15,465 (16 %) SNP were mapped to the *Setaria italica* reference genome. Representative markers covering the reference genome from both marker types were selected for further diversity assessment of the collection. The results revealed the genetic richness of the collection and separated the collection into two main groups. This information will be useful for different purposes including core collection/subset development, trait-marker association analysis and impact assessment of the use of genetic resources from the Genebank.

PRESENTER BIO: Dr. Alemayehu Teressa Negawo is a research officer/laboratory supervisor at International Livestock Research Institute and has been working on molecular studies of forage genetic resources for over three years. He has experience on tissue culture, molecular characterization and genetic transformation of various crops.

IMPROVING THE USE OF GENOMIC TOOLS IN TROPICAL FORAGE GRASS BREEDING WITH DIPLOID RUZIGRASS AS A REFERENCE

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Beef production in Brazil is based on planted pastures, with most of the area occupied by species of *Urochloa*. Breeding in the genus relies on intra- and inter-specific crosses within an agamic complex including three *Urochloa* species, which compose a mixture of sexual diploids and polyploids, among facultative or obligate apomicts. One of these species, ruzigrass (*Urochloa ruziziensis*), is a diploid tropical forage grass known for its high nutritional quality. Ruzigrass plays a crucial role in brachiaria grass breeding as a sexual female parent that can be crossed with apomicts, once its chromosome number is doubled with colchicine. In *Urochloa*, it is estimated that the development and release of a new cultivar can take up to 10 years. There is room for significant improvement in rates of genetic gain by using more efficient breeding methods and reducing breeding cycles. While marker-assisted selection and genomic prediction would be valuable tools for this goal, use of genomic information in tropical grass forage breeding is still limited. Application of molecular tools for QTL mapping, genome sequencing and assembly or marker-assisted selection is rare or non-existent. Our group has focused on acquiring genomic information in diploid ruzigrass, in order to provide useful tools for *Urochloa* breeding and genetics. Previous studies based on shallow Illumina sequencing allowed the development of the first microsatellite markers for the species, assessments of germplasm diversity and structure, assembly and annotation of complete plastid genomes for four *Urochloa* species, as well as characterization of their phylogenetic divergence. Next, we assembled a draft genome for ruzigrass C69 heterozygous clone using PacBio Sequel single-molecule real-time sequencing. The current assembly obtained with FALCON contains 7,628 primary contigs, with NG50 of 412 kbp. Assessment of genome completeness using BUSCO showed 80.7% complete genes, 21.7% of which were duplicated. In addition, RNA-seq data was obtained using Illumina NovaSeq, totaling approximately 500 million reads and 30 Gbp of raw data. Initial transcriptome assemblies obtained with Trinity, SOAPdenovo-Trans, rnaSPAdes, and Velvet/Oases, totaling 11 million transcripts, were used as input for EvidentialGene-R pipeline to generate a high-quality gene set. This produced 108,000 transcripts, which will be used for gene model prediction and genome annotation. Further steps include the optimization of assembly quality parameters, haplotype phasing and polishing, haplotig deduplication, and Hi-C scaffolding. A high-quality genome assembly for ruzigrass will aid research groups in the development and application of genomic tools in breeding and genetics of brachiaria grasses and closely related species. In addition, ongoing research is also focused on establishing the necessary foundations for genomic selection in ruzigrass.

PRESENTER BIO: Dr. Pessoa-Filho is a Research Scientist at Embrapa, applying genomics to improve the use of plant genetic resources by breeding programs. He has experience with the development and use of molecular tools in genetics and breeding of crops such as rice, soybean, cassava and ruzigrass.

RESILIENCE AND STABILITY OF ALFALFA CULTIVARS IN NORTH AMERICA

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Resilient, stable, and productive forage systems are needed to endure increasingly frequent climatic extremes. However, no assessment of resilience of cultivars has been reported. Resilience is the ability of a forage system to withstand a climatic crisis with high yields; stability is the minimal variability of yields across normal years; productivity is the average yield across normal years. The goal of this research was to quantify resilience, stability, and productivity of alfalfa cultivars to identify superior cultivars. Forage yield means from alfalfa cultivar trials from eleven US states and one Canadian province over nineteen years (1995 to 2013) were analyzed using linear mixed models. Quantitative measures for resilience and stability for each cultivar were calculated in each location. Resilience, stability and productivity were different among cultivars across locations, showing that some cultivars were consistently superior for each variable. Cultivar resilience was not associated with stability, which suggests these are two different traits which should not be confused. Disease resistance was positively associated with cultivar resilience. A coordinated plant breeding approach across many locations is proposed to test and improve cultivar resilience in the future, and develop alfalfa cultivars more profitable in the long term.

PRESENTER BIO: Dr. Picasso is an Assistant Professor, researching forage management, perennial grains, agroecology, and climate change. He has conducted interdisciplinary international research on climate change mitigation and adaptation, focusing on ecological intensification of livestock systems. He also studies and develops perennial dual-purpose grain and forage crops like Kernza Intermediate wheatgrass.

VARIABILITY IN VERNALIZATION REQUIREMENTS OF KERNZA INTERMEDIATE WHEATGRASS POPULATIONS

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Intermediate wheatgrass is a cool season forage species (*Thinopyrum intermedium*) which has been bred for greater seed yield in North America and has become the first perennial grain and forage, commercially available under the tradename Kernza. One of the main constraints for the expansion of this new crop to temperate areas is the vernalization (cold) requirement for flowering. However, genetic variability of vernalization in this species has not been explored yet. The goal of this research was to evaluate the vernalization requirements of different populations of Kernza Intermediate wheatgrass, to identify materials adapted to temperate regions. Seeds from eight different Intermediate wheatgrass populations origins (The Land Institute Cycle 4 and 5, and five University of Minnesota Synthetics) were planted in a greenhouse experiment UW – Madison Walnut Street Greenhouse from October 2017 to June 2018. Seedlings were grown in conetainers in the greenhouse at 25°C and 16 hs light (8 h winter daylight plus 8 h low-intensity incandescent light), until plants reached the three-leaf stage and then moved to growth chambers for vernalization treatments (4°C and 10 hs light) from 1 to 7 weeks of incubation, removing plants from the growth chambers once every week. After incubation, all plants were transplanted to pots and grown at 25°C and 16 h in greenhouse. Percent of plants heading, heading time, plant height, spikes per plant, spikelets per spike, spikelets per plant, spike length and above ground biomass were measured for each plant. The experimental design was a randomized complete block design with factorial structure of treatments: factor 1 was seed origin and factor 2 was length of vernalization period. From two to six plants for each seed origin were used in each treatment.

Heading percent increased with the length of vernalization period from 1 to 5 weeks and then reached a maximum. Plants that achieved flowering, reached its maximum number of spikes with only three weeks of primary induction. The population MN02 had higher heading percent than TLIC5, TLIC4MN, TLIC4A and MN01, while MN03, MN04 and MN05, achieved intermediate heading percent. While with optimal vernalization conditions all populations reached high percent of headed plants, with infra-optimal conditions (less than 3 weeks) the synthetic population MN02 showed more headed plants than TLIC5, TLIC4MN and TLIC4WI while all remaining synthetic populations reached intermediate heading percent.

These results confirm that breeding populations of Kernza intermediate wheatgrass differ in vernalization requirements, which could be used for breeding materials adapted to temperate areas.

PRESENTER BIO: Dr. Picasso is an Assistant Professor, researching forage management, perennial grains, agroecology, and climate change. He has conducted interdisciplinary international research on climate change mitigation and adaptation, focusing on ecological intensification of livestock systems. He also studies and develops perennial dual-purpose grain and forage crops like Kernza Intermediate wheatgrass.

NATURALLY OCCURRING STABLE 8X AND CHIMERICAL 4X/8X ZOYSIAGRASS GENOTYPES REVEALED BY FLOW CYTOMETRY

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Zoysiagrass (*Zoysia* Willd.) is increasingly selected for use as a turfgrass in warmer climatic zones. The *Zoysia* spp. are tetraploids, although recently both induced and naturally occurring octaploids were reported. Variable ploidy levels in a turfgrass can have advantages, e.g. sterile hybrids between ploidy levels and changes in morphological texture; or disadvantages, e.g. crossing barriers and genetic instability. Flow cytometry has been widely used in forage and turfgrass breeding programs as an aid in determining ploidy of naturally occurring and induced ploidy changes. This research reports evaluation of ploidy levels and morphology of naturally occurring variants of 'Empire' zoysiagrass and progeny from a naturally occurring octaploid line from Empire.

The ploidy level and internal chimerical ploidy variability of a naturally occurring octaploid Empire zoysiagrass clone, other clones growing in the same field block, and certified Empire were evaluated by flow cytometry. Flow cytometry measurements were determined on pots established from single node cuttings from plugs taken from the field block. Known standards of sorghum or chicken erythrocytes were used to calculate 2c DNA values. Morphological differences in raceme lengths, seed head heights, blade lengths, and blade widths among these clones and a certified Empire clone were also determined.

Empire Clone "E11" showed a consistent G1 peak double the value of certified Empire. Both of these clones had a "normal" G2 peak that consistently had 20% or fewer events than the G1 peak, as usually seen with healthy growing vegetative tissue. In contrast, several clones sampled across the field block showed a "G1 peak" at the value equivalent to Empire and a second "G1 peak" equivalent to E11, with both peaks having similar cell event counts. These flow cytometry results remained consistent through frequent removal of vegetative growth (e.g. mowing) for more than one year. These results strongly suggest the presence of a periclinal L1/L2 chimera in some of the sampled clones. Research is underway to evaluate seedling progeny from the "chimerical clones" and the E11 clone to determine ploidy stability through a sexual seedling generation. Preliminary evaluations of ploidy of seedling progeny from open pollination of E11 are in progress. Morphological differences were identified between certified Empire and the E11 clone. Seed head height, raceme length, blade length and blade width were larger with the octaploid E11 clone compared to Empire. These morphological differences resulting from increased ploidy are consistent with other reported effects of chromosome doubling.

The cause of these changes in ploidy are unknown. It has been speculated that the dinitroaniline herbicides (commonly used in turfgrasses) and also known to be a mitotic poison might be the source. Further research is needed to investigate the type and stability of these apparent chimerical zoysiagrass clones. Breeding behavior of these clones also needs to be investigated. The potential to use the octaploid E11 clone in crosses with tetraploid zoysiagrasses to produce sterile hexaploids is intriguing.

PRESENTER BIO: Dr. Quesenberry is a Professor Emeritus with more than 40 years of experience conducting research on forages and turfgrasses. He has extensive experience with genetics and breeding of various turf and forage crops and has released numerous recognized cultivars of forage grasses and legumes and turfgrasses.

ANNUAL RYEGRASS BREEDING AT THE UNIVERSITY OF FLORIDA

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Annual ryegrass (*Lolium multiflorum* Lam) is a self-incompatible species with a broad range of adaptation. It is widely used as a desirable forage to provide winter grazing in the southeastern United States (U.S.) and as a cover crop in the Midwestern U.S. The yield and quality of annual ryegrass can be affected by Crown Rust (*Puccinia coronata* Corda) and Gray Leaf Spot (*Pyricularia grisea* Sacc.). Annual ryegrass breeding began at the University of Florida in the 1950's and continues to this day. Environmental conditions in North Central Florida are ideal for the development of many diseases that affect ryegrass production. When reliable and consistent disease epidemics and environmental conditions are present in a location, phenotypic recurrent mass selection (particularly in an annual cross pollinated crop) can be a powerful tool to develop disease resistant cultivars. Manipulation of ploidy levels in forage crops like annual ryegrass has been shown to be another important tool for genetic improvement and potential dry matter yield gains. Tetraploid genotypes in the Florida annual ryegrass breeding program were developed in cooperation with German scientists in the 1990s using elite Florida germplasm with high levels of foliar disease resistance and then again at the University of Florida in 2010 using extremely early maturing germplasm. Further recurrent selection in these tetraploid populations has been conducted over the past two decades.

The program has released 26 cultivars of annual ryegrass that are currently on the market, of which 12 are tetraploid and 14 are diploid. All these cultivars have been licensed by the Florida Foundation Seed Producers, Inc. to commercial companies, primarily in the Pacific Northwest, USA. The majority of these remain in commercial production. Cultivars that have particular commercial success include tetraploids such as 'Attain', 'Big Boss', 'Jumbo' and 'Prine'; and two diploids, 'Fria' and 'Winterhawk'. Annual ryegrasses from the University of Florida are high yielding and have a wide range of maturity dates that fit into an array of cropping systems. The recent reemphasis on cover crop utilization in annual grain crops could result in a further increase in utilization of annual ryegrass. A new release in 2017 'Frostproof' has enhanced cold tolerance which could fit into diverse management systems.

