UNIVERSIDADE FEDERAL DE SANTA MARIA
CENTRO DE CIÊNCIAS RURAIS
PROGRAMA DE PÓS-GRADUAÇÃO EM AGRONOMIA

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ÍNDICES DE ESTABILIDADE GENOTÍPICA E SELEÇÃO SIMULTÂNEA MULTIVARIADA: UMA NOVA ABORDAGEM

Santa Maria, RS
2019

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Tese apresentada ao Curso de Doutorado do Programa de Pós-Graduação em Agronomia, área de concentração em Produção Vegetal, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do título de **Doutor em Agronomia.**

Orientador: Prof. Dr. Alessandro Dal'Col Lúcio

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2019

Olivoto, Tiago
ÍNDICES DE ESTABILIDADE GENOTÍPICA E SELEÇÃO SIMULTÂNEA MULTIVARIADA: UMA NOVA ABORDAGEM / Tiago Olivoto.- 2019.
147 p.; 30 cm

Orientador: Alessandro Dal'Col Lúcio
Tese (doutorado) - Universidade Federal de Santa Maria, Centro de Ciências Rurais, Programa de Pós Graduação em Agronomia, RS, 2019

1. AMMI 2. BLUP 3. GGE 4. Interação genótipo-ambiente 5. metan I. Dal'Col Lúcio, Alessandro II. Título.

Sistema de geração automática de ficha catalográfica da UFSM. Dados fornecidos pelo autor(a). Sob supervisão da Direção da Divisão de Processos Técnicos da Biblioteca Central. Bibliotecária responsável Paula Schoenfeldt Patta CRB 10/1728.

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Santa Maria, RS
2019

DEDICATÓRIA

Aos meus pais Luiz Antônio Olivoto e Sidnei Salette Carniel Olivoto, meu irmão Luiz Gustavo Olivoto, pelos ensinamentos de vida, exemplo de caráter e amor incondicional, dedico-lhes este trabalho.

AGRADECIMENTOS

À **Deus**, pela oportunidade da existência, pela saúde e paz e por ter iluminado os caminhos que me trouxeram até aqui.

Aos meus pais **Luiz Antônio Olivoto** e **Sidnei Salette Carniel Olivoto**, meu irmão **Luiz Gustavo Olivoto**, pelo amor incondicional, exemplos de humildade e ética, ensinamentos e conselhos. Como é bom ter vocês por perto.

Ao meu orientador **Alessandro Dal'Col Lúcio**, pela oportunidade de cursar o doutorado junto ao seu grupo de pesquisa, pela orientação e pela confiança depositada em mim. Obrigado pela amizade, pelos conselhos e ensinamentos. Seu apoio foi fundamental para ter conseguido conciliar as atividades do doutorado com as atividades de docência. Obrigado!

Aos eternos mestres **Valmor Antonio konflanz**, por ter despertado em mim o interesse pela pós-graduação e **Velci Queiróz de Souza**, por todo apoio durante os anos de mestrado. Como dizia Newton, "*Se vi mais longe foi por estar apoiado sobre ombros de gigantes*".

À **Universidade Federal de Santa Maria** e ao **Programa de Pós-Graduação em Agronomia** pela infraestrutura física e humana disponibilizadas.

A todos os colegas do Grupo de Pesquisa Experimentação, em especial aos colegas e amigos da "salinha da pós" **Bruno Giacomini Sari**, **Dionatan Ketzer Krysczun**, **Maria Inês Diel** e **Patricia Jesus de Melo**. A convivência com vocês foi sempre momento de muita alegria e inspiração.

Ao **Conselho Nacional de Desenvolvimento Científico e Tecnológico**, pela concessão da bolsa doutorado utilizada pelo período de dois anos.

À **UNIJUI**, na pessoa do Prof. **José Antonio Gonzales da Silva** e a **COODETEC**, na pessoa do Prof. **Volmir Sergio Marchioro** pela disponibilidade de parte dos dados utilizados na demonstração dos métodos propostos nesta tese.

Aos **membros da banca examinadora**, pela valiosa colaboração na redação do documento final.

A todos que direta ou indiretamente contribuíram para meu crescimento pessoal e profissional, possibilitando que este sonho se tornasse realidade.

Meu sincero MUITO OBRIGADO!

Uma resposta aproximada para o problema certo vale muito mais do que uma resposta exata para um problema aproximado.

(John Tukey)

RESUMO

ÍNDICES DE ESTABILIDADE GENOTÍPICA E SELEÇÃO SIMULTÂNEA MULTIVARIADA: UMA NOVA ABORDAGEM

AUTOR: Tiago Olivoto
ORIENTADOR: Alessandro Dal'Col Lúcio

Visando uma melhor compreensão e exploração da interação genótipo-ambiente (IGA) no melhoramento de plantas, tanto o desenvolvimento de novos métodos para análise de adaptabilidade e estabilidade, quanto o aperfeiçoamento dos já existentes são necessários. Este estudo introduz as bases teóricas, a aplicação numérica e a implementação em software estatístico de novos índices de estabilidade genotípica e seleção simultânea multivariada no melhoramento de plantas. A decomposição por valores singulares de uma matriz de dupla entrada contendo os BLUPs (*Best Linear Unbiased Prediction*) dos efeitos da IGA obtidos em um modelo linear de efeitos mistos (LMM) foi utilizada para confeccionar biplots, úteis na identificação dos padrões de uma IGA aleatória. Um novo índice quantitativo de estabilidade genotípica chamado WAASB, baseado na média ponderada dos escores absolutos da decomposição por valor singular da matriz de BLUPs para os efeitos da IGA obtidos em um LMM é proposto. Por definição, quanto menor o valor de WAASB, mais estável é um determinado genótipo. Também são introduzidos os fundamentos teóricos de um índice de superioridade que permite ponderar entre estabilidade (WAASB) e desempenho médio (Y), que foi convenientemente chamado WAASBY. O WAASBY assume valores no intervalo 0−100, sendo 100 atribuído ao ideótipo, ou seja, o genótipo mais estável e com o melhor desempenho médio dentre os considerados nos ambientes de teste. Um índice de estabilidade multivariada (MTSI, *multi-trait stability index*) é utilizado para estender os índices WAASB e WAASBY para uma estrutura multivariada permitindo, assim, a seleção para estabilidade ou a seleção simultânea para estabilidade e desempenho médio com base em diversas variáveis analisadas. A aplicação destes índices é ilustrada utilizando dados reais de ensaios multiambientes com a cultura da aveia-branca (*Avena sativa* L.). O WAASB permitiu a quantificação da estabilidade genotípica e a identificação de grupos de genótipos com diferentes padrões de estabilidade e desempenho médio. Utilizando o índice WAASBY foi possível identificar genótipos que combinam, simultaneamente, alto desempenho e estabilidade de rendimento. No contexto da seleção multivariada, diferenciais de seleção (DS) positivos (1,75% ≤ DS ≤ 17,8%) foram observados para as médias das variáveis que se desejava aumentar e negativo (DS = −11,7%) para uma variável que se desejava reduzir. Os DS negativos obtidos para o índice WAASB (−63% ≤ DP ≤ −12%) indicam que os genótipos selecionados eram mais estáveis. Medidas confiáveis de estabilidade usando o WAASB podem ajudar melhoristas e agrônomos a tomar decisões corretas ao selecionar ou recomendar genótipos. Além disso, o índice de seleção simultânea, WAASBY, será útil quando a seleção considerar pesos diferentes para estabilidade e desempenho médio. O MTSI tem ampla aplicabilidade na seleção simultânea para estabilidade e desempenho médio for baseada em múltiplas características, pois proporciona um processo de seleção único, de fácil interpretação e que considera a estrutura de correlação entre as variáveis. Os índices propostos foram implementados no pacote para software R `metan` (multi-environment trial analysis). A versão de desenvolvimento do `metan` está disponível no Github <https://tiagoolivoto.github.io/metan/> e pode ser instalada diretamente via console R usando `devtools::install_github("TiagoOlivoto/metan")`. O `metan` apresenta uma coleção de funções para verificar, manipular e resumir dados típicos de ensaios multiambientes, analisar ensaios em ambientes individuais usando modelos de efeito fixo e misto, calcular estatísticas de estabilidade paramétricas e não paramétricas, bem como implementar análises multivariadas.

Palavras-chave: AMMI. BLUP. GGE. Interação genótipo−ambiente. metan.

