Harnessing Underutilized Gene Bank Diversity & Genomic & Phenomic Prediction to Improve Annual Ryegrass (*Lolium Multiflorum* Lamarck)

Esteban Rios, PhD^{1,2} Pablo Sipowicz, PhD candidate² Kevin Kenworthy, PhD¹ ⁽¹⁾Agronomy Department, University of Florida ⁽²⁾Plant Breeding Graduate Program, UF-IFAS







PLANT BREEDING





FORAGE BREEDING AND GENETICS

Outline

- Annual ryegrass breeding at UF
- Germplasm sources
- Ploidy and genetic parameters
- Genetic diversity
- Genomic prediction
- Phenomic prediction
- Breeding for climate adaptation



Annual ryegrass breeding program at UF



Dr. Gordon Prine

Objectives

Disease resistance; maturity; cold tolerance (collaborations)



Crown rust (Puccinia coronata)



Grey leaf spot (Pyricularia grisea)



Leaf spot (Bipolaris)

Annual ryegrass breeding program at UF



Dr. Gordon Prine

Recurrent phenotypic selection (8-10 years)





- More than 40 cultivars released
- 2016-2020: seed sales of UF-IFAS cultivars averaged 10 million kg /year, which would plant over 350,000 ha.

Germplasm sources



Germplasm Resources Information Network

139 Pl's: only 14 with known ploidy level



Other sources:

- 15 experimental lines
- 13 commercial cultivars
 - 167 accessions in total

Ploidy level





Rios et al., 2015



Rios et al., 2015

Login for returning member. Don't have an online profile? Register Now

Ploidy level





** : significant differences at P < 0.001

Rios et al., 2015

Genetic parameters



• Rios et al., 2019

Genetic corr

-1.0 -0.5 0.0

elation 0.5 1.0	Leaf length	0.11	0.11	0.10	0.16	0.09	0.09	0.16	0.08	0.14	0.12	0.13	0.10	0.17	0.13	0.08	0.06	1.00
	Leaf width	0.08	0.11	0.09	0.14	0.03	0.11	0.13	0.06	0.09	0.08	0.10	0.06	0.16	0.08	0.09	1.00	0.70
	Plant vigor	0.13	0.16	0.15	0.21	0.13	0.14	0.21	0.13	0.17	0.16	0.17	0.11	0.20	0.18	1.00	0.86	0.83
	SPAD	0.11	0.15	0.12	0.16	0.09	0.14	0.16	0.10	0.11	0.11	0.11	0.10	0.18	1.00	0.21	0.71	0.37
	Establishment	0.14	0.18	0.16	0.21	0.16	0.16	0.19	0.16	0.18	0.17	0.18	0.15	1.00	-0.11	0.31	0.04	0.28
	Leaf stem ratio	0.10	0.08	0.07	0.14	0.06	0.11	0.14	0.06	0.07	0.05	0.07	1.00	0.28	0.61	0.67	0.76	0.48
	Leaf spot	0.12	0.14	0.11	0.17	0.09	0.13	0.15	0.11	0.05	0.11	1.00	-0.82	-0.11	-0.76	-0.58	-0.63	-0.45
	Rust	0.09	0.13	0.11	0.15	0.09	0.13	0.15	0.10	0.09	1.00	0.59	-0.84	0.04	-0.58	-0.36	-0.63	-0.28
	Grey leaf spot	0.11	0.15	0.12	0.17	0.10	0.14	0.16	0.11	1.00	0.74	0.94	-0.87	-0.15	-0.76	-0.61	-0.76	-0.45
	Tiller size	0.11	0.07	0.06	0.13	0.05	0.10	0.15	1.00	-0.70	-0.58	-0.67	0.84	0.31	0.70	0.62	0.80	0.75
	Tillers	0.10	0.12	0.13	0.10	0.13	0.14	1.00	0.11	0.39	0.00	0.36	0.10	0.26	-0.25	-0.29	-0.39	-0.14
	Flag leaf length	0.09	0.09	0.09	0.12	0.10	1.00	0.13	0.55	-0.11	0.01	-0.27	0.34	0.25	0.14	0.47	0.25	0.68
	Flag leaf width	0.09	0.11	0.09	0.14	1.00	0.40	-0.34	0.88	-0.70	-0.61	-0.73	0.77	-0.08	0.70	0.73	0.98	0.68
	Crown	0.07	0.09	0.12	1.00	-0.08	0.45	0.71	0.40	0.17	0.01	-0.11	0.16	0.09	-0.08	-0.11	-0.22	0.13
	Plant height	0.11	0.06	1.00	0.45	0.57	0.55	0.29	0.82	-0.61	-0.47	-0.63	0.75	0.22	0.52	0.42	0.60	0.59
	Biomass	0.11	1.00	0.82	0.82	0.55	0.68	0.49	0.96	-0.48	-0.46	-0.58	0.78	0.41	0.43	0.49	0.50	0.69
	Heading date	1.00	0.49	0.20	0.85	-0.34	0.46	0.66	-0.00	0.43	0.46	0.21	-0.28	0.08	-0.23	-0.40	-0.50	-0.02