Future research will be directed towards adaptation to global climate changes that include enhanced disease resistance, increased drought resistance and adaptability to erratic temperature changes. Genomic selection tools are being evaluated to enhance progress in the breeding program. Several cultivars with enhanced disease resistance, high yields, and diverse maturities will be released in the near future.

PRESENTER BIO: Mr. Reith is the farm manager of the University of Florida Agronomy Forage Research Unit. He has spent the last 23 years working with the ryegrass breeding program which has produced 26 varieties of ryegrass that are currently on the market.

STUDY ON EMS MUTAGENESIS EFFECT OF SHEEPGRASS (*LEYMUS CHINENSIS*)

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Sheepgrass (*Leymus chinensis*(Trin.) Tzvel.) is a rhizomatous perennial C3 species dominating the Eurasian Steppe grasslands. Sheepgrass plays an important role in maintaining the stability of grassland ecosystem, biodiversity and grassland productivity in north China. By using ethyl methyl sulfonate (EMS) chemical mutagenesis breeding, the rich mutant population obtained will provide the research foundation for the new species breeding of sheepgrass. Therefore, by using different concentration and time combinations of EMS to mutate the "jisheng 4" seed of sheepgrass, analyze the EMS mutagenesis effect and determine the optimal germination system of EMS mutagenesis. Screening the optimal seedling system of sheepgrass seeds of EMS mutagenesis, and the seeds of suitable seedling system mutagenesis were grown in greenhouse pot.

In this study, after EMS mutagenesis of different concentrations and time, we determined the optimal mutagenesis system of sheepgrass seeds germination: 1.8% EMS for 12h and 1.5%EMS for 16h. It was determined that the optimal seedling system was 0.5% EMS processing 8h after the screening of the seedling system in the soil. A total of 120 mutant plants that phenotypic traits were significantly altered were obtained in the M1 generation, and the mutation frequency was 18.66%; we obtained 14 dwarf mutants, 1 multi-tillers mutant and 1 broad leaf mutant. Group A (0.5% EMS for 8h) had 66 mutant plants with the highest mutation frequency were 25.38%, and had the most mutation types and the most abundant variation. Group B (0.3%EMS for 12h) showed a variation in leaf color traits. Group C (0.5% EMS for 12h) had 16 mutant plants. There were 13 mutant plants in group D (0.7%EMS processing 8h). Group E (0.3% EMS for 8h) had the least mutation, with a total of 3 mutant plants.

A preliminary study on EMS mutagenesis of sheepgrass was conducted, and we determined the optimal mutagenesis system for germination and seedling of sheepgrass EMS mutagenesis respectively and analyzed sheepgrass seed germination, seedling growth and plant growth comprehensively to provide the basic research idea for the follow-up study of sheepgrass. M1 generation mutation materials with abundant variation were obtained to provide the basis research for building M2 generation mutant library. The construction of EMS mutation population of "Jisheng 4" sheepgrass will provide the basic materials for genetic improvement and breeding research of sheepgrass. It is of great significance for the improvement of degraded grassland and the construction of high yield artificial grassland.

PRESENTER BIO: Dr. Ren Weibo, Professor, director of science and technology management office. He has extensive experience with new variety breeding and evaluation of sheepgrass and fodder oat, the adversity stress response molecular mechanism of grassland plant. He has led more than 12 projects dedicated to the enhancement of forage grass.

SEED SET EVALUATION IN INTER-SPECIFIC HYBRIDS OF LOTUS

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Lotus corniculatus L. (birdsfoot trefoil) and *Lotus uliginosus* Schkuhr (syn. *L. pedunculatus* Cav.; big trefoil) have different growth strategies, root systems and growth habits, but both have high agronomic value in Uruguay. Persistence is generally one of the breeding goals in *L. corniculatus*, while seed production is the main problem in *L. uliginosus*. Inter-specific hybridization opens an opportunity to combine positive attributes from both species, with the objective to widening the genetic basis for breeding and achieving a superior agronomic cultivar. The objective of this research was to assess the seed set as a fertility indicator, in four hybrids groups resulted from the reciprocal crosses between *L. uliginosus* 'INIA Gemma' and *L. corniculatus* 'INIA Draco'. Hybrid groups, G1 and G4, were created from *L. uliginosus* x *L. corniculatus* crosses; while G5 and G6, are the result of the reciprocal cross (*L. corniculatus* x *L. uliginosus*). Groups G1 and G5, were obtained by crossing plants selected by high seed set, while groups G4 and G6, were obtained by crossing plants selected by low seed set. These selections took place in a previous selection process. The four hybrid groups and their parental cultivars were assessed in micro-plots of 20 plants each (manually transplanted in the winter of 2016) at La Estanzuela (Colonia, Uruguay) and Glencoe (Paysandú, Uruguay) INIA's Research Stations, during the summer of 2016-2017. Differences ($p < 0.05$) were observed among hybrid groups for number of flowers and pods per square meter, and seed grams every 20 flower-heads, and total seed production (kg ha^{-1}). As expected, the parental cultivar 'INIA Draco' produced more than three times more seed than 'INIA Gemma'. The G1 group produced four times more seed than group G4, while G5 produced the double than G6 group. The hybrid groups G1 and G4 (*L. uliginosus* as the mother) produced in average 30% of the seed produced in average by the hybrid groups G5 and G6 (*L. corniculatus* as the mother). None of the hybrid groups produced more seed than the parental cultivar *L. corniculatus* 'INIA Draco'; however, the hybrid group G5 had higher seed yield than the parental cultivar *L. uliginosus* 'INIA Gemma'. A maternal effect was clearly observed, not only in the variables assessed, but also in the phenotype that the hybrid plants resembled. Great genetic variability was observed within each hybrid group, and that is the basis for the breeding program that is currently performed looking for improving forage and seed productivity, and persistence.

PRESENTER BIO: Dr. Reyno is a researcher and a plant breeder focused on developing improved varieties of forage crops. He has worked with both grass and legume species and is the current responsible for INIA's Forage Breeding Program (2017-2022), and with specific responsibilities in Lotus, Alfalfa and Bahiagrass breeding programs.

PROGRESS IN HAIRY VETCH COVER CROP BREEDING

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Cover crops are powerful tools for improving agricultural productivity and sustainability. Hairy vetch (*Vicia villosa*) is a widely used legume cover crop that excels in nitrogen production, weed suppression, and soil health improvement. Despite these benefits, *V. villosa* suffers from low vigor, winter kill, non-ideal flowering timing, unpredictable seed dormancy and pod shatter. Our team represents the first coordinated effort to improve *V. villosa* genetics for cover crop use. Uniting USDA-ARS, USDA-NRCS Plant Materials Centers, land grant universities, private research foundations, and pioneering farmers, our team screened and selected genotypes at ten locations across the United States.

Each site screened 30 global populations for winter hardiness, plant vigor and biomass, high biological nitrogen fixation, flower timing, and hard seed. *V. villosa* demonstrated large genotype by environmental interactions for traits of interest. The performance of genotypes largely grouped according to the severity of winter conditions, as measured by freezing-degree days. Consequently, the breeding program has divided selection and testing environments among northern and southern locations.

Over two seasons at six sites in Minnesota, Wisconsin, New York, Maryland, and North Carolina, the top five percent of 12,480 genotypes were selected for fall and spring vigor, winter survival, and flower timing. Four farmers in Pennsylvania, Iowa, and Wisconsin also conducted participatory selection of top-performing genotypes on their farms. Beyond selection in the field, our team aims to identify markers associated with seed dormancy and shatter. After screening 477 maternal lines for hard seed and shatter, we will conduct a genome-wide association study on divergent lines.

Preliminary results show large gains in selection for traits of interest for cover crop use. To compare breeding lines to check varieties, thirteen sites across the United States have planted advanced line trials of improved *V. villosa*. Results should show lines with superior cover crop performance and potential for release.

PRESENTER BIO: Dr. Riday is a forage legume breeder with over 20 years of breeding and research experience. His research and breeding work includes work in the following species: red clover, alfalfa, kura clover, birdsfoot trefoil, hairy vetch, white clover, other winter annual cover crop legumes, and other tropical annual legumes.

USING MACHINE LEARNING TO IDENTIFY AND ANALYZE RED CLOVER SPACE PLANTS IN BREEDING NURSERIES

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We have developed a program capable of identifying individual space-planted red clover breeding nursery plants in digital images. The program takes advantages of pre-trained neural networks and other machine learning methods. The program is very robust and can identify red clover plants with a 99% accuracy, even when plants of interest are in close proximity to each other, overlap slightly, or are surrounded by complex vegetation, such as turf grass and weeds. The correct within plot plant position was determined 93% of the time. Images fed into the program do not require the camera used to take the images to be in exactly the same position in relationship to the red clover plants from image to image. For identified plants, the program can count the number of leaves on the plant. This plant leaf count value was found to be highly correlated with field based human visual ratings (a proxy for biomass yield) of these same plants with an r^2 of over 68%. Future work will improve the program's accuracy and expand the number of traits phenotyped.

PRESENTER BIO: Dr. Riday is a forage legume breeder with over 20 years of breeding and research experience. His research and breeding work includes work in the following species: red clover, alfalfa, kura clover, birdsfoot trefoil, hairy vetch, white clover, other winter annual cover crop legumes, and other tropical annual legumes.

GENETIC PARAMETERS FOR NUTRITIVE VALUE IN BERMUDAGRASS GERMPLASM DETERMINED BY NIRS

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Variation in nutritive value (NV) can be due genotype, management and environment. A bermudagrass (*Cynodon* spp.) collection is available at the USDA National Plant Germplasm System (NPGS), but there is limited information on nutritive value (NV) traits present in this germplasm. The objective of this study was to estimate variance components and genetic parameters for NV in bermudagrass grown under two nitrogen (N) rates (0 and 100 kg N ha⁻¹). A set of 287 bermudagrass accessions: i) 146 Plant Introductions (PIs) from the USDA core collection, ii) 137 PIs from the USDA-NPGS, and iii) controls: 'Coastal', 'Jiggs' and 'Tifton-85'. A row-column design with two replicates for each nitrogen rate was established in Ardmore, OK in 2015. Four NV traits were measured five times between July 2016 and August 2017. Approximately 450 g of fresh biomass was harvested, dried in a forced-air oven at 55°C by 72 hours and ground in a Wiley mill to pass a 1-mm screen. Crude Protein (CP), Neutral Detergent Fiber (NDF), Phosphorus Content (P), and In-Vitro Dry Matter Digestibility (IVTDMD) were determined using near infrared reflectance spectrophotometry (NIRS) using the NIRS Consortium Equations for grass hay. Linear mixed models were implemented in ASReml to estimate variance components and calculate genetic parameters. Genotype exhibited the largest variance component across all traits, but the environment effect (N rate) was statistically significant for all traits, although much smaller than for genotype. Repeatability (H^2) varied between 0.35±0.02 (CP) and 0.54±0.02 (NDF). For all traits, high ($r_{gh}>0.79$) genotype by harvest correlations and high ($r_{ge}>0.87$) genotype by environment correlations were observed. High negative genetic correlations were found between NDF and IVTDMD, while CP showed a high positive correlation with P and IVTDMD. Genetic correlations among traits within each environment (N rate) were higher than for inter-environment correlations. Nitrogen fertilization improved NV in bermudagrass, and the N fertilized plots exhibited increases in mean values for CP (12%), P (2.8%), IVTDMD (2.2%) and a reduction of -1.4% for NDF. Several PI's were placed at the top of the ranking for each NV trait. Large genetic variability exists for NV traits indicating that selective breeding could be achieved in this germplasm. The high r_{gh} and r_{ge} correlations showed that selection could be performed with fewer evaluations and locations.

PRESENTER BIO: Dra. Lopez is a the Biological Scientist at the Forage Breeding and Genetics Lab, Agronomy Department, University of Florida. Dr. Lopez has extensive experience in the area of plant breeding and genetics.