ABSTRACT

GENOTYPIC STABILITY INDEXES AND MULTIVARIATE SIMULTANEOUS SELECTION: A NEW APPROACH

AUTHOR: Tiago Olivoto
ADVISOR: Alessandro Dal'Col Lúcio

In order to better understand and explore the genotype-environment interaction (GEI) in plant breeding, the development of new methods for adaptability and stability analysis, as well as the improvement of existing ones, is necessary. This study introduces the theoretical foundations, shows the numerical application and the implementation into a statistical software of new indexes for genotypic stability and multivariate simultaneous selection in plant breeding. The singular value decomposition of a two-way matrix containing the BLUPs (Best Linear Unbiased Prediction) of the GEI effects obtained in a linear mixed-effect model (LMM) was used to produce biplots useful in identifying the patterns of a random structure of GEI. A new quantitative index of genotypic stability called WAASB, based on the weighted average of the absolute value decomposition scores of the BLUPs matrix for the effects of IGA obtained in an MLM is proposed. By definition, the lower the WAASB value, the more stable a given genotype is. It is also introduced the theoretical foundations of a superiority index that allows weighting between stability (WAASB) and mean performance (Y), which was conveniently called WAASBY. The WAASBY assumes values in the range of 0−100, with 100 being assigned to the ideotype, i.e., the genotype that was most stable and that best performed on average among those considered in the test environments. A multi-trait stability index (MTSI) is used to extend the WAASB and WAASBY indexes to a multivariate structure, thus allowing selection for stability or simultaneous selection for stability and mean performance based on several traits. The application of these indexes is illustrated using real data from multi-environment trials with white oat (*Avena sativa* L.) crop. The WAASB allowed the quantification of genotypic stability and the identification of genotype groups with different patterns for stability and mean performance. Using the WAASBY index it was possible to identify genotypes that combine simultaneously high performance and yield stability. In the context of multivariate selection, positive selection differentials (SD) (1.75% ≤ SD ≤ 17.8%) were observed for trait means that wanted to increase and negative (SD = −11.7%) for one variable that wanted to reduce. The negative DS obtained for the WAASB index (−63% ≤ SD ≤ −12%) suggesting that the selected genotypes were more stable. Reliable stability measures using WAASB can help breeders and agronomists make the right decisions when selecting or recommending genotypes. Besides, the simultaneous selection index, WAASBY, will be useful when selection considers different weights for stability and mean performance. The MTSI has broad applicability in simultaneous selection for stability and mean performance based on multiple traits since it provides a unique selection process that is easy-to-handle and considers the correlation structure between traits. The proposed indices were implemented in the R `metan` (multi-environment trial analysis) software package. The development version of `metan` is available on Github <https://tiagoolivoto.github.io/metan/> and can be installed directly via console R using `devtools::install_github("TiagoOlivoto/metan")`. The package `metan` presents a collection of functions for verifying, manipulating and summarizing typical multi-environment trial data, analyzing single-environment trials using both fixed- and mixed-effect models, computing parametric and non-parametric stability statistics, and implementing multivariate analysis.

Keywords: AMMI. BLUP. Genotype−environment interaction. GGE. metan.

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1 INTRODUÇÃO

É comum que em programas de melhoramento genético, a classificação/recomendação das linhagens/cultivares seja baseada em seu desempenho médio em relação a uma cultivar testemunha. No caso de espécies autógamas, linhagens em avançado estádio de homozigose (geralmente a partir da geração F_8) são avaliadas em ensaios preliminares, onde um grande número de linhagens (ex. 100−200 linhagens) são avaliadas em um único local e comparadas com cultivares testemunhas. Nesta fase, a pressão de seleção é de aproximadamente 15−20%.

No segundo ano, as linhagens selecionadas são avaliadas em mais de um local, em ensaio delineado, geralmente em blocos completos casualizados. A pressão de seleção nesta fase é menor, em torno de 50−60%. Como este ensaio já se caracteriza como um ensaio multi-ambiente, informações quanto a estabilidade das linhagens já podem ser utilizadas como um dos critérios de seleção.

Com um número reduzido de linhagens em mãos e uma maior quantidade de sementes disponíveis, as linhagens selecionadas são avaliadas em uma série de locais, geralmente em mais de um estado, caracterizando como ensaios regionais ou nacionais. Nestes ensaios, normalmente, as variáveis analisadas são aquelas exigidas do ensaio VCU (valor de cultivo e uso) de cada espécie, aliado a informações quanto ao desempenho produtivo e em relação a doenças.

Após o lançamento comercial das cultivares, as mesmas são avaliadas em ensaios brasileiros visando avaliar, em diferentes ambientes, o potencial de rendimento, qualidade de grãos e outras características agronômicas. Estes ensaios permitem tanto a identificação de cultivares estáveis e de alta performance quanto a indicação de cultivares à ambientes específicos.

Tanto a seleção de linhagens nos ensaios preliminares quanto a classificação das cultivares nos ensaios de competição são baseadas, quase que exclusivamente, no desempenho médio dos materiais. Assim, informações quanto a estabilidade destas linhagens/cultivares dificilmente são consideradas como critério de seleção. É justo afirmar que um dos principais motivos para a estabilidade não ser amplamente utilizada como um dos critérios de seleção é a inexistência de um índice que contemple, conjuntamente, a estabilidade e o desempenho médio das linhagens/cultivares com base em diversas variáveis, proporcione um processo de

seleção único, de fácil interpretação e que esteja implementado em software estatístico conhecido.

O aumento do volume de dados fenotípicos e a rápida evolução dos computadores pessoais estimularam o desenvolvimento de novas abordagens estatísticas para descrição e predição mais precisas dos fenômenos da interação genótipo−ambiente (IGA). Uma melhor modelagem da IGA contribuirá, inegavelmente, para uma maior eficiência dos programas de melhoramento de plantas.

Os primeiros métodos estatísticos para análise de adaptabilidade e estabilidade visando melhor compreender a IGA foram propostos na década de 1920 e popularizados na década de 1960, sendo baseados em princípios de regressão linear. Na segunda metade do século XX, métodos baseados em princípios de análise de variância ganharam popularidade. Atualmente, a maior parte dos métodos de adaptabilidade e estabilidade disponíveis é baseada nestes dois princípios. Com a evolução na capacidade de processamento e a facilidade de acesso a computadores pessoais na década de 1960, métodos mais sofisticados −que até então não eram aplicados na prática devido à alta complexidade de operações algébricas necessárias− ganharam popularidade.

Existem atualmente mais de uma dezena de métodos para a análise de adaptabilidade e estabilidade. Modelos com ótimas ferramentas gráficas para a interpretação da IGA como o modelo AMMI (*Additive Main Effect and Multiplicative Interaction*) e GGE (*Genotype plus Genotype vs Environment interaction*) se firmaram como métodos populares nos últimos anos. Estes métodos são baseados em um modelo de efeitos estritamente fixos, ou seja, ambos efeitos principais (aditivos) para genótipos e ambientes e efeitos multiplicativos para a IGA são considerados fixos. Métodos baseados em modelos mistos que exploram a melhor capacidade preditiva do BLUP (*Best Linear Unbiased Prediction*) têm sido usados, mas a pobreza gráfica deste método pode estar limitando sua expansão para um grande número de usuários.

À luz do grande número de trabalhos científicos observados nos últimos anos com relação a análise de estabilidade −uma pesquisa bibliométrica é apresentada ao final da revisão bibliográfica− é óbvio que o estudo da IGA se tornará ainda mais importante em um futuro próximo do que já era no passado. Assim, visando uma melhor compreensão e exploração da IGA em pesquisas futuras, tanto o

desenvolvimento de novos métodos quanto o aperfeiçoamento dos já existentes são necessários.

Esta tese introduz as bases teóricas, a aplicação numérica e a implementação em software estatístico de novas metodologias para a análise de adaptabilidade e estabilidade no melhoramento de plantas. O documento apresenta a seguinte estrutura: Em um primeiro momento é apresentada uma introdução ao assunto. Posteriormente, uma revisão bibliográfica é utilizada como base para a identificação e caracterização dos problemas que levaram à definição das hipóteses e dos objetivos da pesquisa. Nos três capítulos seguintes são apresentados três artigos científicos mostrando a proposta de índices univariados (i) e multivariados (ii) de estabilidade genotípica e seleção simultânea, bem como (iii) a implementação destes índices em um pacote para o software estatístico R, convenientemente chamado `metan` (***m**ulti-**e**nvironment **t**rial **an**alysis*).

1.1 REVISÃO BIBLIOGRÁFICA

1.1.1 Interação genótipos x ambientes

Melhoristas e geneticistas se esforçam continuamente para aumentar a frequência de alelos favoráveis em novas cultivares e conhecer quanto do progresso de seleção atingido em um ambiente pode ser transferido para outros ambientes é de fundamental importância. Fatores imprevisíveis são observados em diferentes ambientes (ALI et al., 2019; CURCIC et al., 2018; KALRA; KUMAR, 2018; OZKAN; AKCAOZ, 2002; QIAO et al., 2018; QIAO; YU; WU, 2018; RENGASAMY, 2010), que podem ser caracterizados por locais, anos ou épocas de cultivo ou qualquer combinação destes fatores. Como as plantas respondem a uma série de estímulos bióticos (ALONSO; RAMOS-CRUZ; BECKER, 2019; MAJEED; MUHAMMAD; AHMAD, 2018; SIGNORELLI et al., 2019), abióticos (ATKINSON; URWIN, 2012; ELLI et al., 2018; GENGMAO et al., 2015; MITTLER, 2006; OLIVOTO et al., 2018), bem como à interação entre estes estímulos (ANDERSON et al., 2004; BAI et al., 2018; BORSANI; VALPUESTA; BOTELLA, 2001; BOYKO et al., 2010; HODGE, 2014; OLDROYD, 2013; XIONG; SCHUMAKER; ZHU, 2002; YASUDA et al., 2008), um determinado genótipo pode ter um bom desempenho em um determinado ambiente mas apresentar baixo desempenho em outros.

Se a classificação dos genótipos mudar significativamente entre os ambientes (Figura 1E), uma interação significativa entre genótipo e ambiente (IGA) é observada. Essa interação é conhecida como interação qualitativa ou do tipo *crossover* e desempenha um papel fundamental na formulação de estratégias para o melhoramento de plantas. Outra forma de interação caracterizada apenas pela expansão ou contração de escala no conjunto de ambientes sem que ocorra alteração na ordem de classificação também pode ocorrer (Figuras 1C-D). Este tipo de interação é conhecida como quantitativa ou *non-crossover*. Neste sentido, compreender a IGA e buscar novas formas de explorá-la são passos importantes para um uso mais eficiente dos recursos visando o aumento na produção de alimentos (KANG, 1997).