THE STE STE 189 Fed led lend Led set all not part set at here the



Pablo Sipowicz

Genetic diversity





Sipowicz et al., unpublished







Annual ryegrass

Allele

frequency (m)



Alfalfa

Cross-pollinated species Training population Family phenotypic value (FV) Family pooled

phenotypic and

genotypic data

Genome-wide Family

Prediction (GWFP)

Rios et al., 2021

✓ Half-sib progeny → common strategy in forage and turfgrass species







April-May 2016

October 2016

January 2017

February 2017

Germplasm

237 half-sib families; 2 checks (Marshall, Frostproof) Phenotypic data

100

120

80

Dry Weight (g)

Canopy height (CH), yield (Y), heading date (HD), and tiller width (TW) were measured in 2018 and 2023.



10

15

20

25

Stems Dry Weight (g)

30

35









40

60

Genomic data

DNA extracted from bulked samples.

Sequence Capture (SC) with 200,000 probes; 3500 SNPs from

Models

G matrix was estimated from allele frequencies using AGHmatrix GBLUP model fitted using ASReml in R.





Pablo Sipowicz

Sipowicz et al., unpublished

$P = G + E + G^*E \dots M + \dots + G \times E \times M$

lgnore

- Matrix A based on pedigree
- Matrix G based on molecular markers
- Matrix *P based on the phenome

*P: endophenotypes capture the expressed genotypes and the complex regulatory networks that occur in the different layers between genome and phenotype. Source for phenome NIRS and/or hyperspectral imagery. Ricent et al., 2018 <u>https://doi.org/10.1534/g3.118.200760</u>

$$y = X\beta + Zg + e \quad g \sim N(0, P^{-1}\sigma_g^2)$$

Sipowicz et al., unpublished





Pablo Sipowicz

Genomic vs Phenomic prediction



Pablo Sipowicz





3500 Genomic predictors

Sequence capture genotyping from bulked samples.

 $y = X\beta + Zg + e$ $g \sim N(0, G^{-1}\sigma_g^2)$



Near Infrared Spectrometer



Spectral profile of annual ryegrass samples



3300 Phenomic predictors

 $y = X\beta + Zg + e$ $g \sim N(0, P^{-1}\sigma_q^2)$

Sipowicz et al., unpublished

Genomic vs Phenomic prediction 1.00 Pablo Sipowicz 0.75 0.50 0.25 Predictor - 0.00 Genomics Phenomics -0.25 -0.50 ٠ -0.75 -1.00 Dry Matter Heading Date Tiller Width Canopy Height

Sipowicz et al., unpublished

Breeding for climate adaptation

Compare 2x vs 4x with the same genetic background

Wild and cultivated populations







Breeding for climate adaptation

WUE under normal and elevated CO₂

- CO₂: 540 and 800 ppm
- Genotypes: Wild and Cultivar at 2x vs 4x
- Water: field capacity and 50% field capacity



Jennifer Timmers Maria Mailhos Nicolas Caram





2.5

2.0

5

1.0

0.5

0.0

Leaf biomass

540 ppm

P = 0.028





Thanks!

Forage Breeding and Genetics Lab

Dr. Yolanda Lopez, Pablo Sipowicz, Jack Harling, Habib Akinmade, Sandip Aryal, Dr. Barry Morton, Karina Duran

Former postdocs: Dr. Dev Paudel, Dr. Mario Andrade, Dr. Claudio Fernandez, Dr. Rebecca Ulbricht, Dr. Cleber Lopes de Souza

Former students: Janam Acharya, Jennifer Timmers

Former visiting students: Dr. Beatriz Gouveia, Dr. Antonio Porto, Dr. Carlos Pereira

Undergraduate students: Maryjo Valle, Adina Grossman





FLORIDA DAIRY FARMERS