GENOME-WIDE FAMILY PREDICTION FOR AGRONOMIC TRAITS IN ALFALFA

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Alfalfa (*Medicago sativa* L.) is the most important perennial forage in the world, and its estimated crop value in the U.S. (US\$9 B) is just behind corn, soybean and wheat. The widespread use of alfalfa in subtropical environments is compromised by the lack of adapted and improved cultivars. Genomic selection is revolutionizing plant breeding, and the use of genome-wide family prediction (GWFP) in alfalfa could accelerate cultivar development, particularly for traits that must be phenotyped in swards. The objective of the study was to test the predictive ability of GWFP models in alfalfa. A reference alfalfa population was created using 35 parents (six males and 27 females) and resulted in 145 full-sib and 36 half-sib families. The field experiment was designed as a row-column (14x32) with augmented representation of three controls ('Bulldog805', 'FL99' and an advanced breeding line from UF). The population was phenotyped for dry matter yield (DMY) at the family/plot level during two harvests in 2018. DNA extractions were performed by pooling at least 20 individuals per family and genotyped using sequence capture. After data trimming (quality score of 20), demultiplexing, and barcode removal, reads were aligned to the alfalfa draft genome using Mosaik v.2.2.3. Single nucleotide polymorphisms (SNPs) were called using FreeBayes v.1.0.1 considering the option for tetraploid species. A total of 88K biallelic markers presented population sequence depth higher than 40, SNP quality score higher than 30, and mapping quality above 10, and were used in population genetic and in GWFP analyses. Markers were used in allele frequency formats for each family (ratio between the number of reads of reference allele and the number of reads for the alternative allele). Population genetic analyses were conducted using the R package adegenet v. 1.3-1. GWFP was fitted in a one-step single-trait linear mixed model using the BGLR package in R. Phenotypic predictive ability (PA) was based on a 10-fold cross-validation within each harvest. The linkage disequilibrium presented in the population decays over 433Kb considering the mean distance at the threshold of $r^2=0.2$. More than 21% of the variation was captured in the first two axes in a principal component analysis (PCA). In addition, discriminant analysis of the PCA suggested the presence of population structure, where the six subgroups identified correspond to the six male parents used in the crosses. The population structure information was considered to perform the cross-validation step in the GWFP analyses. The average PA for dry matter yield in each harvest was above 0.6. These results showed potential for using GWFP in breeding programs where individual plants are of limited interest and phenotypes are scored in field plots, such as breeding programs for out-breeding species. A lower cost for genotyping family pools would encourage the application of GWFP in forage breeding programs.

PRESENTER BIO: Dr. Esteban Rios is an Assistant Professor in the Agronomy Department, University of Florida. Dr. Rios conducts research in the area of forage breeding and genetics with focused on alfalfa, bermudagrass, ryegrass and clover breeding, integrating classical and modern breeding tools.

COMPARATIVE TURFGRASS PERFORMANCE OF *LOLIUM PERENNE* GERMPLASM UNDER LIMITED IRRIGATION

Joseph Robins

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One hundred perennial ryegrass germplasm accessions and cultivars were evaluated for ground cover and green color retention under 60 % evapotranspiration irrigation replacement at two field sites in Utah, USA. The perennial ryegrasses were also exposed to two mowing treatments (51 mm and 76 mm) and two traffic treatment (traffic and no traffic). Based on broad sense heritability, there was significant genetic variation within the perennial ryegrass germplasm for both ground cover ($H^2 = 0.72 \pm 0.16$) and green color retention (0.64 ± 0.13). All binary interactions among the four main effects (location, traffic, mowing, and population) differed from zero for ground cover, and all but the traffic-by-mowing and population-by-mowing interactions differed from zero for green color retention. Within the individual traffic-mow combinations, broad-sense heritability estimates ranged from 0.52 ± 0.18 to 0.95 ± 0.16 and 0.25 ± 0.17 to 0.73 ± 0.19 for ground cover and green color retention, respectively. Many of the accessions exhibited higher ground cover and/or green color retention than the included check cultivars. Thus, this study provides evidence of ample untapped genetic variation within perennial ryegrass germplasm accessions to improve its turfgrass quality and performance.

GENOME EDITING IN ALFALFA (*MEDICAGO SATIVA*) TO HYPER-ACCUMULATE PHOSPHATE

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Rock phosphate, the main source of phosphate (P) for crop fertilizers, is a finite resource that is predicted to be depleted in 50-100 years. P is a critical nutrient in agriculture and its application can dramatically improve plant productivity. However, many soils have excess amounts of P from application of animal manures and runoff of phosphate from agricultural lands is the major source of non-point water pollution in the Midwestern US. The goal of this project is to create mutations by gene editing in the ubiquitin E2 conjugating enzyme PHO2, involved in P signaling and P homeostasis in alfalfa so that plants hyper-accumulate phosphate. Such plants could be used to reduce soil P levels and reclaim P for use as a fertilizer. From a draft diploid *Medicago sativa* genome scaffold sequence and the alfalfa transcriptome database (AGED), three PHO2 genes were identified. The genes, two of which are >99% homologous (a/b), each have seven exons interspersed by six introns. The open reading frames are 912 amino acids except when an alternate splice site is used in a/b gene transcript resulting in a 902 amino acid sequence. Alfalfa plants grown under P limiting conditions expressed low levels of the a/b transcripts with higher levels seen for PHO2c, while application of higher P induced increased expression mainly of the a/b transcripts. Under high P conditions, roots and shoots accumulated 4.1x and 2.5x more P than in low P conditions, respectively. An initial CRISPR/Cas9/Cys4 reagent targeting all three genes was generated and used to transform alfalfa cv. RegensY. A total of 67 verified transgenic plants were screened by acrylamide gel shift assays, cloning, and sequencing to identify plants with mutations. Mutations ranging from a 1 bp insertion to a 25 bp deletion were identified in a total of 10 plants and some plants had multiple targets hit. Recently, a second attempt at CRISPR/Cas9 mutation utilized a cassette vector system with either the tRNA or Cys4 splicing system and exonuclease components. Initial screening results indicate that the tRNA splicing system may have yielded greater numbers of mutations. TaqMan probes were designed to identify plants with changes in the target sites and were verified by restriction digestions, cloning, and sequencing. Data on inheritance of mutations and phosphate accumulation in edited plants will be presented. The results of these experiments demonstrate that editing of multiple targets can be accomplished in alfalfa, although the tetraploid inheritance of genes complicates analysis.

PRESENTER BIO: Dr. Deborah Samac is a Research Geneticist with USDA-Agricultural Research Service in Saint Paul, Minnesota, USA. She conducts research on resistance to disease in alfalfa and diversity in alfalfa pathogen populations. Her research group also uses biotechnology approaches including CRISPR/Cas9 genome editing for alfalfa improvement.

RESISTANCE TO APHANOMYCES ROOT ROT IN ALFALFA (*MEDICAGO SATIVA L.*)

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Aphanomyces root rot (ARR), caused by the oomycete *Aphanomyces euteiches*, is one of the most important yield-limiting factors in production of legumes. In Europe, it is the main limiting factor for pea production while in the U.S. it is one of the most important diseases of alfalfa and pea. Alfalfa varieties with resistance to ARR became widely available in the 1990s; however, failure of resistant varieties identified a second race of the pathogen. Recent reports of failure of varieties with resistance to both race 1 and race 2 suggest that additional pathogenic races are present in alfalfa production fields. To gain a better understanding of the distribution of pathogen races, 53 soil samples from alfalfa production fields in Minnesota and 40 soil samples from New York were used in a bioassay to identify the races present. In both states, race 2 isolates were most common. A significant number of soils were classified as “atypical” because the race of *A. euteiches* could not be determined with the bioassay. Pure cultures of *A. euteiches* were isolated from diseased alfalfa seedlings grown in nine of the atypical soils and individual strains were used to inoculate the differential varieties. None of the 44 individual strains could overcome the resistance in the check variety WAPH5 that has resistance to race 1 and race 2. Zoospores were found to be attracted primarily to the root hair zone of alfalfa seedlings and penetrated root cells within one hour of inoculation. Cells of resistant plants reacted to penetration with a rapid and localized hypersensitive response characterized by cell death and browning of penetrated cells. Cells of the pathogen were limited to only epidermal cells or a small number of cortical cells in resistant seedlings. Highly upregulated genes in resistant plants at 24 hours after infection included genes involved in the phenylpropanoid biosynthetic pathway leading to production of phytoalexin (medicarpin), and cell wall degrading enzymes chitinase and beta-1,3-glucanase. The production of phenylpropanoid compounds and suberization of the stele was seen microscopically in resistant plants at 24 hours after inoculation. Similar responses were seen for resistance to race 1 and race 2 strains. To identify genetic loci involved in resistance, a biparental F₁ mapping population was used for genotyping-by-sequencing followed by genotype calling. SNP markers significantly associated with resistance to race 1 strains were identified on chromosome 1. Using the genome sequence for cultivated alfalfa at the diploid level, three candidate NBS-LRR disease resistance genes were identified within 38 kbp of the most significant marker ($P=2.76 \times 10^{-23}$). Significant SNP markers associated with resistance to race 2 strains were located on chromosome 2 ($P=1.7 \times 10^{-9}$). These results indicate that a small number of genes are involved in the hypersensitive resistance mechanism in the variety 53V52. Ongoing QTL mapping aims to identify markers tightly linked to resistance that may be useful in marker-assisted selection for breeding alfalfa varieties with high levels of resistance to ARR. The results of this study support the use of diverse *A. euteiches* strains when selecting disease resistant parental materials to achieve durable resistance to ARR.

PRESENTER BIO: Dr. Deborah Samac is a Research Geneticist with USDA-Agricultural Research Service in Saint Paul, Minnesota, USA. She conducts research on resistance to disease in alfalfa and diversity in alfalfa pathogen populations. Her research group also uses biotechnology approaches including CRISPR/Cas9 genome editing for alfalfa improvement.

GGE BILOT ANALYSIS IN MULTI-ENVIRONMENT TRIALS AND HARVESTS FOR LEAF DRY MATTER YIELD IN *PANICUM MAXIMUM* JACQ. (*MEGATHYRSUS MAXIMUS* JACQ.)

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Among the main forage grass crops in Brazil, *Panicum maximum* Jacq. (*Megathyrsus maximus* Jacq.) stands out due to its excellent yield performance, adaptation and good forage quality. In forage evaluations, if the most important traits are strongly influenced by the GxE interaction, a larger number of environments may be necessary. The objective of this research was to verify the effect of GxE interaction, as well as to obtain information about the number of harvests per environment required for genotype selection in the context of multi-environment trials (MET) for leaf dry matter yield (LDMY). The trials were planted in 5 locations in Brazil: Campo Grande, MS; Planaltina, DF; Presidente Médici, RO; Rio Branco, AC and Valença, RJ. Evaluation was performed with 23 genotypes: 14 genotypes selected from the genebank, 4 selected hybrids and 5 cultivars as checks (Aruana, Milênio, Mombaça, Tanzânia and Massai), in 11 harvests in two years, under wet and dry seasons. A randomized complete block design (RCBD) was used with three replications. Plots were 6 rows of 4m in length, occupying an area of 12 m², with spacing of 0.5m between rows and 2m of distance between plots. Statistical analysis was performed by the Mixed Models approach, with the asreml R package. Visual grouping of the locations associated with the genotypes were obtained through the GGE Biplot analysis performed by GGEbiplotGUI R package and the scores of the first two principal components (PC1 and PC2) were used to display two-dimensional biplot. Pair-wise Spearman correlations of cumulative harvests were computed to obtain the ideal number of harvests. Strong evidence of the presence of the GxE interaction was observed. By the PC analysis, it was possible to differentiate three mega-environments. The MS, AC and DF locations constituted one, while RO and RJ were the other two mega-environments. The genotypes: 6 and 2 for the RJ; 14 and 15 for the MS, AC and DF; 21 and 16 for the RO, performed better at these environments, respectively. The harvests performed in the dry season tended to decrease the average, genetic variability and the correlations between harvests, showing inability to discriminate genotypes. To perform genotype selection for the LDMY trait, seven harvests per environment were representative and enough to discriminate genotypes. These results show us the importance of studying the GxE interaction effects in MET analysis, to support the forage breeders in the context of genotype selections and improve the *P. maximum* breeding programs.

PRESENTER BIO: Dr. Santos is a young Forage Breeder hired by Embrapa Beef Cattle in 2014. Currently, he is working with Dr. Liana Jank in the *Panicum maximum* Breeding Program developing cultivars for the market and also doing some research in inheritance of traits, population improvement and marker assisted selection.