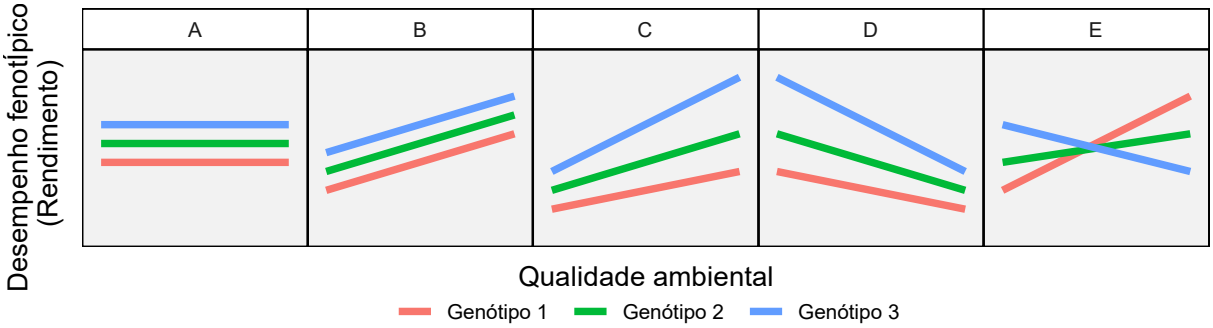

Figura 1. Representação esquemática de três genótipos que ilustram várias formas de plasticidade e interação genótipo-ambiente (IGA). Nenhuma plasticidade em (A) *versus* plasticidade em (B−E). Nenhuma IGA em (A−B) *versus* várias formas de IGA em (C−E).

1.1.2 Análise de adaptabilidade e estabilidade

1.1.2.1 Baseados em regressão

O desejo dos melhoristas em modelar adequadamente a IGA levou ao desenvolvimento de procedimentos conhecidos como análises de estabilidade, onde as ideias originais de Mooers (1921) de comparar o desempenho de um genótipo em relação à um índice ambiental precedem até mesmo a análise de variância. Mais tarde, Yates & Cochran (1938) deram destaque a análise de estabilidade baseada em regressão.

Um quarto de século mais tarde, Finlay & Wilkinson (1963) usaram a técnica de regressão para examinar a estabilidade do rendimento de vários genótipos de cevada, embora afirmassem que melhores ajustes eram obtidos com rendimentos transformados para a escala logarítmica. Estes autores também consideraram que

simplesmente comparar os *slopes*[1] da regressão não era suficiente: o desempenho geral de um genótipo também precisava ser levado em consideração. O *slope* da linha de regressão para cada genótipo foi, então, plotado em relação ao seu rendimento médio no ambiente. Os genótipos com *slope* próximo de 1 e alto rendimento médio foram considerados bem adaptados a todos os ambientes. À medida que o rendimento médio diminuiu, os genótipos com *slopes* altos ou baixos foram considerados especificamente adaptados a ambientes favoráveis ou desfavoráveis, respectivamente.

A análise de estabilidade baseada em regressão foi certamente popularizada por Eberhart & Russell (1966). Estes autores consideraram os desvios da linha de regressão como outro componente importante da estabilidade, sendo uma cultivar considerada estável aquela com *slope* próximo de 1 com uma pequena soma de quadrados de desvios da regressão. Eles pareciam não ter consciência, no entanto, de que essa soma de quadrados é dependente do *slope* da regressão, tanto em um sentido matemático quanto biológico (HARDWICK; WOOD, 1972). Assim, inevitavelmente, altos desvios serão observados ao ajustar linhas de regressão a dados que só podem ser adequadamente representados em várias dimensões.

1.1.2.2 Baseados em regressão bissegmentada

Como o método de regressão linear não é capaz de detectar o genótipo teoricamente ideal, ou seja, aquele com sensibilidade relativamente baixa nos ambientes desfavoráveis e que responda positivamente em ambientes favoráveis, métodos de regressão bissegmentadas foram propostos. Os pioneiros desta técnica foram Verma; Chahal; Murty (1978). O princípio envolvido neste método é o de realizar duas regressões separadamente. Na primeira, considera-se apenas os ambientes favoráveis. Na outra considera-se somente os ambientes desfavoráveis. Um reduzido número de ambientes, no entanto, pode limitar a utilização deste método.

Modificações nos modelos foram posteriormente realizadas, destacando-se o método proposto por Cruz; Torres; Venconvsky (1989). Neste método, dois

[1] Optou-se por utilizar o termo inglês *slope* ao invés de “coeficiente angular” devido a simplificação da escrita, além deste termo ser mundialmente conhecido para indicar a inclinação de uma reta de regressão linear de primeiro grau.

segmentos de reta são ajustados a uma única equação, possibilitando sua realização em um número relativamente reduzido de ambientes.

1.1.2.3 Baseados em análise de variância

Métodos para explorar a IGA baseados em princípios de análise de variância foram propostos e vêm sendo utilizados até os dias de hoje. O método tradicional é baseado no desdobramento da soma de quadrados dos efeitos de ambiente mais os efeitos da IGA, em soma de quadrados dos efeitos de ambiente dentro de cada genótipo. A variação ambiental de cada genótipo é utilizada como estimativa de estabilidade, onde o genótipo que apresentar a menor soma de quadrado médio nos vários ambientes é considerado o mais estável.

Plaisted & Peterson (1959) computaram uma análise de variância para cada par de genótipos a fim de estimar a variância de interação para cada combinação de dois genótipos. A média das variâncias de interação obtidas para cada genótipo foi utilizada como um indicador da contribuição desse genótipo para a IGA total. Uma desvantagem desta técnica é o número de análises requeridas. Se existirem n genótipos, $n(n-1)/2$ análises de variância precisam ser computadas.

Wricke (1965) propôs que a contribuição de um genótipo para a soma de quadrados da interação em uma análise de variância bidirecional fosse usada como uma medida de sua instabilidade.

Annicchiarico (1992), por outro lado, propôs um índice de confiança cujo resultado é expresso em porcentagem em relação à média dos ambientes analisados. A um nível α de confiança, a adaptabilidade e estabilidade são computadas por meio do desvio padrão entre as médias porcentuais dos ambientes. Quanto maior for este índice, maior será a confiança na recomendação da cultivar. Schmildt et al. (2011) propuseram que o erro padrão da média ao invés do desvio padrão fosse utilizado apresentando, segundo os autores, as vantagens de valorizar mais os desvios apresentados entre os ambientes, por levar em consideração o número de ambientes em análise.

1.1.2.4 Métodos AMMI e GGE

Métodos que combinam diferentes técnicas estatísticas também foram desenvolvidos para análise de estabilidade. Por exemplo, Gollob (1968) propôs um método que combina os recursos da análise de fatores e análise de variância em um único método. Naquela época, esse método era conhecido como FANOVA. Atualmente, esse mesmo método é conhecido pela sigla AMMI, *Additive Main Effect and Multiplicative Interaction*. O método AMMI tem boas propriedades preditivas (GAUCH, 1988; GAUCH; ZOBEL, 1988) e permite visualizar os padrões da IGA em biplots (GABRIEL, 1971). Ambientes ou genótipos marginais, bem como agrupamentos de genótipos e agrupamentos de ambientes podem ser facilmente detectados (GAUCH; ZOBEL, 1997). Até mesmo a adaptação pode ser investigada em biplots AMMI (VOLTAS et al., 1999). Como se esperaria, as abordagens com modelo AMMI são notavelmente populares (EBADI; HALLAJIAN; KORDROSTAMI, 2019; GEBEYEHU et al., 2019; MANIRUZZAMAN et al., 2019; PERSAUD; SARAVANAKUMAR; PERSAUD, 2019; RADAELLI et al., 2020; SINGH et al., 2019).

Índices quantitativos de estabilidade baseados na análise AMMI têm sido utilizados, à saber, o valor de estabilidade AMMI (PURCHASE; HATTING; VAN DEVENTER, 2000), a soma dos valores absolutos dos IPCAs[2] e a média dos quadrados dos autovetores (SNELLER; KILGORE-NORQUEST; DOMBEK, 1997) e o valor absoluto da contribuição relativa dos IPCAs para a interação (ZALI et al., 2012).

O método GGE, *Genotype plus Genotype-Environment interaction* (YAN; KANG, 2003) inclui no biplot ambos efeitos de genótipo (g_i) e IGA (ga_{ij}) que são as duas fontes de variação relevantes para a avaliação das cultivares (GAUCH; ZOBEL, 1996; KANG, 1988). Em um biplot GGE, um eixo médio do ambiente pode ser construído como a média das representações vetoriais dos ambientes. As projeções dos genótipos nesse eixo médio aproximam o efeito principal genotípico. Presume-se que a distância entre uma representação de genótipo no biplot GGE e sua projeção no eixo médio do ambiente dê uma estimativa de sua estabilidade (YAN; RAJCAN, 2002). Esse tipo de estabilidade no biplot GGE será próximo à estabilidade com base no primeiro escore genotípico em um biplot AMMI. Assim como o modelo AMMI, os modelos GGE desfrutam de uma grande popularidade na literatura aplicada sobre IGA

[2] IPCA, Interaction Principal Component Axis

(BADU-APRAKU; AKINWALE, 2019; DE OLIVEIRA et al., 2019; KOUNDINYA et al., 2019; TENA et al., 2019).

Várias discussões foram publicadas sobre a comparação das análises dos modelos AMMI e GGE (GAUCH, 2013; GAUCH; PIEPHO; ANNICCHIARICO, 2008; YAN et al., 2007; YAN; TINKER, 2006; YANG et al., 2009), sem que os autores chegassem a um acordo. Segundo afirmam Yan et al. (2007), o modelo GGE é superior ao AMMI na análise de genótipos, porque explica mais do efeito $g_i + ga_{ij}$ e possui ferramentas gráficas para avaliação de ambientes de teste, o que não é possível na análise AMMI. Por outro lado, Gauch; Piepho; Annicchiarico (2008) argumentam que a razão fundamental pela qual o AMMI é mais apropriado para a pesquisa agrícola é que a parte ANOVA do AMMI pode separar os efeitos principais de g_i e os efeitos de ga_{ij}, que apresentam aos pesquisadores problemas e oportunidades muito diferentes, enquanto a separação do g_i da ga_{ij} é uma impossibilidade matemática para análise GGE.

AMMI e GGE possuem ótimas ferramentas gráficas, mas falham em alguns aspectos como, por exemplo, não permitir uma estrutura de modelo de efeito misto, tendência crescente na análise de ensaios de cultivares (VAN EEUWIJK; BUSTOS-KORTS; MALOSETTI, 2016). Assim, novas abordagens são necessárias para o aperfeiçoamento destes métodos.

1.1.2.5 Baseados em modelos mistos

A teoria do BLUP (*Best Linear Unbiased Prediction*) como procedimento ideal para predição de efeitos aleatórios foi difundida a partir da década de 1970 por Charles Henderson, nos Estados Unidos (HENDERSON, 1950, 1975, 1976) e Robin Thompson, na Inglaterra (THOMPSON, 1973, 1976, 1977), entre outros. Na primeira década do segundo milênio, trabalhos pioneiros no campo de modelos lineares mistos utilizando BLUP foram realizados no Brasil (BUENO FILHO; VENCOVSKY, 2000; BUENO FILHO; VENCOVSKY, 2009). Desde então, modelos com componentes fixos e aleatórios vêm ganhando cada vez mais espaço na avaliação de genótipos em ensaios multiambientes, pois permitem a estimativa de parâmetros genéticos e ambientais (FOLLMANN et al., 2019; OLIVOTO et al., 2017c), apresentando, geralmente, melhor capacidade preditiva que modelos de efeito fixo (PIEPHO, 1994). Da mesma forma, modelos mistos reduzem os ruídos de análises realizadas com

dados desbalanceados e também de variáveis que não assumem aditividade, características frequentemente observadas nos ensaios de melhoramento genético (HU, 2015).

Baseando-se nas premissas de que quanto menor for o desvio padrão do comportamento genotípico entre ambientes, maior será a média harmônica de seus valores genotípicos entre estes ambientes e que os valores genotípicos preditos são expressos como proporção da média geral de cada local, a seleção pelos maiores valores da média harmônica dos valores genotípicos (MHVG) implica, simultaneamente, em seleção para produtividade e estabilidade (RESENDE, 2004).

A adaptabilidade, ou seja, a capacidade do genótipo aproveitar vantajosamente as condições ambientais, pode ser avaliada pela performance relativa do dos valores genotípicos (PRVG) na série de ambientes, mesmo conceito aplicado no índice de confiabilidade genotípico de Annicchiarico (1992), mas utilizando os BLUPs.

A seleção simultânea para produção, estabilidade e adaptabilidade, no contexto de modelos mistos pode, então, ser realizada pela média harmônica da performance relativa dos valores genéticos preditos (MHPRVG). Estudos utilizando a MHPRVG tem obtido sucesso na identificação de genótipos produtivos e estáveis (ALVES et al., 2018; AZEVEDO PEIXOTO et al., 2018; CANDIDO et al., 2018; COLOMBARI FILHO et al., 2013; DE PAULA et al., 2014; DE VASCONCELOS et al., 2019; DIAS et al., 2018; ROSADO et al., 2019; TORRES et al., 2018).

Estudos têm demonstrado que o BLUP supera a capacidade preditiva dos modelos AMMI e GGE (OLIVOTO et al., 2019a; PIEPHO, 1994, 1998), e que em um futuro próximo, a análise da IGA dependerá menos de modelos lineares-bilineares e mais de modelos mistos (VAN EEUWIJK; BUSTOS-KORTS; MALOSETTI, 2016). A identificação dos padrões de IGA, bem como a delineação de mega ambientes utilizando o BLUP, no entanto, são dificultadas pela ausência de uma abordagem gráfica.

1.1.2.6 Baseados em análise não paramétrica

Métodos não paramétricos que são mais robustos a desvios das premissas usadas na análise paramétrica como normalidade dos erros e dos efeitos da interação foram propostos para avaliar a estabilidade genotípica. Lin; Binns (1988) propuseram um índice de superioridade geral do genótipo definido como o quadrado médio da

distância entre a resposta do genótipo e a resposta máxima média de todos os locais. Como a resposta máxima é o limite superior em cada local, um pequeno quadrado médio indica superioridade geral do genótipo em teste. Este método foi adaptado por Carneiro (1998) qual propôs a identificação de genótipos superiores nos grupos de ambientes favoráveis e desfavoráveis.

Vários procedimentos foram propostos com base na comparação da classificação dos genótipos em cada ambiente, sendo considerados estáveis genótipos com classificação semelhante entre ambientes. Por exemplo, Huehn (1979, 1990) propôs três índices de estabilidade, sendo $S_i^{(1)}$ a média da diferença absoluta da classificação do genótipo testada em *n* ambientes; $S_i^{(2)}$ a variância das classificações entre os *n* ambientes; e $S_i^{(3)}$ soma dos desvios absolutos da classificação dos genótipos em relação a estabilidade máxima. Para um genótipo *i* com estabilidade máxima, obtém-se $S_i^{(1)} = S_i^{(2)} = S_i^{(3)} = 0$.

Fox et al. (1990) propuseram uma medida de superioridade não paramétrica usando uma classificação estratificada dos genótipos. Neste método, as classificações são obtidas em cada local separadamente. A proporção de locais em que o genótipo ficou entre os três primeiros é expressa como a medida de superioridade.

1.1.2.7 Seleção simultânea para estabilidade e performance

A seleção bem-sucedida de cultivares estáveis e de alto desempenho é fundamental para os programas de melhoramento. Kang (1988) propôs que a soma das classificações obtidas para o rendimento (*rY*) e para a variância de Shukla ($r\sigma_S^2$ (SHUKLA, 1972) fosse utilizada como um índice de seleção simultânea, $SSI = rY + r\sigma_S^2$. O genótipo com o menor SSI é o mais desejado.

De maneira semelhante, o índice de estabilidade genotípico (GSI) (FARSHADFAR, 2008) combina a soma das classificações obtidas para o rendimento (*rY*) e para o valor de estabilidade AMMI (*rASV*), $GSI = rY + rASV$, visando a seleção simultânea para estabilidade e desempenho na análise AMMI (ADJEBENG-DANQUAH et al., 2017; BOCIANOWSKI; NIEMANN; NOWOSAD, 2019; BOSE et al., 2014; DE OLIVEIRA; DE FREITAS; DE JESUS, 2014; FARSHADFAR; MAHMODI;

YAGHOTIPOOR, 2011; SABAGHNIA; SABAGHPOUR; DEHGHANI, 2008). Menores valores deste índice são desejáveis.

Embora os métodos de Kang (1988) e Farshadfar (2008) sejam de fácil interpretação, a ambiguidade destes índices pode resultar em falsos positivos ou falsos negativos na seleção de genótipos estáveis e de alto desempenho. Muitos pesquisadores podem não perceber, mas *GSI = 50* pode ser o resultado de, por exemplo, 50 = 5 + 45 ou 50 = 45 + 5. Em outras palavras, genótipos com padrões totalmente distintos de estabilidade e desempenho médio são considerados semelhantes. Portanto, devemos ter em mente que a recomendação de um genótipo estável, mas de baixo desempenho (50 = 45 + 5) é completamente diferente da recomendação de um genótipo com bom desempenho médio, mas inconsistente de ambientes para ambientes (50 = 5 + 45). Neste último caso, a recomendação para ambientes específicos deve ser explorada.

Outro aspecto importante que precisa ser considerado é que estes índices consideram pesos iguais para estabilidade e desempenho médio. Melhoristas podem, no entanto, preferir atribuir um maior peso ao rendimento ou à estabilidade, o que seria lógico para uma variável como o acamamento de plantas, por exemplo. Neste caso, genótipos com menor média de acamamento seriam preferidos, independentemente da constância desta variável de ambiente para ambiente.

1.1.2.8 Estabilidade multivariada

O primeiro índice para seleção multivariada[3] foi proposto por Smith (1936) para melhoramento de plantas e Hazel (1943) para melhoramento animal. Mais recentemente, um índice baseado em modelos mistos e análises de fatores foi proposto, apresentando grande potencial de utilização no melhoramento de plantas (ROCHA; MACHADO; CARNEIRO, 2018).

É muito comum que a análise de estabilidade seja realizada para uma única variável, geralmente o rendimento de grãos (BORNHOFEN et al., 2018; MOHAMMADI et al., 2018). A confiabilidade na recomendação de genótipos, no entanto, poderia ser maior se as inferências fossem realizadas com base em diversas variáveis. Estudos recentes (ADJEBENG-DANQUAH et al., 2017; BOCIANOWSKI; NIEMANN;

[3] O termo “Seleção multivariada” diz respeito a seleção de genótipos baseada em diversas variáveis.

NOWOSAD, 2019; KOUNDINYA et al., 2019; NDUWUMUREMYI et al., 2017; SHAHRIARI; HEIDARI; DADKHODAIE, 2018; VEENSTRA et al., 2019) que avaliaram diversas variáveis obtiveram algum sucesso quando a seleção simultânea para estabilidade e desempenho médio foi realizada, separadamente, para cada variável.