PERFORMANCE AND CORRELATION FOR LEAF DRY MATTER YIELD OF DIVERSE GERMPLASM OF *PANICUM MAXIMUM* JACQ. UNDER TWO SOIL FERTILITY LEVELS IN THE BRAZILIAN CERRADO

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Brazilian Cerrado is one of the main biomes for beef cattle production in the world. Soils of Cerrado are acid and with low availability of phosphorous and bases such as calcium, magnesium and potassium. Thus, high input of lime and fertilizers are used to increase the yield of crops and forage species. *Panicum maximum* Jacq. (syn. *Megathyrsus maximus* Jacq.) is a tropical forage grass known to be highly demanding on soil fertility. However, considering the Embrapa Beef Cattle breeding program, little information is available on the performance and tolerance of the germplasm for soils of Cerrado. The objective of this work was to evaluate different sources of germplasm under two levels of soil fertility. For this, leaf dry matter yield (LDMY) of six commercial cultivars, ten sexual plants and eight apomictic accessions from the genebank was evaluated at the Experimental Station of Embrapa Beef Cattle, Campo Grande, Brazil, in a randomized complete block design with three replicates under a haplic ferralsol with two fertility levels. Lime and phosphorous were added to the soil to reach the following levels: 2 ppm of phosphorous and 35% of base saturation, named low fertility soil (LFS); and >6 ppm of phosphorous and >45% of base saturation, named high fertility soil (HFS). Plots were two rows of five plants occupying an area of 4.5 m². LDMY was evaluated in six harvests in the rainy season and one harvest in the dry season from February 2017 to February 2018. Data, in ton.ha⁻¹, were analyzed following a mixed model approach for different environments and harvests available in the SELEGEN-REML/BLUP software. Mean LDMY decreased from 2.33 to 1.47 ton.ha⁻¹ under HFS and LFS, respectively, showing a 37% mean loss due to low fertility. Genotypic variance was highly significant, but genotype by soil interaction was not significant based on the LRT test. Correlation between genotypes across soil fertility levels was 0.91, showing low effects of soil fertility in the ranking of genotypes. Tolerance to low fertility, as the percentage of decrease in LDMY from HFS to LFS, varied from -31 to -46%, showing a narrow variation for tolerance for low fertility among the genotypes. Correlation between genotype performance and tolerance was -0.87, showing that genotypes with higher performance showed lower losses due to reduced fertility. The results showed the importance of phosphorous and base saturation for *P. maximum* production on Cerrado soils. Moreover, the genotypes used currently by farmers or germplasm used by the breeders presented the same ranking in the two levels of fertility.

PRESENTER BIO: Dr. Santos is a young Forage Breeder hired by Embrapa Beef Cattle in 2014. Currently, he is working with Dr. Liana Jank in the *Panicum maximum* Breeding Program developing cultivars for the market and also doing some research in inheritance of traits, population improvement and marker assisted selection.

AUTOTETRAPLOID *UROCHLOA RUZIZIENSIS* BREEDING

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The forage grass *Urochloa ruziziensis* autotetraploid and with sexual mode of reproduction is an important component of the *Urochloa spp.* breeding programs. The intrapopulation breeding objectives are to increase biomass and seed yield, and nutritional forage quality aiming to cross-pollinate with apomictic species. As with other tropical forage grass species, the association between flowering and seed yield components, the mode of inheritance and the effects of population structure on phenotypic breeding are scarce. Twenty-one characteristics evaluated in fifty-nine half-sib progenies of seven subpopulations were analyzed using a mixed model methodology. The genetic variation within subpopulations was higher than among subpopulations for all characteristics, based on the magnitude of heritability. The characteristics index of flowering, number of panicles, seed weight, *seed-set* and all those associated with the production of biomass, excluding regrowth in the dry season, showed additive genetic variation statistically significant between and within populations. The narrow sense heritability within subpopulations varied from 0.08 to 0.30 and from 0.17 to 0.29 for agronomic characteristics evaluated during wet and dry season, respectively. Nutritional quality and reproductive characteristics presented lower magnitude narrow sense heritability within (0.01 to 0.22) and among subpopulations (0.01 to 0.16). The highest magnitudes of heritability among the subpopulations were observed for the total number of panicles (0.16), *seed-set* (0.15), green matter yield (0.22) and DM yield (0.21) during dry season. The genetic variability available in experimental breeding populations is derived from the structuring of subpopulations and progeny and from Mendelian segregation effects within progeny. All three pieces of information can and should be capitalized on genetic improvement programmes based on phenotyping. The null commonality ($Com = 0.01$) evidenced between the filled seed production and biomass production is advantageous for the selection and genetic gain in both characteristics, i.e., there is not causal relationship between them which is useful for selection and breeder decision-making.

PRESENTER BIO: Dr. Simeão is a Researcher with more than 18 years of experience researching tropical forage. She has extensive experience with genetic breeding of apomictic and sexual forage grass as well as *Stylosanthes spp.* and *Arachis spp.* forage legumes.

APPLICATION OF MULTISPECTRAL IMAGE SENSORS FOR EVALUATION OF PERENNIAL RYEGRASS PERSISTENCE IN FIELD PLOTS

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Perennial ryegrass (*Lolium perenne* L.) is one of the most important forage grass species in temperate regions of the world, but it can have poor persistence due to low tolerance of abiotic and biotic stresses. Thereby creating a challenge for livestock producers to utilise their agricultural lands more productively and intensively within sustainable limits. Breeding perennial ryegrass cultivars that are both productive and persistent is a target of forage breeding programs. This then allows farmers to select better cultivars that would deliver the highest profitability over the lifetime of a sward. Conventional methods for the estimation of pasture persistence depend on manual ground cover estimation or counting the number of survival plants in a given area. Those methods are sometimes subjective, time-consuming or required skilled labour. Application of multispectral imaging for plant phenotyping offers a non-destructive, precise method for agricultural research. This study aimed to develop a sensor-based phenomic method to evaluate persistence of perennial ryegrass cultivars in field plots. In this study, 72 perennial ryegrass plots were used to estimate persistence. Plot size was 1.5 m x 1.5 m and 100 plants were planted in each plot with 15 cm inter-row spacing. Plot-level perennial ryegrass persistence was significantly varied based on dry matter production and plant survival rate. The multispectral images were taken after the final harvest (3 years after sowing) using 3DR Solo quadcopter and Parrot SEQUOIA multispectral sensor. The software packages eCognition Developer 9 and QGIS 2.18 were used to analyse multispectral images to estimate plot-level ground cover and the normalized difference vegetation index (NDVI). Estimated ground cover data were compared with plant survival rate and dry matter production to validate the image-based data extraction. Analysis of multispectral images generates fast, accurate phenomic data to estimate perennial ryegrass persistence. With further development this technique could be used to explain spatial and temporal changes of perennial ryegrass persistence for large-scale pastures.

PRESENTER BIO: Dr Kevin Smith is Professor of Pasture Agronomy and Breeding with the University of Melbourne and Fellow with Agriculture Victoria Research.

COMBINING NDVI AND PLANT HEIGHT AS A PROXY TO ENABLE HIGH-THROUGHPUT PHENOTYPING OF HERBAGE YIELD IN PERENNIAL RYEGRASS BREEDING PROGRAM

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Increasing the yield of perennial ryegrass remains a crucial factor underpinning the profitability of grazing industries, and therefore is a priority of breeding programs. Methods for assessment of dry matter yield include visual scores, sample clipping and/or mowing of all plots. These methods are often destructive, costly and time-consuming. The use of multispectral imaging and light detection and ranging (LiDAR) technologies offer a non-destructive, high-throughput and efficient way of phenotyping to accelerate perennial ryegrass screening in the field. We developed an unmanned aerial system (UAS) equipped with multispectral imaging systems and a ground vehicle system for fresh and dry matter yield phenotyping of perennial ryegrass aiming to measure yield of single plants. A spaced plant field trial containing 50,000 individual plants from 50 varieties/breeding lines was established in June 2016. A subset of 475 plants from five breeding lines were used to measure NDVI, fresh and dry matter in this experiment. Out of the 475 plants, 92 were used for plant height measurement using LiDAR. Normalized difference vegetative index (NDVI) was extracted from single plants based on a predefined georeferenced polygon area using PiX4D and QGIS software, and plant height from LiDAR scan. We used simple linear and non-linear regression models to predict biomass from NDVI, plant height, and the combination of NDVI and plant height (NDVI*plant height). The coefficient of determination between NDVI vs. fresh and dry matter yield indicated a strong non-linear relationship. The NDVI*plant height measurement was observed to be 20% more accurate in prediction of fresh and dry matter yield than using either NDVI or height alone. We, therefore, recommend combining NDVI and plant height to enable high-throughput phenotyping of herbage yield in perennial ryegrass breeding programs.

PRESENTER BIO: Dr Kevin Smith is Professor of Pasture Agronomy and Breeding with the University of Melbourne and Fellow with Agriculture Victoria Research.

USE OF NEW GENOMIC TOOLS TO ASSESS AND TRACE GENETICALLY MODIFIED FORAGES

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Implementation of molecular biotechnology in forage species, such as transgenic technologies, can improve agricultural profitability through the achievement of higher productivity, more efficient use of natural resources and decreased environmental impact. As part of the regulatory process, all new genetically modified (GM) cultivars are required to go under rigorous safety assesses prior to commercial release. Novel approaches to assess and trace a high-energy transgenic ryegrass have been developed, using emerging technologies such as Oxford Nanopore sequencing and digital PCR. A portable MinION devise was used to comprehensively characterize a transgene of 3,300 base pairs length. Detailed description of the transgenic event and genetic modification, including the insertion site and flanking regions was achieved in a single read from the long-read sequencer of over 32,000 bases. Moreover, digital droplet PCR (ddPCR) allowed the absolute quantification of recombinant DNA copies in different agricultural commodities such as seed, herbage and silage at low levels, with accurate detection at low levels (0.5% presence). The use of these technologies for GM characterization and identification, are not only important to ensure compliance and traceability, but also to enable necessary regulated GM labelling. Additional plant molecular diagnostics platforms are being developed, to enable rapid detection of GM forages on farm, which will allow an on-site detection at the DNA level, rather than at the gene product level. These technologies have shown to be more sensitive and accurate than conventional methods, which could reduce regulatory delays of these potentially large and transformative industry.

PRESENTER BIO: Dr Kevin Smith is Professor of Pasture Agronomy and Breeding with the University of Melbourne and Fellow with Agriculture Victoria Research.

VALUING GENETIC GAIN – WHAT'S THE LIKELY ON FARM VALUE OF NEW CULTIVARS

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In contrast to livestock where the relative of improved genetics is routinely available when producers make purchasing decisions, this kind of information is rarely available when livestock producers make cultivar choices prior to resowing.

Forage value indices to describe the relative on-farm value of perennial ryegrass cultivars have recently been released in Ireland, New Zealand and most recently in Australia. In these indices the differences in cultivar performance for traits such as seasonal dry matter production are combined with the relative value of that marginal increase in dry matter to combine the differences in these traits across cultivars in an overall index.

In Australia an index has been developed for each of 4 key dairy regions, based on dry matter production in 5 seasons (Autumn, Winter, Early Spring, Late Spring). The value of an extra kg/ha in these seasons varies from \$0.11AUD in Late Spring in Tasmania to \$0.43AUD in Winter in Gippsland. When combined with the BLUPs for cultivar performance this equates to a predicted increase in farm profit of \$131 - \$159AUD/ha/yr if the best performing cultivars are chosen in each region. This information is available to dairy farmers on the Dairy Australia website (<https://www.dairyaustralia.com.au/FVI>).

In this paper we describe the derivation of the index and research that is underway to increase the number of traits in the Forage Value Index.

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A HIGH THROUGHPUT BIOASSAY TO EVALUATE THE EFFECTIVENESS OF DIFFERENT ASEXUAL *EPICHLÖE* SPP. ENDOPHYTE STRAINS FOR CONTROL OF APHID PESTS OF PERENNIAL RYEGRASS (*LOLIUM PERENNE*)

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Perennial ryegrass (*Lolium perenne* L.) is one of the most important temperate pasture grass species globally and is the primary feed base for dairy and livestock industries in Australia and New Zealand. Insect pests represent the greatest source of biotic stress on temperate pastures in Australia and New Zealand with foliar and root aphids key pest species of significance due to direct feeding and virus-associated damage. A high-throughput *in-planta* bioassay was developed to evaluate use of different asexual *Epichloë* spp. endophyte strains for control of *Rhopalosiphum padi* (Bird cherry-oat aphid), *Diuraphis noxia* (Russian wheat aphid), *Metopolophium dirhodum* (Rose grain aphid) and *Aploneura lentisci* (Root aphid). Bioassays were established whereby seedlings were exposed to individual aphids and monitored for fecundity (# nymphs / day), development (time taken to reach reproduction) and survival (number of aphids that survived on each treatment) over a 30-day period. Treatments represented commercial perennial ryegrass-fungal endophyte symbiota, compared to endophyte free ryegrass and barley (preferred host). Results indicate that aphid fecundity and survival rate are reduced when aphids fed on perennial ryegrass seedlings with an endophyte, compared to endophyte free ryegrass and barley. Further analysis using Electrical Penetration Graphs (EPG) provides an indication as to the mechanism of aphid control for each perennial ryegrass-endophyte symbiotum tested. Thus, utilising endophytes in grazing systems, particularly those under aphid pressure, is a means to control aphid populations. The bioassay also identified variability between endophytes, indicating that the bioassay can be used to benchmark the insecticidal activity of commercial and pre-commercial ryegrass-endophyte symbiota. Overall, the bioassay has proven to be a rapid method of evaluating the insecticidal activity of perennial ryegrass-endophyte symbiota, and an important tool for evaluating novel symbiota.