Combinar a seleção para estabilidade e desempenho médio de diversas variáveis em um único índice pode ser uma tarefa desafiadora. Esta seleção é definida aqui como ***seleção simultânea multivariada***[4]. Tal índice precisaria apresentar, preferencialmente, as seguintes propriedades: (i) ser baseado em modelos com base teórica consolidada; (ii) ser de fácil interpretação; (iii) para cada variável, permitir a escolha do sentido da seleção, ou seja, se o maior ou menor valor é melhor; (iv) para cada variável, permitir a ponderação entre estabilidade e desempenho médio; (v) lidar com os problemas de multicolinearidade que frequentemente são observados em uma estrutura multivariada (OLIVOTO et al., 2017a, 2017b); e (vi) não apresentar nenhum grau de ambiguidade. Até onde se sabe, nenhum índice que apresente estas características existe.

1.1.3 Produção científica sobre análise de adaptabilidade e estabilidade

Conhecer indicadores como a distribuição geográfica e temporal das publicações científicas em uma determinada área de estudo é importante. Assim, uma pesquisa bibliométrica foi realizada com o objetivo de compreender a dinâmica das publicações relacionadas a análise de adaptabilidade e estabilidade no último meio século (1969−2019). Esta pesquisa foi realizada no banco de dados SCOPUS utilizando palavras−chave como "*AMMI*", "*GGE*", "*genotype-environment interaction*", "*ecovalence*", "*HMRPGV*" e "*adaptability and stability analysis*". A análise bibliométrica foi realizada com o pacote R `bibliometrix` (ARIA; CUCCURULLO, 2017) e os gráficos confeccionados com o pacote `ggplot2` (WICKHAM, 2016).

Foram encontrados 6590 documentos publicados em 902 fontes (Revistas, livros, etc.) por 19.351 autores. A máxima produção científica anual foi observada em 2017 (515 documentos). A taxa de crescimento observada da produção científica foi de 11,22%, mas foram nos últimos dez anos que a maior quantidade (~64%) dos

[4] ***Seleção simultânea multivariada*** diz respeito a seleção para estabilidade e desempenho médio quando diversas variáveis são analisadas em cada genótipo.

documentos foi publicada. A média total de citações por documento mostrou uma clara redução após 2005.

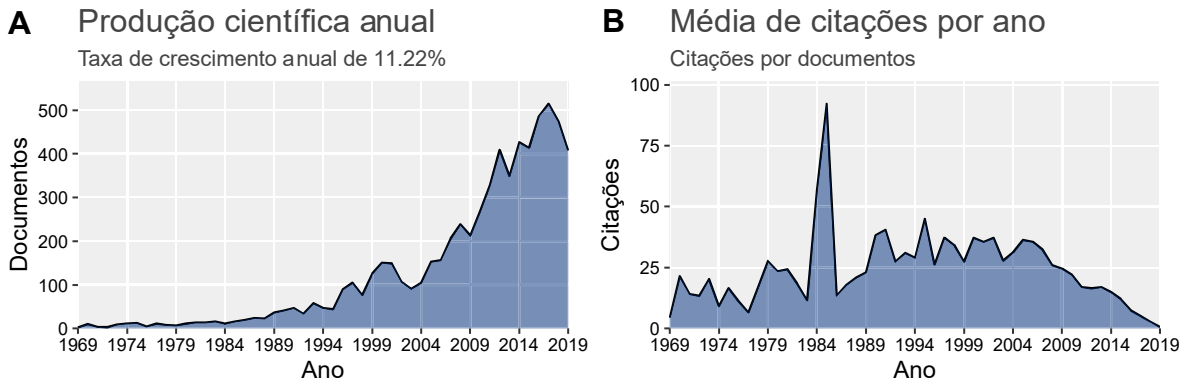

Figura 2. Evolução da produção científica anual (A) e média de citações por documentos (B) de trabalhos relacionados a adaptabilidade e estabilidade publicados entre 1969 e 2019.

Os Estados Unidos da América (USA) se destacou como o país mais produtivo, com 780 documentos publicados, seguidos pelo Brasil (371) e Índia (338). Embora seja um dos países com maior quantidade de publicação, o baixo número de citações total (87% menos que o primeiro colocado) e citações por documento (80% menos que o primeiro colocado), indicam que dentre os documentos listados, os trabalhos Brasileiros apresentam baixo impacto comparado com trabalhos de países desenvolvidos. Cabe ressaltar que documentos publicados em revistas Brasileiras não indexadas no SCOPUS não foram contabilizados nesta pesquisa, mas podem apresentar ampla aplicabilidade local.

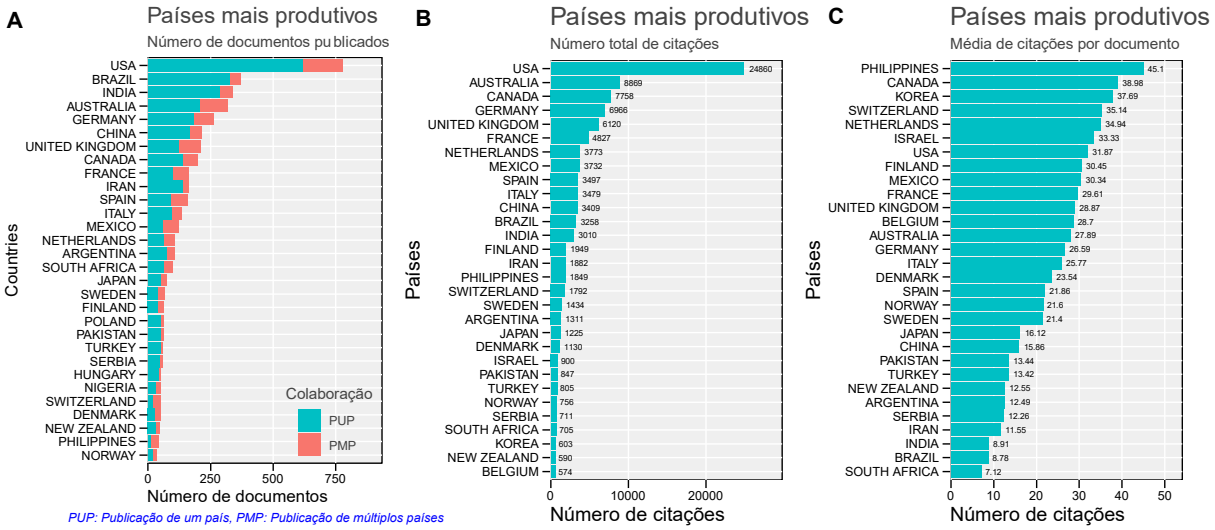

Figura 3. Classificação dos 30 primeiros países quanto ao número de documentos publicados (A), número total de citações (B) e número de citações por documento (C) de trabalhos relacionados a adaptabilidade e estabilidade publicados entre 1969 e 2019.

As 50 principais palavras-chave são mostradas na figura abaixo. "*Interação genótipo por ambiente*", "*Interação genótipo ambiente*" e "*Estabilidade*" foram os

termos mais frequentemente usados como palavras-chave nos artigos publicados, com mais de 400 ocorrências.

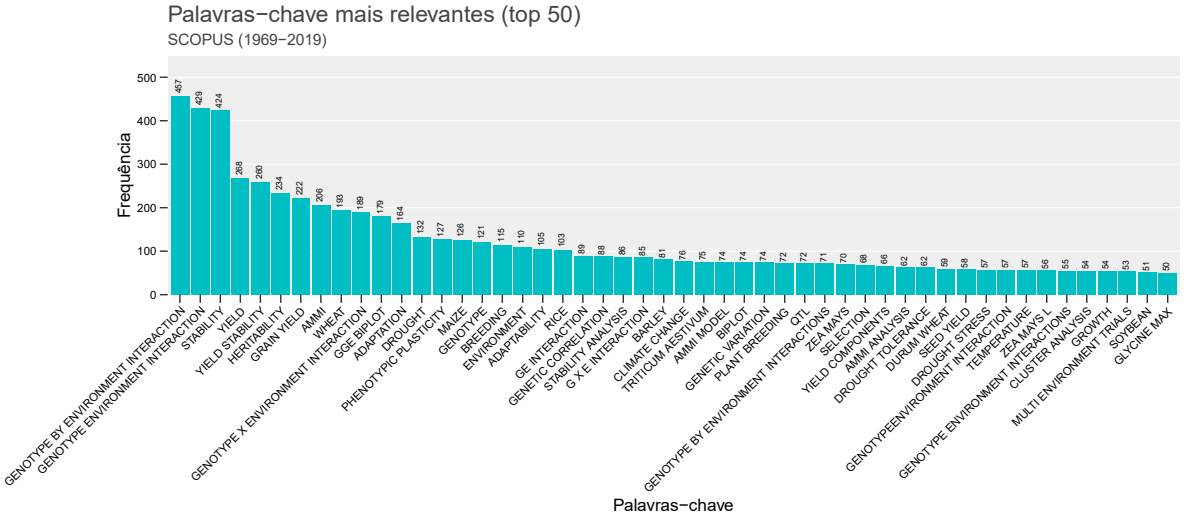

Figura 4. Palavras chaves mais utilizadas em documentos relacionados a análise de adaptabilidade e estabilidade publicados entre 1969 e 2019.

1.2 PROBLEMÁTICA E JUSTIFICATIVA

A análise AMMI possui boas ferramentas gráficas para modelar a IGA, mas falha em alguns aspectos, como acomodar uma estrutura de modelo linear de efeito misto. O BLUP fornece estimativas mais confiáveis que o AMMI, mas ferramentas gráficas para lidar com uma estrutura de IGA aleatória são necessárias. Assim, índices que combinam as principais vantagens destes métodos seriam bem-vindos na literatura.

A seleção e/ou recomendação de genótipos poderia ser mais eficiente se baseada em dados de diversas variáveis, mas identificar genótipos que combinam alto desempenho e estabilidade em um conjunto de variáveis tem sido uma tarefa difícil. É fato que a literatura atual é carente de um índice para seleção simultânea multivariada, confiável e de fácil interpretação.

Esforços têm sido direcionados em comparar os métodos AMMI e BLUP no que diz respeito a capacidade preditiva, mas nenhuma proposta para combinar as características destes métodos ganhou popularidade. Considerando o grande número de estudos que utilizam isoladamente estas técnicas, um método que combinasse as propriedades gráficas do AMMI e a capacidade preditiva do BLUP ganharia popularidade rapidamente. Da mesma forma, a proposta de um índice de seleção simultânea multivariada preencherá uma lacuna existente na literatura por permitir a

seleção para estabilidade e desempenho médio considerando diversas variáveis. Inúmeros estudos que propõe métodos falham em não oferecer uma maneira acessível para que outras pessoas possam aplica-lo. Assim, a criação de um pacote estatístico que contém funções para implementação dos índices propostos será de grande utilidade para a comunidade científica.

1.3 HIPÓTESES

- É possível combinar as vantagens dos métodos AMMI e BLUP em um único índice para análise de estabilidade.
- Combinando técnicas uni- e multivariadas, é possível o desenvolvimento de um índice para seleção simultânea multivariada.

1.4 OBJETIVOS

1.4.1 Objetivo geral

As hipóteses formuladas fundamentaram o seguinte objetivo geral: propor novos índices uni- e multivariados para a análise de estabilidade e seleção simultânea para estabilidade e desempenho médio e realizar a implementação destes índices em software estatístico.

1.4.2 Objetivos específicos

- Combinar as principais vantagens dos métodos AMMI e BLUP para permitir a criação de biplots para mostrar os padrões de uma interação genótipo-ambiente aleatória.
- Desenvolver índices para quantificar a estabilidade em modelos de efeitos fixos e mistos.
- Desenvolver índices de seleção simultânea para estabilidade e desempenho médio uni e multivariados em modelos de efeito fixo e misto.
- Implementar os índices propostos em um pacote estatístico para o software R.

2 ARTIGO I - MEAN PERFORMANCE AND STABILITY IN MULTI-ENVIRONMENT TRIALS I: COMBINING FEATURES OF AMMI AND BLUP TECHNIQUES

Submetido para o periódico: Agronomy Journal

ISSN: 0002-1962

Qualis Capes na data de defesa da tese: A1

Fator de impacto na data de defesa da tese: 1,897

Situação: Publicado

DOI: 10.2134/agronj2019.03.0220

Material suplementar disponível online em:

https://dl.sciencesocieties.org/publications/aj/supplements/0/agronj2019.03.0220-supplement1.pdf

Páginas: 40

Número de Figuras: 3

Número de Tabelas: 6

Core ideias

- The predictive accuracy of BLUP and AMMI was investigated using four real datasets.
- BLUP was found to outperform AMMI in all datasets analyzed.
- A genotypic stability index that inherits the principles of AMMI and BLUP was proposed.
- A superiority index that allows weighting between mean performance and stability was proposed.
- An R package with useful functions for MET analysis is presented.

Mean performance and stability in multi-environment trials I: Combining features of AMMI and BLUP techniques

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Abbreviations: AMMI, Additive Main effects and Multiplicative Interaction; ASV, AMMI stability value; AVRC, index and the ranks of the mean yields; BLUP, Best Linear Unbiased Prediction; EV, averages of the squared eigenvector values; GEI, genotype-by-environment interaction; HMGV, harmonic mean of genotypic values; HMRPGV, harmonic mean of relative performance of genotypic values; IPCA, interaction principal component axis; LMM, linear mixed-effect model; MET, multi-environment trials; NF, no fungicide; RCBD, randomized complete block design; RMSPD, root mean square prediction difference; SPIC, sums of the absolute value of the IPCA scores; SVD, singular value decomposition; WAASB, weighted average of absolute scores from the SVD of the matrix of BLUPs for the GEI effects generated by an LMM; WAASBY, weighted average of WAASB and response variable; WF, with fungicide; Za, absolute value of the relative contribution of IPCAs to the interaction.

2.1 ABSTRACT

Additive Main Effect and Multiplicative Interaction (AMMI) and Best Linear Unbiased Prediction (BLUP) are two popular methods for analyzing multi-environment trials (MET). AMMI has nice graphical tools for modeling genotype-vs-environment interaction (GEI) but fails in some aspects, such as accommodating a linear mixed-effect model (LMM) structure. BLUP provides reliable estimates but new insights to deal graphically with a random GEI structure are needed. This article compares the predictive success of BLUP and AMMI, shows how to generate biplots for modeling GEI in MET analysis using LMM, and proposes a new quantitative genotypic stability measure called WAASB, which is based on the Weighted Average of Absolute Scores from the singular value decomposition of the matrix of BLUPs for the GEI effects

generated by an LMM. We also introduced the theoretical basis of a superiority index that allows weighting between mean performance and stability, which was conveniently called WAASBY. BLUP was found to outperform AMMI in the analysis of four real MET trials. The application of our indexes is illustrated using an oat MET dataset. It was shown that reliable measures of stability using WAASB may help breeders and agronomists to make correct decisions when selecting or recommending genotypes. In addition, the simultaneous selection index, WAASBY, will be useful when the selection should consider different weights for stability and mean performance. Some advantages over existing statistics are discussed. Finally, the implementation of the procedures of this article in future studies is facilitated by an R package containing all required functions.

2.2 INTRODUCTION

Breeders and geneticists continually strive to increase crop productivity in order to meet the growing world demand for food. In the final stage of a plant breeding program, much effort and resources need to be invested in the evaluation of the genotypes. Generally, a few hundred genotypes are investigated in a large number of environments, resulting in the well-known multi-environment trials (MET). MET makes it possible to identify genotypes that display a small temporal variability −which is desired by and is beneficial to growers−, or cultivars that are consistent from location to location −which is desired by and is beneficial to seed companies and breeders− (Yan and Kang, 2003).

The data from these trials result in a matrix M of dimension genotype × environment. Since plants respond to a host of environmental signals (both biotic and

abiotic), a given genotype can perform relatively well in a given environment, but relatively poorly in others. If the genotypes' ranking changes significantly across environments, a significant genotype−vs−environment interaction (GEI) is observed. This interaction is known as qualitative or crossover type interaction and plays a key role in formulating strategies for crop improvement. Another form of interaction may also occur from just expansion or contraction of scale over the range of environments without a change in rank order. This form is known as quantitative or non-crossover type. At this step, one of the main challenges of MET analysis arises: to understand the GEI, seeking new ways of exploiting it and using it to benefit the selection of highly productive genotypes targeted to specific environments or that are broadly adapted.

The breeders' desire to modeling these interactions appropriately has led to the development of procedures called stability analysis. Yates and Cochran (1938) suggested a multiplicative operator for modeling the GEI that consists of a simple regression of a genotype's performance on the environmental mean −known as joint regression analysis− and that was based from ideas presented by Mooers (1921). Years later, this approach was popularized by Finlay and Wilkinson (1963) and Eberhart and Russell (1966). Methods that combine different statistical techniques have been also developed for stability analysis. For example, Gollob (1968) proposed a method which combines the features of factor analysis and analysis of variance into a single method. At that time, this method was known as FANOVA. Nowadays, this same method is known by the acronym AMMI, Additive Main Effect and Multiplicative Interaction (Gauch, 1988).

AMMI analysis is used mainly in a fixed-effect model framework. In some cases, it may be reasonable considering genotypes or environments (or both) to be random effects (Smith et al. 2005). When one factor is fixed and others random, we have a

linear mixed-effect model (LMM). More specifically, the BLUP (Best Linear Unbiased Prediction) offers the potential to improve the predictive accuracy of random effects (Smith et al. 2005). A study comparing the predictive success of BLUP and AMMI suggested that BLUP should be used in order to obtain reliable estimates in MET (Piepho 1994). Since the 1990s, LMM has been more frequently used to analyze MET. Between 2013 and 2015, for example, the larger number of papers proposing methods to deal with GEI were related to LMM (van Eeuwijk et al., 2016).

From the practical point of view, BLUP and AMMI can be seen as two distinct approaches to achieve the same goal: to distinguish the GEI pattern from the random error. From the statistical point of view, these models are vastly different. AMMI analysis retains most of the GEI pattern in the first interaction principal component axis (IPCA) resulting from the singular value decomposition (SVD) of the nonadditive effects matrix, while most of the random error is retained in the last IPCAs. BLUP, on the other hand, initially estimates the effects of the ANOVA model and then attributes weights to these effects and could thus be considered a shrinkage estimator (Piepho 1994). These two models are frequently used alone in the evaluation of METs. For example, some studies were successful in estimating genotypic values in MET using BLUP (Olivoto et al. 2017; Nardino et al. 2016) while others were successful in modeling GEI patterns using AMMI (Bocianowski et al., 2019; Veenstra et al., 2019). Taking into account the importance of AMMI and BLUP, the question that becomes apparent at this point is if the benefits of these two important techniques could be incorporated into a single method. Thus, there would seem to be value in an investigation to combine the graphical tools of AMMI and the predictive accuracy of BLUP.