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APPLICATIONS OF UNMANNED AIRCRAFT SYSTEM FOR GENOMIC SELECTION OF DROUGHT TOLERANCE IN PERENIAL RYEGRASS

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Drought is a serious challenge to global agriculture and food security. Genomic selection in forage plant species have been proposed as a molecular breeding tool for increasing genetic gain efficiently in particular for quantitative traits such as yield and drought tolerance. Current assessment of yield is either by destructive harvest or by non-destructive visual score. Both methods have their limitations in dealing with large scale field trials. High-throughput and accurate phenotyping will enable reliability and accuracy of prediction and efficiency of selection. We applied unmanned aircraft systems and a multispectral sensor for drought tolerance phenotyping of perennial ryegrass, aiming to develop genomic prediction equations. A spaced plant field trial containing 644 individually genotyped perennial ryegrass plants under two treatment conditions with two replicates was established in 2016. The drought treatment was applied through a rainout-shelter structure and the control plots were under natural rainfall. Weekly aerial images were taken using a 3DR Solo quadcopter with Parrot SEQUOIA multispectral sensor. The normalized difference vegetation index (NDVI) values for individual plants were acquired through analysis of the multispectral images through Pix4D and QGIS software packages. The experiment was repeated in 2017, correlations and plants have been selected and provided to industry for further evaluation and testing.

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FUNCTIONAL METABOLOMICS ANALYSIS OF LOLITREM B AND ITS BIOSYNTHETIC INTERMEDIATES IDENTIFIES CATECHOLAMINERGIC ACTIVATION PROFILES AND NON-TREMORGENIC METABOLITES

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The neuroactive mycotoxin lolitrem B causes a neurological syndrome in grazing livestock resulting in hyperexcitability, muscle tremors, ataxia and, in severe cases, clonic seizures and death. Lolitrem B is the endpoint in a biosynthetic pathway of indole diterpenoid toxins present in fodder, yet the neuroactive status of the pathway intermediates remains undefined. To define the effects of lolitrem B and, pathway intermediates terpendoles B, C and E in the brain, a functional metabolomic study was undertaken in which coordination and tremor were quantified and metabolomic profiling undertaken to determine quantification and relative abundance of both toxin and key neurotransmitters in various brain regions. Marked differences were observed in the duration of tremor and coordination between pathway members, with some showing protracted effects and others none at all. Quantification of lolitrem B using Liquid Chromatography Mass Spectrometry (LCMS) identified presence of lolitrem B in liver and kidney, cerebral cortex, thalamus and brainstem but not in cerebellum. Metabolomic profiling by LCMS of brain isolated from intoxicated animals showed significant variation in targeted neurotransmitter and amino acid profiles over time. This study demonstrates for the first time bioaccumulation of lolitrem B in the brain, with absence of detectable levels of toxin in the cerebellum, as determining a dynamic catecholaminergic response over time. This data indicates that the indole diterpenoid toxins induce alterations in catecholamine pathways in the brain as well identifying pathway intermediates with non-tremorgenic profiles. This study identifies a functional metabolomic approach for physiological profiling of neurotoxic agents in the brain.

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HARNESSING THE POWER OF LONG READ SEQUENCING IN FUNGAL ENDOPHYTE GENOME ASSEMBLY

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Although many fungal endophyte genomes have been sequenced with the use of high throughput next generation sequencing, most of them remain incomplete due to having a large number of highly fragmented contigs. One main reason for the fragmented assemblies is the inability to resolve repetitive elements using the short reads (2x150bp) generated through short read (Illumina platform) sequencing. Genome assembly is further challenging in hybrid endophyte genomes such as fescue grass derived *Epichloë* endophytes which have contributions from multiple progenitors. For example, resolving the genome assembly and generating an annotated draft genome for the highly reticulated heteroploid *E. coenophiala* genome which has contributions from three progenitor genomes was impossible using only short reads generated through Illumina platform sequencing. Thus, long read sequencing with a median read size of 10,000bps opens up the avenue to generate complete genome assemblies for both haploid as well as heteroploid *Epichloë* endophytes. In order to sample genomic diversity across the various taxonomic groups, asexual *Epichloë* endophytes representing the diversity of taxa associated with *Lolium* spp. have been subjected to long read sequencing using PacBio and Minlon sequencing platforms, obtaining 10,000bps mean read length with reads up to 140kb long. Genome assemblies were generated with at least 50X coverage depth and initial assemblies were polished using the paired-end sequences generated through Illumina HighSeq 3000. Annotated draft genomes were generated to multiple *Epichloë* genomes predicting protein-coding genes. The combined platform approach, of long read genome assembly and genome sequence refinement using short read sequences, thus allows pan-genome analysis to a degree of resolution not previously possible, enabling enhanced understanding of the genomic variation amongst various *Lolium* spp. endophytes; improved insights into mitochondrial and nuclear genomic changes that have occurred during endophyte evolution; an increased ability to associate genetic variation with phenotypic differences, such as presence/absence of key genes in alkaloid biosynthesis, and the diversity of effector proteins that contribute to compatible endophyte-grass associations.

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INVESTIGATING THE EFFECTS OF FUNGAL ENDOPHYTES ON THE PASTURE PEST *HETERONYCHUS ARATOR* (AFRICAN BLACK BEETLE)

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The major insect pest of Australian cool temperate pastures is the root-feeding insect *Heteronychus arator* (African black beetle, ABB). Significant pasture damage can occur from damage by ABB, even at low densities (>10 individuals per square meter), and when infestation occurs, re-sowing of the whole paddock is required. Mitigation of the effects of pasture pests, and in particular subterranean species such as the larval form of ABB, can be challenging. Early detection is limited by the ability to visualise an above-ground effect and chemical control of insects in soil is often ineffective. Efforts have been made to develop pastures that maximize production by enhancing persistence in response to pasture invertebrate pests via use of fungal endophytes, symbionts which produce a suite of bioactive secondary metabolites. Several well-characterised secondary metabolites have been shown to benefit the host plant through deterrence of insect pests from feeding and insecticidal activity (e.g. peramine, lolines, ergopeptines). Determining the optimal level of alkaloid required to produce feeding deterrence *in planta* is imperative. A colony of ABB was established from populations collected in Victoria, Australia. Genetic analysis (based on the mitochondrial CO1 gene) of Victorian populations of ABB detected two genetically distinct populations. An *in vitro* bioassay, based on feeding an artificial diet containing endophyte derived insecticidal alkaloids, to test alkaloid activity against ABB was developed. Individuals were fed a range of alkaloids to determine a dose response (feeding deterrence). This bioassay informed the levels of insecticidal alkaloids required *in planta* for efficient control of ABB in the field. The *in vitro* bioassay was then extended to test perennial ryegrass – endophyte symbiota for control of ABB. This assay has been implemented as a phenomics tool for benchmarking pre-commercial and commercial symbiota and can be used to provide recommendations on endophyte performance against ABB to industry earlier than previously possible for pasture breeding.

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NEAR-INFRARED SPECTROSCOPY FOR RAPID ASSESSMENT OF SEED QUALITY TRAITS IN *LOLIUM*

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Perennial ryegrass (*Lolium perenne* L.) is an important forage grass in which specific cultivar traits, including yield, quality and persistence, can be influenced by symbiotic infection by *Epichloë* fungal endophytes. The selection of seed traits is important for the delivery of optimal economic value within farming systems. However, endophyte presence and identity cannot be discerned by the naked eye. This work aims to provide a rapid, non-destructive method to accurately differentiate between perennial ryegrass-endophyte associations. Near-infrared spectroscopy (NIR) coupled with multivariate statistical analysis was employed to discriminate between perennial ryegrass seeds from a range of different cultivars infected with a suite of endophytes representing the diversity of perennial ryegrass endophytes (both commercial and novel endophyte strains) including Standard Toxic Endophyte (SE). Endophyte-negative controls (without endophyte, WE) were also employed in the study. To develop the method, each association was scanned on multiple occasions to determine the reproducibility of scanning data and build a reference collection. For each scanning event, every sub-sample was scanned twice (a minimum of six replicates per sample in total). The reproducibility of scanning small and large sample sizes was also determined. Once optimised, Principal Component Analysis (PCA) illustrated clear clustering of each different endophyte strain within a cultivar, indicating good spectral reproducibility between samples and scans. Nested partial least squares discriminant analysis (PLSDA) models revealed that accurate and robust prediction of cultivars and endophyte strains within a cultivar could be achieved. Significant findings of this study are: 1) the perennial ryegrass cultivars examined in this study can be accurately predicted; 2) most endophyte strains within a cultivar can be accurately predicted; 3) endophyte presence/absence can be accurately predicted; 4) SE endophyte presence can be accurately predicted; 5) closely related endophyte strains from the same taxon, such as AR37 and NEA12 (*LpTG-3* strains), are clearly discriminated and accurately predicted. This study confirms that NIR can be used to accurately predict seed quality traits, such as cultivar and endophyte strain within a cultivar. Investigations into the use of NIRS to predict other seed quality traits, including single seed traits, is continuing.

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TALL FESCUE ENDOPHYTES FOR PERENNIAL RYEGRASS: DISCOVERING ENDOPHYTES WITH NOVEL BIOACTIVITY AND BROAD HOST SPECIFICITY

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Symbiotic association between temperate grasses and endophytic *Epichloë* fungal species leads to enhanced resistance to several biotic stress factors, including protection from mammalian and insect herbivory as well as resistance to nematode pests and some fungal pathogens. The ability of endophyte infected tall fescue grasses to synthesise secondary metabolites such as peramine and the lolines *in planta* plays a major role in providing protection from insect pests of grasses. However, endophytes usually residing in perennial ryegrass plants are unable to produce loline alkaloids. In the current study novel tall fescue endophytes that produce only insecticidal alkaloids, peramine and the lolines, were identified and characterised. Genetic analysis revealed that these endophytes belong to the *FaTG-3* taxonomic group. Furthermore, in an *in vitro* bioassay for antimicrobial bioactivity these endophyte strains exhibit strong bioactivity against a collection of plant pathogenic fungi and bacteria. *FaTG-3* strains are ideal endophytes, as they have the optimal alkaloid profile for pest (peramine and lolines) and disease control (unknown), and do not produce the compounds associated with toxicity in grazing livestock (epoxy-janthitrems, lolitrem B and ergovaline). To introduce those favourable properties of fescue grass derived endophytes into perennial ryegrass, *FaTG-3* endophytes were inoculated into perennial ryegrass and tall fescue hosts. Inoculation ability and vegetative stability was determined in both tall fescue and perennial ryegrass host backgrounds. Intergenerational stability of perennial ryegrass-*FaTG-3* symbiota was also determined. Production of lolines and peramine and associated insecticidal activity of *FaTG-3* endophytes was assessed in novel tall fescue and perennial ryegrass symbioses and compared to current commercial cultivar-endophyte symbioses. Novel endophyte strains that naturally co-exist with tall fescue grasses have been discovered that are suitable for deployment in perennial ryegrass pasture improvement.