Our hypothesis in this study is that the shrunken GEI effects matrix generated by a BLUP-based mixed model can be subjected to an AMMI-like analysis using SVD procedure. Thus, the purposes of this study were to: (i) evaluate the predictive ability of AMMI and BLUP using real data with different GEI patterns; (ii) introduce a genotypic stability measure and a superiority index that allow weighting between performance and stability (iii) compare these measures with worldwide-known parametric and nonparametric indexes in terms of genotype ranking; and (iv) introduce an R package that includes user-friendly functions for MET analysis, and for implementing the procedures proposed in this study.

2.3 MATERIAL AND METHODS

2.3.1 Basic concepts of AMMI and BLUP

Consider a set of multi-environment trials where g genotypes are tested in each of e environments. For convenience, we will consider that in each environment the genotypes are arranged in a randomized complete block design (RCBD) with b replications. The simplest linear model with interaction effect used in the statistical analysis of this data is given in Eq. [1].

$$y_{ijk} = \mu + \alpha_i + \tau_j + (\alpha\tau)_{ij} + \gamma_{jk} + \varepsilon_{ijk} \quad [1]$$

where y_{ijk} is the response variable (e.g., grain yield) of the *k*th block of the *i*th genotype in the *j*th environment (*i* = 1, 2, ..., *g; j* = 1, 2, ..., *e; k* = 1, 2, ..., *b*); μ is the grand mean; α_i is the main effect of the *i*th genotype; τ_j is the main effect of the *j*th environment; $(\alpha\tau)_{ij}$ is the interaction effect of the *i*th genotype with the *j*th environment; γ_{jk} is the

effect of the *k*th block within the *j*th environment; and ε_{ijk} is the random error assuming $\varepsilon_{ijk} \sim NID(0,\sigma^2)$, where *NID* means normally, identically and independently distributed.

For cases in which a complex GEI structure is observed, a more accurate estimate of can be obtained using AMMI analysis (Gauch, 1988). Generally, the mean response of individual genotypes averaged over b replications within each environment is computed and used to fill a *g*× *e* matrix. Briefly, the estimate of y_{ij} is given in two steps according to Eq. [2].

$$y_{ij} = \mu + \alpha_i + \tau_j + \sum_{k=1}^{p} \lambda_k a_{ik} t_{jk} + \rho_{ij} + \varepsilon_{ij} \quad [2]$$

Firstly, the additive effects of genotype (α_i) and environment (τ_j) are fitted by standard ANOVA procedures; then, the nonadditive −or residual− effects matrix is decomposed as $y_{ij} - \mu - \alpha_i - \tau_j = \sum_{k=1}^{p} \lambda_k a_{ik} t_{jk}$, where λ_k is the singular value for *k*th IPCA; a_{ik} is the *i*th genotype eigenvector for axis *k*; t_{jk} is the *j*th environment eigenvector for axis *k*. A residual, ρ_{ij}, remains if not all the *p* IPCA are used i.e., *p* = min(*g* − 1; *e* − 1). The scores for genotypes and environments are then used in biplots (Kempton, 1984), allowing a graphical interpretation of the GEI effects.

In MET trials, modelling genotypic effects as random may be preferable despite the fact that it would be classified as fixed using traditional definitions (Stroup and Mulitze, 1991). To illustrate the methodology, we will assume α_i and $(\alpha\tau)_{ij}$ to be random effects; thus the model in Eq. [1] can be conveniently rewritten in a standard linear mixed model (Yang, 2007):

$$\mathbf{y = Xb + Zu + e} \quad [3]$$

Where **y** is an $n[=\sum_{j=1}^{e}(gb)]\times 1$. vector of response variable (e.g., grain yield) $\mathbf{y} = \left[y_{111},\ y_{112},\ \ldots,\ y_{geb}\right]'$; **b** is an $(eb)\times 1$ vector of unknown and unobservable fixed effects $\mathbf{b} = [\mu + \gamma_{11}, \gamma_{12}, \ldots, \gamma_{eb}]'$; **u** is an $m[= g + ge]\times 1$ vector of unobservable random effects $\mathbf{u} = \left[\alpha_1, \alpha_2, \ldots, \alpha_g, (\alpha\tau)_{11}, (\alpha\tau)_{12}, \ldots, (\alpha\tau)_{ge}\right]'$; **X** is an $n\times(eb)$ design matrix of 0s and 1s relating **y** to **e; Z** is an $n\times m$ design matrix of 0s and 1s relating **y** to **u**; **e** is an $n\times 1$ vector of random errors $\mathbf{e} = \left[y_{111},\ y_{112},\ \ldots,\ y_{geb}\right]'$; and the prime (′) represents the vector transposition. Random vectors **u** and **e** are assumed to be normal and independently distributed with zero mean and variance-covariance matrices **G** and **R** respectively, such that

$$\begin{bmatrix}\mathbf{u}\\ \mathbf{e}\end{bmatrix} \sim N\left(\begin{bmatrix}\mathbf{0}\\ \mathbf{0}\end{bmatrix}, \begin{bmatrix}\mathbf{G} & \mathbf{0}\\ \mathbf{0} & \mathbf{R}\end{bmatrix}\right)$$

The matrices **G** and **R** are allowed to take different covariance structure (Piepho, 1998), but here we will take a simple form of **G** and **R** for a comparison with the AMMI model:

$$\mathbf{G} = \begin{bmatrix}\hat{\sigma}_{\alpha}^{2}\mathbf{I}_{g} & 0\\ 0 & \hat{\sigma}_{\alpha\tau}^{2}\mathbf{I}_{ge}\end{bmatrix}$$

and $\mathbf{R} = \hat{\sigma}_{\varepsilon}^{2}\mathbf{I}_{n}$, where $\hat{\sigma}_{\alpha}^{2}$, $\hat{\sigma}_{\alpha\tau}^{2}$, and $\hat{\sigma}_{\varepsilon}^{2}$ represent variances for genotype, genotype-vs-environment interaction and random errors, respectively; $\mathbf{I}_g$, $\mathbf{I}_{ge}$, and $\mathbf{I}_n$ are the identity matrices of order g, $g\times e$, and n, respectively.

The vectors **b** and **u** are then estimated using the well-known mixed model equation (Henderson, 1975).

$$\begin{bmatrix}\hat{\mathbf{b}}\\ \hat{\mathbf{u}}\end{bmatrix} = \begin{bmatrix}\mathbf{X}'\mathbf{R}^{-1}\mathbf{X} & \mathbf{X}'\mathbf{R}^{-1}\mathbf{Z}\\ \mathbf{Z}'\mathbf{R}^{-1}\mathbf{X} & \mathbf{Z}'\mathbf{R}^{-1}\mathbf{Z}+\mathbf{G}^{-1}\end{bmatrix}^{-}\begin{bmatrix}\mathbf{X}'\mathbf{R}^{-1}\mathbf{y}\\ \mathbf{Z}'\mathbf{R}^{-1}\mathbf{y}\end{bmatrix} \qquad [4]$$

where the superscript "$^{-1}$" and "$^{-}$" represent the inverse and generalized inverse of the matrices, respectively. Generally, variance components are usually unknown and thus their estimates ($\hat{\mathbf{G}}$ and $\hat{\mathbf{R}}$), often obtained by REstricted Maximum Likelihood (REML) using the Expectation-Maximization algorithm (Dempster et al. 1977) are substituted into Eq. [5]. The significance of the random effects may be tested by a likelihood ratio (LR) test, which for the model in Eq. [4], compares the −2(Res)log likelihoods for two models, one with all random terms (full model) and other without one of the random terms (reduced model). The probability is then obtained by a two-tailed chi-square test with one degree of freedom (χ^2_1).

Considering balanced data, the effect the *i*th genotype ($\hat{g}_i$) within $\mathbf{u_g}$ is given in standard ANOVA notation as follows:

$$\hat{g}_i = h_g^2(\bar{y}_{i.} - \bar{y}_{..}) \qquad [5]$$

where $h_g^2 = \left(\hat{\sigma}^2_{\alpha\tau} + e\hat{\sigma}^2_{\alpha}\right) / \left(\hat{\sigma}^2_{\alpha\tau} + \hat{\sigma}^2_{\varepsilon} + e\hat{\sigma}^2_{\alpha}\right)$ is the shrinkage effect for the genotype effect. The BLUP of the *i*th genotype is then given by $BLUP_i = \mu + \hat{g}_i$. The effect of the *i*th genotype in the *j*th environment ($\hat{g}_{ij}$) within $\mathbf{u_{ge}}$ is given as follows:

$$\hat{g}_{ij} = h_g^2(\bar{y}_{i.} - \bar{y}_{..}) + h_{ge}^2(y_{ij} - \bar{y}_{i.} - \bar{y}_{.j} + \bar{y}_{..}) \qquad [6]$$

where h_g^2 is as defined above and $h_{ge}^2 = \hat{\sigma}^2_{\alpha\tau} / \left(\hat{\sigma}^2_{\alpha\tau} + \hat{\sigma}^2_{\varepsilon}\right)$ is the shrinkage effect for GEI. The BLUP of the *i*th genotype in the *j*th environment is then given by $BLUP_{ij} = \bar{y}_{.j} + \hat{g}_{ij}$. It is easy to see that the smaller the error term, the smaller the shrinkage effect, becoming one (no shrinkage effect) if error term is zero.