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TAXONOMIC CHARACTERISATION OF *BRACHIARIA* GRASS ASSOCIATED ENDOPHYTES

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Brachiaria is a pan-tropical grass genus comprising c. 100 species, of which several are economically important forage pasture crops. Seed-transmitted endophytic *Acremonium* fungi have been reported to form mutually beneficial associations with *Brachiaria brizantha*. Although widely used for pasture-based agriculture in the tropics, *brachiaria* exhibits several shortcomings that constrain both its use and genetic improvement. Endophytes provide an excellent mechanism for novel trait delivery for *brachiaria* improvement. A total of 83 fungal endophytes were isolated from pseudostem material of 30 different *Brachiaria/Urochloa* accessions representing nine species. Initial morphological and ribosomal DNA (rDNA) sequence analysis of the internal transcribed spacer (ITS) region revealed the presence of 11 diverse species of fungi among these isolates, with multiple strains present within most species. Selected representatives from each species were subjected to whole genome sequence analysis, and extended phylogenetic characterisation was performed on both rDNA (18S and 28S) and nuclear DNA (*tub2*, *tef1*, *act1*,) regions. In this study, fungal species previously reported to have endophytic or non-obligate endophytic (i.e. saprophytic-endophytic) lifestyles with other host plant species have been identified in *brachiaria* grasses, however the newly discovered *brachiaria* associated strains are genetically novel. Based on the combined morphological and molecular taxonomic characterisation, four of the eleven fungal species have not previously been described and are characterised for the first time in this study. Further, several of these *brachiaria* associated fungi were also identified in seed and/or root material of economically important *brachiaria* forage species (e.g. *B. humidicola*, *B. decumbens* and *B. brizantha*), providing evidence for endophytic or non-obligate endophytic lifestyles of those species.

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THE GENETIC BASIS FOR EPOXY-JANTHITREM BIOSYNTHESIS IN PERENNIAL RYEGRASS ENDOPHYTES

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Epoxy-janthitrems are a class of indole diterpenes with structural similarity to lolitrem B. Two taxa of asexual *Epichloë* endophytes have been identified that produce epoxy-janthitrems, *LpTG-3* (*Lolium perenne* Taxonomic Group 3; e.g. NEA12, AR37) and *LpTG-4* (e.g. E1). The epoxy-janthitrems associated with endophyte-infected perennial ryegrass provide superior protection against a wide range of pasture pests and have also been shown to be tremorgenic. Epoxy-janthitrems are not well understood when compared to other alkaloid groups synthesised by *Epichloë* endophytes. For example, while the genes and biosynthetic pathway for lolitrem B have been described and extensively characterised, the epoxy-janthitrem biosynthetic pathway and associated gene complement have not been described. The aim of the present study was to identify and functionally characterise candidate genes for janthitrem biosynthesis in asexual *Epichloë* endophytes. Whole genome sequence analysis was used to identify candidate genes for janthitrem biosynthesis that are unique to the NEA12 genome. Protein sequences of LtmE and LtmJ from Standard Endophyte (SE) were used to search the NEA12 predicted protein database. A single LtmE NEA12 homologue was identified. This gene has homology to aromatic prenyl transferases, from *P. janthinellum* (JanD) and *P. paxilli* (PaxD), associated with synthesis of the indole diterpenes shearinine K and paxilline respectively. Following this nomenclature, the candidate gene was named *jtmD*. The location of *jtmD* and consequently the janthitrem biosynthesis gene cluster was determined in the assembled NEA12 reference nuclear genome. Contig 3 contains cluster 1 and cluster 2 of the LTM cluster as well as four genes, *PP01*, *PP02*, *jtmD* and *jtmO*, that are unique *LpTG-3* and *LpTG-4* endophytes. Expression of each of the genes was confirmed using transcriptome analysis of perennial ryegrass-NEA12 and perennial ryegrass-E1 symbiota. Sequence analysis confirmed the genes are functionally similar to those involved in biosynthesis of related indole diterpene compounds. Based on the identification and fragmentation of metabolites, a pathway for biosynthesis of the epoxy-janthitrems in *LpTG-3* and *LpTG-4* endophytes is proposed. The *jtmD* gene was functionally characterised using RNAi, followed by liquid chromatography-mass spectrometry (LCMS) analysis of transgenic endophytes *in planta*. RNAi silencing of *jtmD* and *in planta* assessment in host-endophyte associations confirms the role of *jtmD* in epoxy-janthitrem production.

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EXTENSIVE CLONALITY IN A BAHIAGRASS GERMPLASM COLLECTION FROM URUGUAY

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Populations of apomictic species are composed of single or a combination of clones. Individual plants in species like bahiagrass (*P. notatum* Flüge) may also propagate vegetatively by stolons and compete with one another resulting some populations dominated by individuals originating from a single seedling, a life form that is associated with extremely long lifespans. Consequently, long lived apomictic individuals composed of a very large number of ramets have ample opportunity to produce and disseminate a very high number of clonal seed further extending the range of a single clone to new locations. The rate of formation of new clones will depend on the reproductive system of each species and clone, its remaining ability to reproduce sexually or the availability of sympatric sexual biotypes. The resulting overall rate of origination of new clones is usually unknown and extensive germplasm collections may in extreme cases be majoritarily composed of a single widespread clone. In these cases germplasm evaluation, conservation, use and management in general may become extremely costly and inefficient. In order to assess the degree of variability and optimize the management and evaluation of the germplasm collection kept in INIA (Uruguay) we analyzed a total of 166 individuals from 74 different locations. Different individuals in a single collection point were chosen based on observable differences and distance. All individuals were analyzed with a set of four microsatellite markers specifically designed for the species which had previously been assessed for Mendelian behavior in a commercial sample of sexual diploid *Pensacola bahiagrass*. We identified a total of 29 multilocus genotypes (MLG) with an average 2.7 alleles per locus. Considering differences in individual alleles in otherwise identical MLG as putative mutations, a total of ten single genotypes and nine putative clones with more than one representative were identified. The most widespread clone contained 85 individuals from different locations in the whole country; the second most frequent one included 31 individuals mostly from eastern locations, and the rest of the putative clones included 2-7 individuals. Of the smaller clones the third most frequent one was also clustered in north-western locations. Especially in the case of highly apomictic grasses, specifically designing collection strategies that take into account the expected structure of variability of a wild species based on its reproductive biology can result in much a more efficient germplasm management strategy. Based on this analysis a whole collection can be reduced by almost ten times for evaluation, although the exploration of mutational variability within a promising widespread clone should not be ruled out. These results can also greatly simplify the choice of parents for crossing programs.

PRESENTER BIO: María Paz Catanzaro is an Assistant Professor and PhD Student at the University of Buenos Aires working in grass taxonomy and phylogeny. She has participated in several projects on grasses and weed science.

EFFECTS OF PLOIDY ON PHOTOSYNTHESIS, TRANSPIRATION EFFICIENCY, AND GROWTH OF ANNUAL RYEGRASS UNDER VARIABLE ATMOSPHERIC CARBON DIOXIDE AND WATER CONDITIONS

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With agricultural intensification, there is increasing concern over water use by agricultural cropping systems, a concern that is further exacerbated by climate variability. Stomata play an important role in water use efficiency (WUE) and regulate gas exchange between plants and the environment. In numerous instances, it has been observed that tetraploid plants have bigger stomata than diploids, which has been shown to decrease transpiration, increase biomass, and increase growth tolerance to limited water. The objectives of this research were to investigate the effects ploidy level on photosynthesis, transpiration efficiency, and growth of diploid and tetraploid annual ryegrass under controlled conditions. The plant material consisted of diploid and tetraploid seed for the cultivar “Marshall” and the USDA plant introduction 241586 (tetraploid seed was obtained by treating diploid seed with colchicine, growing the confirmed autotetraploids and harvesting seed from them). Diploid and tetraploid lines were grown under ambient (~400 ppm) and elevated (~800 ppm) atmospheric carbon dioxide, and optimal and water-limited conditions for approximately 90 days. Results from fall 2017 showed significant effects of ploidy on stem, root to shoot ratio, and leaf to stem ratios ($p < 0.05$). Additionally, it was found that there was an interaction effect of ploidy and CO₂ on the leaf to stem ratio ($p < 0.05$). The tetraploid lines exhibited higher root:shoot and leaf:stem ratios, but the diploid lines produced more stem biomass than tetraploids. There was also an interaction effect on the carboxylation rate between ploidy and water ($p < 0.05$). On the diploid level, plants that were well watered had a higher carboxylation rate than those that were water stressed. This was reversed on the tetraploid level with water stressed plants having a higher carboxylation rate than those that were well watered. The second replication in the spring of 2018 had a similar effect of ploidy on above ground biomass (stems and leaves), roots, and tiller count. Tetraploid lines produced higher amounts of aboveground biomass, roots, and tillers ($p < 0.05$).

PRESENTER BIO: Jennifer is a PhD student in her third year at the University of Florida. Her area of interest is in sustainable forage and grazing management systems. Her doctoral research focuses on ryegrass resilience in water stressed and elevated CO₂ environments.

COMBINING ABILITY OF *BRACHIARIA* HYBRIDS CONSIDERING AGRONOMIC TRAITS

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The General Combining Ability (GCA) of the parents, as well as the Specific (SCA) of the crosses can be obtained using a diallel scheme and analysis. These estimates are useful to breeders in the choice of the parents and the definition of crosses in a breeding program. However, information from this kind of analysis is still limited for *Brachiaria*. The aim of this study was to identify superior parents and verify genes action of traits through the evaluation of GCA and SCA estimates in *Brachiaria* hybrids. A partial diallel design was used to cross three apomictic and ten sexual parents, resulting in 29 full-sib families, which were evaluated in an incomplete blocks design experiment with plots of five plants. Field Green Weight (FGW, Kg.ha⁻¹) and Regrowth Ability (REG, scores) traits were measured in seven cuts. The data were analyzed by a mixed model approach in the R environment using the ASReml package and performing the modeling of the residual variances and covariance matrix (R). The R matrix structure that provided the smallest Bayesian Information Criterion (BIC) was the unstructured one for both traits. The sexual parents for REG trait and the apomictic parents, both for FGW and REG, presented a null GCA variance by the likelihood ratio test (LRT) test with 5% of probability. However, the sexual parents GCA variance for FGW was nonzero, thus allowing for select of the parents that contribute to increase the frequency of favorable alleles in future offspring for this trait. Thereby, the sexual parents B13, R69 and R33 were the selected ones since they presented the higher estimates of GCA for FGW. The SCA variance was nonzero for both traits, and the largest predictions magnitudes of the SCA was for B13 x Basilisk and R69 x Mulato II crosses for both traits. In summary, genetic variation was observed for the REG and PGW traits with the indication of additive and non-additive genes action, which provides subsidies for the breeding program and hybrid production.

PRESENTER BIO: Beatriz is a Ph.D. student at Genetics and Plant Breeding Graduate Program at the University of Lavras in Brazil. She is conducting her experimental work at the Embrapa National Beef Cattle Research Center in Campo Grande, MS, Brazil.

COMPLEXITIES OF A BREEDING PIPELINE: DETERMINING THE VALUE OF ENDOPHYTES IN SUMMER DORMANT TALL FESCUE

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Tall fescue (*Festuca arundinacea*) is a valuable and broadly adapted forage grass occupying approximately 14 million hectares across the eastern United States. The success of tall fescue is attributed to the presence of *Epichloë coenophiala*, a dominant symbiont that colonizes the above ground plant tissue. There are four interspecific hybrid *Epichloë* taxa that associate with tall fescue, *E. coenophiala*, *Epichloë* sp. FaTG-2, *Epichloë* sp. FaTG-3, or *Epichloë* sp. FaTG-4. Each can be distinguished based on genetic variation that equates to differences found at the alkaloid loci for production of ergot alkaloids, indole-diterpenes, lolines, and peramine. These alkaloids provide protection to the plant host from herbivory, but some have also earned a bad reputation, such as the ergot alkaloids that are toxic to grazing livestock. However, a number of elite cultivars have been developed with improved persistence and the inclusion of an *Epichloë* species that is considered safe for grazing livestock. Currently, we are trying to advance the production of tall fescue into Oklahoma and Texas where hot and dry summers can deplete or eliminate stands of tall fescue. Mediterranean (summer dormant, SD) tall fescue has a survival strategy by going dormant during summer thus offering multi-year persistence in stressed environments, unlike the widely planted summer active (continental) tall fescue. We have developed a number of clonal-pair populations that differ by presence or absence of endophyte strain to determine if endophyte-infected Mediterranean tall fescue will provide the same benefits with an endophyte that are seen with continental tall fescue. Seeds have been produced from nine populations of clone pairs each E+ and E- line and we have established sward plots to evaluate persistence of endophyte-infected vs endophyte-free material under intensive grazing and mechanical harvesting. However, breeding with endophytes presents an extra layer of complexity to preserving the integrity of both the symbiont and host throughout the breeding pipeline. We routinely track endophyte infection rate and strain identity, testing both seed and tillers through the greenhouse to the field, using PCR markers developed to distinguish strains based on genetic variation of alkaloid biosynthesis. Currently, research is under way to evaluate summer dormant, clonal pair, tall fescue populations in hot, low rainfall regions to determine the impact of the associated endophyte as a value-added trait in future cultivar development.

PRESENTER BIO: Michael Trammell is a Senior Plant Breeder with more than 20 years of experience in plant breeding and forage research. He has released several commercially available cultivars that include tall fescue, white clover and forage crabgrass.

GENETIC VARIABILITY IN THE USDA *CYNODON* GERMPLASM COLLECTION FOR BERMUDAGRASS STEM MAGGOT AND AGRONOMIC TRAITS

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Bermudagrass (*Cynodon* spp. L.) is an important warm-season perennial grass grown for forage and turf in Southeastern U.S. The bermudagrass stem maggot (*Atherigona reversura*; BSM) is a new invasive fly species, native to southeastern Asia, that has caused a significant economic impact due to its causal reduction in forage yield and quality. The objectives of this study were to calculate genetic correlations in a set of 287 bermudagrass genotypes for BSM and agronomic traits (dry matter yield, leaf width, nutritive value) in Citra, Florida. The population was composed of: i) 146 PI's from the bermudagrass core collection; ii) 137 PI's from the USDA-NPGS; and iii) commercial cultivars ("Tifton 85", "Coastal", "Jiggs", and "Florida 44"). The experiment was designed as a row-column with two replicates and augmented representation of the cultivars. The population was evaluated twice (June and August 2015) for the following traits: i) BSM damage on a scale of 0 to 9 (scale: 0 = no visible damage; 9 = more than 90% damage); ii) Dry Matter Yield (DMY); iii) Nutritive Value (NV) using wet chemistry [crude protein (CP), phosphorus (P), in-vitro dry organic matter digestibility (IVOMD) and neutral detergent fiber (NDF)]; and once (June 2nd 2015) for leaf width (LW). Linear mixed models with repeated measures were implemented in ASReml using genotypes as a random effect. The estimated genotypic values were used to compute Pearson's correlation (r) among traits and Principal Components Analysis (PCA). Very low correlation ($r < 0.10$) was observed between BSM/LW and BSM/DMY, while a moderate correlation ($r = 0.54$) was observed for LW/DMY. Similarly, low correlation coefficients ($-0.11 < r < 0.16$) were observed among BSM and all NV traits, while moderate/high correlations ($-0.69 < r < 0.59$) were found among NV traits. Genetic variability exists for BSM in the USDA germplasm; however, complete resistance is nonexistent and the mechanism(s) conferring tolerance remain unknown. Large genetic variability is present for BSM and agronomic traits among commercial cultivars, with Tifton85 providing high DMY, high NV and slight BSM tolerance.

PRESENTER BIO: Maryjo Valle is an undergraduate student pursuing a degree in Plant Science – Plant Genetics in the College of Agricultural Life Sciences at UF. She is a student researcher working in Dr. E. Rios lab. Her research focuses on determining if resistance in annual ryegrass to *Pyricularia grisea* is due to a variation in ploidy number and the effect cuticle thickness has on the pathogen's penetration.

BERMUDA 2000 – A NEW BERMUDAGRASS FORAGE CULTIVAR FOR FLORIDA

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Bermuda 2000 (B2000) is a hybrid bermudagrass entry with potential to be used as forage in Florida. It was introduced in Florida in 2000 but it has limited agronomic data published in the literature. The objective of this study was to evaluate herbage accumulation and nutritive value of B2000 in Florida. The studies were conducted in Ona, FL and Marianna, FL from April to October 2015 and 2016. Treatments were different bermudagrass entry/cultivars, 'Jiggs', 'Tifton 85', 'Coastal', 'Tifton 44', and B2000 at Ona and 'Jiggs', 'Tifton 85', 'Coastal', 'Tifton 44', 'Alicia', 'Russell', and B2000 at Marianna, distributed in a randomized complete block design with four replicates. Plots were 3 x 2 m and were harvested at 15 cm stubble height with 4 wk regrowth interval. Plots were fertilized with 60 kg N, 15 kg P₂O₅, and 60 kg K₂O after every harvest. There was a significant cultivar x month interaction for herbage accumulation at Ona and Marianna. In Ona, Jiggs and B2000 had greater herbage accumulation than Tifton 85 and Coastal in April while Tifton 44 did not differ from Jiggs, B2000, Tifton 85, and Coastal. In addition, Jiggs and B2000 had greater herbage accumulation than the other cultivars in October and November. Tifton 44 had the greatest crude protein concentration and there was no difference among Tifton 85, Jiggs, and B2000. Conversely, Tifton 85 had the greatest in vitro organic matter digestibility (IVDOM) and there was no difference among Tifton 44, Jiggs, and B2000. In Marianna, B2000 and Jiggs had greater herbage accumulation than the other cultivars in May but there was no difference in total annual herbage accumulation among cultivars/entry. Tifton 85 had greater IVDOM concentration, followed by B2000, Coastal, Jiggs, and Tifton 44. Alicia and Russell had the least IVDOM concentrations. B2000 has superior spring growth in Florida and has potential to be released as a bermudagrass cultivar.

PRESENTER BIO: Dr. Joao Vendramini is an Associate Professor, Agronomy - Forage Specialist at the University of Florida. His research is dedicated to forage management with emphasis on sub-tropical production systems. The major area of interest is forage-livestock interface and the impact of forage management on forage and animal production, and environmental quality.

PASPALUM ATRATUM AND P. MALACOPHYLLUM POLLEN CRYOPRESERVATIONA. P. Fávero¹, B. B. Z. Vigna¹ and N. B. Dinato²¹Embrapa Pecuária Sudeste, São Carlos, SP, Brazil²Genetics and Evolution Department, Federal University of São Carlos, São Carlos, SP, Brazil

The genus *Paspalum* belongs to the Poaceae family, is native from Americas, and 214 species are found in Brazil under different ecological conditions, presenting high genetic diversity. Among these species, two stand out: *P. atratum*, which is a species with excellent forage production, great speed of establishment and regrowth, good acceptance by cattle and horses and has a small requirement in soil fertility, besides having a high production of seeds; and *P. malacophyllum*, that presents high palatability, being well accepted by the animals and presents tolerance to flooding. The majority of *Paspalum* cultivars that had been released as forage grasses were based on selection of apomictic ecotypes. *Paspalum* synthetic sexual tetraploid genotypes were obtained in breeding programs in Argentina and USA aiming hybridizations. Besides the countries previously mentioned, Brazil has also a *Paspalum* breeding program for forage and lawn purposes. However, the hybridization between parents just occurs if the flowering time is synchronized, which sometimes does not occur. Flowering time of different genotypes can be synchronized by manipulating photoperiod and temperature, however, the pollen preservation until the female parent starts to flower is the more feasible approach. The pollen cryopreservation success depends on different factors, like the cell water content, and, when it is high, pollen dehydration is necessary. This study aimed to determine the *P. atratum* and *P. malacophyllum* pollen stainability after 12-month storage in liquid nitrogen. Pollen was collected under greenhouse conditions at Embrapa Pecuária Sudeste and exposed to two dehydration agents (saturated solution of LiCl (75%) and silica gel) as the humidity of the material should be around 20% to a successful freezing. Pollen dehydration was performed in a gerbox using the dehydration agents placed in an open petri dish with a grid supporting another open petri dish containing the pollen grains. The gerbox were kept closed during three different periods (30, 60 and 120 minutes), in three replications, at 25 °C. After this time, the pollen grains were placed in gelatine capsules inside cryotubes and immersed in liquid nitrogen. The non-dehydrated treatment was also evaluated. After slow thawing, which consists of pollen thawing by the maintenance of the samples for 30 minutes in freezer, then 30 minutes in refrigerator and then 30 minutes at room temperature, pollen stainability was evaluated using tetrazolium solution 0,25%. Fresh pollen was also evaluated as control. The non-dehydrated pollen samples presented 34% of stainability after 12-month storage. Pollen that was dehydrated with LiCl for 30 minutes and silica gel for 120 minutes and stored for 12 months into liquid nitrogen had the same stainability (70%) as the fresh pollen, which can be considered a well succeeded way of *Paspalum* pollen long term conservation.

PRESENTER BIO: Dr. Vigna is a Researcher at Embrapa and has more than 10 years of experience with genetic resources and molecular genetic breeding of various forage species (*Urochloa* spp., *Paspalum* spp., alfalfa).

TRANSCRIPTOME ANALYSIS OF *PASPALUM VAGINATUM* UNDER DROUGHT CONDITION

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Paspalum vaginatum Swartz, also known as seashore paspalum, is a halophytic, diploid, self-incompatible, warm-season perennial grass, well adapted to coastal regions in tropical and subtropical environments, as Argentina, Brazil and United States. This grass tolerates several stresses as salinity, drought, low temperatures, among others. Due to its unique growth characteristics, it is a plant with great potential for studies of tolerance to drought and salinity. Transcriptome analysis can help to understand the mechanisms that make plants able to adapt to different environments. The objective of this study is to evaluate the gene expression profile of *P. vaginatum* Sw. in response to drought. The drought experiment was performed in a greenhouse at Embrapa Pecuária Sudeste (São Carlos, Brazil) where the accession *P. vaginatum* Sw. BGP 114 was cultivated in triplicates during Brazilian spring (Nov/2016). Leaf samples of each biological replicate were collected in two conditions, without water stress (28% soil water content) and after eight days under drought stress (4% of soil water content), and had their mRNA extracted. Samples were sequenced using Next Generation Sequencing (NGS) technology on Illumina HiSeq 2500 equipment and paired-end reads (2x100bp). The assessment of quality of the *de novo* transcriptome assembly, the differential gene expression analyzes and the functional annotations of the genes were performed following Trinity pipeline. The sequencing generated around 80 millions of reads per sample and more than 207 million bases were assembled from the *de novo* assembly, 135.997 transcripts were identified from 44.842 predicted genes and N50 stats of 2.259 with median contig length of 1.193 bp. Bowtie2 were used for alignment of reads to the Trinity assembly with great result of 91.39% mapped as proper pairs. Diamond Blast software resulted in 17.381 of 40.817 proteins which were covered by more than 90% of their protein lengths. This latter analysis, additionally to the others bioinformatics tools as FastQC, ExN50 stats and Transrate (score of 0.393), showed satisfactory results by inferring good quality of reads and assembly. BUSCO3 searched in embryophyta dataset from *OrthoDB* v9 database returned a good result of 91.37% of transcriptome completeness. Differential gene expression analyzes using EdgeR software identified 3.280 differentially expressed genes (DEG) and Trinotate software suite were used for functional annotation of the transcriptome. Searches in UniProt databases and PubMed for the top ten annotated DEG showed relation to biological processes and molecular functions. The annotated genes *CCA1* and *LHY* were previously reported in *A. thaliana* and was suggested that they regulate the expression of genes (ex: *TOC1*) involved in drought, salt and heat responses such as stomata closure. *MGL3* gene was also annotated and encodes for LEA (Late Embryogenesis Abundant), which is already known in high activity under drought condition. The analyzes of physiological, genetic data and the regulation of genes will be essential for a better understanding of the mechanisms related to drought and development of new stress-tolerant cultivars.

PRESENTER BIO: Dr. Vigna is a Researcher at Embrapa and has more than 10 years of experience with genetic resources and molecular genetics breeding of various forage species (*Urochloa* spp., *Paspalum* spp., alfalfa).

HERITABILITY OF RESILIENCE TO DEFICIT IRRIGATION IN TALL FESCUE

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Pasture and forage system sustainability is threatened by increasingly severe biotic and abiotic stressors and changing climatic conditions. Therefore, identifying genetic variability and developing plant materials that have enhanced resilience to climatic extremes and widely variable environmental conditions is critical to maintaining productivity and vitality of pasture and rangeland systems. Measures of resilience have been reported, but the genetic control of resilience has not been well defined. Therefore, this experiment examined the genetics of resilience to drought in tall fescue (*Schendonorus arundinaceus* [Schreb.] Dumort, nom. Cons.).

Heritabilities and genetic correlations between five levels of irrigation were estimated for forage mass in a tall fescue half-sib population grown in Logan, Utah, USA. Sward plots were established in a line-source sprinkler experiment with an irrigation gradient established, such that the range closest to the sprinkler received 100% of evapotranspiration (ET) replacement and each successive distal range experiencing greater deficit irrigation (i.e., 100, 78, 57, 36, and 15% ET replacement, respectively). Plots were mechanically harvested five times per growing season, and yearly forage mass determined for three consecutive years.

Deficit irrigation greatly reduced forage mass by up to 62% with yearly forage mass of 12796, 11650, 8786, 6677, and 4887 kg/ha for the 100, 78, 57, 36, and 15% ET replacement levels, respectively. However, heritability for tall fescue forage mass was moderate and similar among irrigation levels (0.62, 0.61, 0.46, 0.56, and 0.62 for the 100, 78, 57, 36, and 15% ET replacement levels, respectively). Phenotypic correlations among irrigation levels ranged from 0.70 to 0.35, with forage mass from the 100 and 15% ET irrigations levels being the least correlated. In contrast, genetic correlations between irrigations levels were moderate to high (0.53 to 0.89) with a genetic correlation of 0.78 between the 100 and 15% ET irrigations levels. These results indicate that tall fescue forage mass response to increasingly deficit irrigation is highly heritable and under similar genetic control regardless of irrigation level. However, the low phenotypic correlations suggested the possibility of variation for resilience to deficit irrigation. Therefore, coefficients and the heritability of resilience across irrigation levels will be estimated, and the potential to breed for greater drought resilience in tall fescue discussed.

PRESENTER BIO: Dr. Waldron is a research plant geneticist with the USDA Agricultural Research Service and has 25 years of experience conducting genetic research on forages and turf. During his tenure, he has initiated innovative breeding programs in tall fescue, forage kochia, western wheatgrass, bluebunch wheatgrass, and low-maintenance turf.

THE STUDY ON DROUGHT RESISTANCE OF *MEDICAGO SATIVA* L. USING TRANSCRIPTIONAL SEQUENCING

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Medicago sativa is widely planted in arid and semi-arid areas of China. But, drought is one of the main limiting factors to its production. It is very important to study the effects of drought stress on *Medicago sativa*. In this study, transcriptional sequencing was carried out to analyze the effect of gene expression in *Medicago sativa* under PEG drought stress.

Materials and method: The 22 day seedling of *Medicago sativa* L. cv. Zhongcao No.5, which was bred by our group were treated with 12% PEG-6000 solution for 6 h (T1) and 18 h (T2), respectively, and treated with distilled water as the control (CK). Three replicates per treatment, 40 plants per replicate. After treatment, roots of each replicate were collected respectively for transcriptome sequencing by Gene Denovo Biotechnology Co.

Results: A total of 94739 unigene were obtained from 9 samples. The longest transcript was 16588bp with an average length of 749bp. Sequence annotation against four databases resulted in the successful annotation of 65846 unigenes. A total of 28789 differentially expressed genes between PEG stress and control were identified, among which, 9346 at 6 h and 19443 at 18h. Prior to sending your abstract, be certain to proof it one last time, including all author information, as abstracts are published exactly as submitted. We look forward to receiving your abstract submission.

KEGG pathway analysis showed that these genes were mainly located in phenylpropanoid biosynthesis, flavonoid biosynthesis, glutathione metabolism, plant circadian rhythm, biosynthesis of secondary metabolites, flavone and flavonol biosynthesis metabolism Pathway. Transcripts known to be involved in the drought stress response were confirmed by qRT-PCR to have expression patterns similar to those measured by RNA-Seq.

Conclusion: This de novo assembly of *Medicago sativa* transcriptome from root of droughted and non-droughted plants provides a rich source for gene identification. A total of 28789 differentially expressed genes were detected. Comparison of gene expression levels between control and treatments identified candidate genes for further analysis of the genetic basis of drought tolerance in *Medicago sativa*.

PRESENTER BIO: Dr. Wang is a Professor with more than 20 years of experience researching forage breeding. She has extensive experience with genetic breeding of legume crops and has led more than several projects dedicated to the enhancement of alfalfa.

GENOMICS-ASSISTED BREEDING FOR ENHANCING RESISTANCE TO BIOTIC AND ABIOTIC STRESSES IN ALFALFA

Long-Xi Yu

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Objectives: Alfalfa production is challenged by endemic and emerging diseases, and adverse environmental factors. Developing alfalfa varieties with resistance to these stressors is imperative for sustainable alfalfa production in the United States. Conventional breeding procedures to develop alfalfa cultivars are time consuming and cost ineffective. To accelerate the breeding process, identification of DNA markers associated with disease, drought and salt tolerance is a first step toward developing resilience and resistance to stressors in alfalfa.

Materials and Methods: Three panels of alfalfa populations were used for evaluating disease and drought/salt resistance in the field and greenhouse. Genome-wide association studies with genotyping by sequencing were used for mapping resistance loci.

Results: Verticillium wilt (VW) is an alfalfa disease that reduces forage yields up to 50 percent. Current breeding strategies rely greatly on phenotypic recurrent selection that allows slow and inefficient genetic improvement progress. In collaboration with S & W Seeds Company, we identified a group of markers and putative candidate genes associated with VW resistance. These genes encode leucin rich repeat kinases and ABC transporter. We developed a DNA marker linked to these genes and obtained high co-segregation with the resistance allele in the S&W populations. With further validation, the marker can be used for marker-assisted selection for breeding alfalfa cultivars with improved resistance to the disease. Drought and salt resistance are important breeding targets for enhancing alfalfa productivity in arid and semi-arid regions. We identified genetic loci associated with drought and salt tolerance in alfalfa using genome-wide association studies. Most markers loci associated with drought and salt resistance in this work overlap with the previously reported quantitative trait loci (QTL) associated with biomass under drought in alfalfa. We used a Basic Local Alignment Search Tool (BLAST) and revealed homology to several annotated genes with functions in stress tolerance. With further validation, these markers may be useful for marker-assisted breeding for new alfalfa varieties with drought and salt resistance.

Conclusion: Quantitative traits such as biotic and abiotic stress resistance are most likely under the control of multiple genes and interact with environmental factors. Identification of resistance loci that contribute to variation in such complex traits, is a primary challenge in plant breeding and population genetics. In the present study, we used an integrated framework that merges a QTL mapping approach to map disease and abiotic stress resistance loci in alfalfa. This framework provides a statistical basis for analyzing marker-trait association using linkage disequilibrium. We identified a group of SNP markers and candidate genes associated with Verticillium wilt resistance and drought and salt tolerance. Validation of the markers is in progress. Once validated, these SNP markers can be used for marker-assisted selection.

PRESENTER BIO: Dr. Long-Xi Yu is Research Geneticist in USDA-ARS. Dr. Yu is interested in applying genomic tools for enhancing resistance to biotic and abiotic stress in forage crops. He has conducted research in plant molecular genetics for 30 years with 80 publications. He served as editorial board for 6 peer-reviewed Journals.

DETECTION OF QUANTITATIVE TRAIT LOCI ASSOCIATED WITH DROUGHT TOLERANCE IN ST. AUGUSTINEGRASS

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St. Augustinegrass (*Stenotaphrum secundatum*) is a warm-season grass species commonly utilized as turf in the southeastern US. Improvement in the drought tolerance of the St. Augustinegrass has significant value within the turfgrass industry. Understanding quantitative trait loci (QTL) in genomic regions associated with drought tolerance will allow for advancement of breeding strategies to identify St. Augustinegrass germplasm with improved performance for this trait. A multi-year and multi-environment study was performed to identify QTL in a 'Raleigh' x 'Seville' mapping population segregating for phenotypic traits associated with drought tolerance. Phenotypic data was collected from a field trial and a two-year greenhouse study, which included relative water content (RWC), chlorophyll content (CHC), leaf firing (LF), leaf wilting (LW), green cover percent (GCP) and normalized difference vegetative index (NDVI). QTL were detected for all traits and distributed on seven of nine linkage groups. Several hotspot regions were found where QTL overlapped for multiple traits. Sequence analysis revealed several drought response genes within these regions. The QTL identified in this study have potential to be used in the future to identify genes associated with drought tolerance and for use in marker-assisted breeding.

PRESENTER BIO: Dr. Yu is a postdoctoral researcher in NCSC turfgrass breeding and genetics program. He got PhD from Nanjing Agricultural University. He is currently working on the projects of improve turfgrass performance using genomic and genetic approaches.

PRELIMINARY STUDY ON UNMANNED AERIAL VEHICLE (UAV) BASED HIGH-THROUGHPUT PHENOTYPING OF PLANT HEIGHT IN SEEDED CENTIPEDEGRASS AND ORNAMENTAL PLANTS

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Promising results have been reported using UAV-based imagery to estimate plant height as a high-throughput phenotyping trait in some grain and row crops. We are not aware of a similar investigation reported in turfgrass or ornamental plants. Our objective was to assess the use of an affordable UAV-based platform on estimating the plant height in centipedegrass (*Eremochloa ophiuroides*) and ornamental plants such as red trispecific *Pennisetum* hybrids, Pride of Barbados (*Caesalpinia pulcherrima*), little bluestem (*Schizachyrium scoparium*), *Pennisetum alopecuroides*, and *Miscanthus sinensis*. UAV equipped with a high resolution RGB camera was used to collect digital images on all studied plants with a flight altitude of 30 meters. A 3D dense point cloud and a digital surface model were obtained for each study site, and the plant height was estimated along with ground measurements. Results showed that plant height can be estimated accurately for red trispecific *Pennisetum* hybrids ($R^2=0.90$, RMSE=12.6 cm) and Pride of Barbados ($R^2=0.86$, RMSE=7.4 cm). Over-estimation was observed for *P. alopecuroides* ($R^2=0.86$, RMSE=10.0 cm) and under-estimation was observed for *M. sinensis* ($R^2=0.64$, RMSE=7.3). Plant heights in centipedegrass and little bluestem were poorly estimated using UAV and RGB cameras due to the low height of centipedegrass (20 – 40 cm) and narrow leaf width in little bluestem. This preliminary study validates the use of an affordable RGB camera and UAV in high-throughput phenotyping of plant height in red *Pennisetum* trispecific hybrids and Pride of Barbados with more horizontal and greater leaf widths.

PRESENTER BIO: Dr. Zhang is a Postdoc Research Associate working in turfgrass management and stress physiology using remote sensing technology.

THE FLOWERING REGULATION OF ORCHARDGRASS DURING VERNALIZATION

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Orchardgrass (*Dactylis glomerata* L.) is a cold season perennial gramineae grass, which is native to northern Africa, western and central Europe, and temperate Asia. Vernalization and the transition from vegetative to reproductive growth involve multiple pathways, vital for controlling floral organ formation and flowering time. In this study, we generated a comprehensive and continuous transcriptome of orchardgrass to elaborate dynamic changes of TFs expression among six stages which responding to vernalization and flowering regulating. Transcription factors (TFs) related to WRKY, NAC, AP2/EREBP, Alfin-like, AUX/IAA, MADS-BOX, bHLH, ABI3/VP1 and CCAAT family during vernalization and floral bud development were enriched. The expression patterns of TFs revealed intricate temporal variations, suggesting relatively separate regulatory programs of TF modules.

WRKY, MADS-BOX, and ABI3/VP1 transcription factor families were found to preferentially accumulate during vernalization, floral organ formation, and heading stages, which could be part of the transcriptional regulatory complex regulating stress response and flower development. Several WRKY transcription factors expressed in before heading stage and heading stage, such as WRKY 12, which participates in cell wall formation and promotes flowering. MADS-box transcription factor suppressor of overexpression of CO1 (SOC1) signals from the GA-dependent pathway influences the flowering time during short days. ABI3/VP1 transcription factor family encoded the B3 domain-containing protein, were the key components of auxin signaling include the AUX/IAA coreceptors and ARF transcription factors. Our results showed that the type of transcription factors was strongly expressed in vernalization stage and heading stage, as well as the MADS-box transcription factors. The basic Helix-Loop-Helix (bHLH) family, comprising transcription factors that are known to be active during flower development, peaked in heading stage. Our study found bHLH TFs significant enriched during booting stage and heading time, suggesting this type of transcription factor family may play a similar role in the regulation of flower formation and heading. In addition, the AUX(AUXIN)/IAA transcription factors also have a high expression level in heading stage. Our results also showed that the WRKY transcription factors have the similar expression pattern with NAC transcription factor family. We found NAC genes concentrated expression in before heading and heading stage in our data, meaning that this type of TFs was produced to join the pathways that lead to flower organ formation and flowering. Our analysis of genes that are associated with transcription regulation showed that TFs play an important role during vernalization and floral bud formation. Expression profiles during different developmental stages suggest temporal specialization of TFs for flowering time control.

PRESENTER BIO: Prof. Xinquan Zhang is a professor with more than 30 years of experience researching forage and turfgrass in Sichuan Agricultural University. He has extensive experience with genetic breeding of various forages and has led more than several projects dedicated to the enhancement forage and turfgrass.

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