2.3.2 Source and characterization of data

The data used in this study comes from experiments with oat (*Avena sativa*, L.), soybean (*Glycine max*. L.), wheat (*Triticum aestivum*, L.) and maize (*Zea mays*, L.). Different datasets were used for covering different GEI patterns, providing more security in the selection of the most predictively accurate model. A total of 118 genotypes and 47 environments were studied. The *Oat* dataset came from trials conducted at the Department of Agrarian Studies of the Regional University of Northwestern Rio Grande do Sul, in Augusto Pestana, RS, Brazil. Ten oat genotypes conducted in 16 environments were evaluated. The environments were defined by the combinations of eight growing seasons (2010 - 2017) and two fungicide managements [with fungicide (WF) and without fungicide (NF)]. The characterization of the 10 oat cultivars is in Supplemental Table S1. The *Soybean* dataset came from trials conducted at Federal Institute of Education, Science, and Technology of Farroupilha, in São Vicente do Sul, RS, Brazil. A total of 13 soybean genotypes were evaluated in five environments. Each environment was considered the combination of cultivation years (2013 and 2014) and sowing seasons (three in 2013 and two in 2014). The *Wheat* dataset came from 17 trials carried out in 2014 with 40 wheat genotypes from the wheat breeding program the Central Cooperative for Agricultural Research (COODETEC). Further details can be found in Bornhofen et al. (2018). The *Maize* dataset came from trials with 55 maize genotypes growing in nine environments. This dataset was used by Silva et al. (2015) and is publicly available in the online version of such article <https://doi.org/10.1371/journal.pone.0131414>. For all datasets, the genotypes in each environment were organized in a randomized complete block

design (RCBD) with three blocks and one replication per block. The measured response variable was the grain yield (GY, Mg ha^{-1}).

2.3.3 Statistical analysis

All statistical analyses were performed using R 3.5.2 software (R core Team 2018). The functions used in this article were organized in an R package called **metan** −**m**ulti-environment **t**rial **an**alysis− (Olivoto, 2019) that will be detailed later in this article.

2.3.3.1 Cross-validation procedure

In order to evaluate the predictive accuracy of the AMMI and BLUP models, a cross-validation procedure was performed according to Piepho (1994). The original data was randomly split into training set −two complete and randomly selected blocks per environment−, and validation set −the remaining block per environment. Depending on the experiment, *n* AMMI models (AMMI0, AMMI1, ..., AMMIF) were fitted to the modeling data according to Eq. [3]. The validation using BLUP considered the same steps and was based on Eq. [4]. Even though these 10 cultivars constitute a relatively small set of cultivars, they represent the majority of the area cultivated with oat in southern Brazil. Thus, it is reasonable to assume that they constitute a random sample of a population. For all models (AMMI*n* and BLUP) the predictive success was compared in relative terms using the root mean square prediction difference (RMSPD) between the model estimates and validation data, as follows:

$$RMSPD = \left[\left(\sum_{i=1}^{n}\left(\hat{y}_{ij} - y_{ij}\right)^2\right) / n\right]^{0.5} \quad [7]$$

where $\hat{y}_{ij}$ is the model predicted value of the *i*th genotype in the *j*th environment and y_{ij} is the observed value of the *i*th genotype in the *j*th environment in the validation set. For all models (AMMI-model family and BLUP), this procedure was repeated 1000 times. A boxplot was used to show the distribution of the 1000 RMSPD of each model. The codes used in this section are in S3.1.

2.3.3.2 Combining the advantages of AMMI and BLUP

As discussed earlier, only the *Oat* dataset will be used in this section. This data is publicly available (Olivoto, 2018) and will be used to reproduce all the examples. In the traditional AMMI model usage, a matrix with the residual of the additive model is decomposed into *k* IPCAs using SVD. Let $\mathbf{A_{ge}}$ be the matrix of BLUPs for the GEI effects generated by an LMM [$h^2_{ge}(y_{ij} - \bar{y}_{i.} - \bar{y}_{.j} + \bar{y}_{..})$ or $\hat{\mathbf{u}}_{\mathbf{ge}}$]. Similar to the AMMI analysis, $\mathbf{A_{ge}}$ was decomposed as follows

$$\mathbf{A_{ge}} = \mathbf{U_{gp}} \mathbf{\Lambda_{pp}} \mathbf{V_{pe}^T} \quad [8]$$

where $\mathbf{\Lambda_{pp}}$ is a diagonal matrix containing *p* singular values, in decreasing order, where $\mathbf{p}$ is the rank of the matrix $\mathbf{A_{ge}}$ with $\mathbf{p} \leq \min(g - 1; e - 1)$. The matrices $\mathbf{U_{gp}}$ and $\mathbf{V_{ep}}$ are orthonormal matrices with singular vectors of $\mathbf{AA^T}$ and $\mathbf{A^TA}$, respectively, being the orthonormal basis for the genotypes and environment effects, respectively.

The coordinate points for genotypes ($\mathbf{G_{gp}}$) and environments ($\mathbf{E_{ep}}$) in the *p* possible interaction axes were then estimated as $\mathbf{G_{gp}} = \mathbf{U_{gp}} \mathbf{\Lambda_{pp}^{0.5}}$, and $\mathbf{E_{ep}} = \mathbf{V_{pe}} \mathbf{\Lambda_{pp}^{0.5}}$. The rows of the submatrices $\mathbf{G_{g2}}$ and $\mathbf{E_{e2}}$ were the points for the coordinates of genotypes and environments, respectively, and were used in an AMMI2-like biplot to model the GEI patterns. Considering that genotypes or environments have random

effects −thus characterizing a mixed model− the GEI will always be random, which allows the estimation of $\mathbf{A_{ge}}$ and, consequently, the estimation of scores for genotypes and environments.

Aiming at identifying possible mega-environments as well as visualizing the "which-won-where" pattern a graphic with the nominal yield ($\hat{y}^{*}_{ij}$) as a function of the environment IPCA1 scores ($\mathbf{E_{e1}}$) was produced. In this graphic, each genotype is depicted by a straight line with the equation $\hat{y}^{*}_{ij} = \mu_i + IPCA1_i \times IPCA1_j$, where $\hat{y}^{*}_{ij}$ is the nominal yield for the *i*th genotype in the *j*th environment; μ_i is the grand mean of the *i*th genotype; $IPC1_i$ is the IPCA1 score of the *i*th genotype and $IPCA1_j$ is the IPCA1 score of the *j*th environment. The winner genotype in a given environment has the highest nominal yield in that environment (Gauch and Zobel, 1997).

2.3.3.3 The genotypic stability index

In the traditional AMMI model usage, when the proportion of the variance explained in IPCA1 is relatively low, there may be a biased interpretation regarding the stability of the genotypes using the AMMI1 biplot since GEI patterns are still explained in the remaining IPCA axis. To handle this problem, we propose a new stability index called WAASB, that is the **W**eighted **A**verage of **A**bsolute **S**cores from the singular value decomposition of the matrix of **B**LUPs for the GEI effects generated by an LMM, estimated as follows:

$$WAASB_i = \sum_{k=1}^{p} \left| IPCA_{ik} \times EP_k \right| \Big/ \sum_{k=1}^{p} EP_k \qquad [9]$$

where $WAASB_i$ is the weighted average of absolute scores of the *i*th genotype (or environment); $IPCA_{ik}$ is the score of the *i*th genotype (or environment) in the *k*th IPCA,

and EP_k is the amount of the variance explained by the kth IPCA. The genotype with the lowest WAASB value is considered the most stable, i.e. the one that deviates least from the average performance across environments. Aiming at identifying highly productive and stable genotypes, we propose swapping the well-known AMMI1 biplot by a biplot with the abscissa represented by the WAASB values and the ordinate by the response variable. This biplot has the advantage of using all the estimated IPCA axes to identify the stability in a bi-dimensional plot.

To identify whether and how the ranks of genotype are altered when different numbers of IPCA are used in the WAASB estimation, the genotype's ranks were obtained considering the WAASB estimated with 1, 2, ..., p IPCA. When using only one IPCA, WAASB = |IPCA1|. The ranking was increasing; so, the genotype with the smallest WAASB value had the first−order rank. A heatmap graph was used to show the ranks of the genotypes in the different scenarios of WAASB estimation. The codes used in the two last sections are in S3.5.

2.3.3.4 A superiority index that allows weighting between performance and stability

To select genotypes that combine high performance and stability we introduced the WAASBY index, which is a superiority index that allows weighting between performance (in our example, GY) and stability (WAASB index). The first step is rescaling both GY and WAASB to 0−100 so that they can be directly compared. Since the best values for GY is the maximum value and for WAASB is the lowest value, the transformations were performed according to the following equations:

$$rG_i = \frac{100 - 0}{G_{\max} - G_{\min}} \times (G_i - G_{\max}) + 100 \qquad [10]$$

and

$$rW_i = \frac{0-100}{W_{max} - W_{min}} \times (W_i - W_{max}) + 0 \quad [11]$$

Where rG_i *and* rW_i are the rescaled values for GY and WAASB, respectively, for the *i*th genotype; G_i and W_i are the response variable (GY) and the WAASB values for *i*th genotype. Then the WAASBY index was calculated according to Eq. [13]:

$$WAASBY_i = \frac{(rG_i \times \theta_Y) + (rW_i \times \theta_S)}{\theta_Y + \theta_S} \quad [12]$$

where $WAASBY_i$ is the superiority index for the *i*th genotype that weights between performance and stability, and θ_Y and θ_S are the weights for response variable and stability assumed to be 65 and 35 in this study, respectively. In addition, twenty-one scenarios varying θ_Y and (100/0, 95/5, 90/10, ..., 0/100) were planned. For each scenario, the first-order rank was attributed to the genotype with the highest WAASBY value. The objective here is to show how the ranking of genotypes is altered depending on the weight assigned to the stability and response variable. To assist with intuitive interpretation, a heat map graph was produced. The codes used in this section are in S3.6.

2.3.3.5 Relationship between stability measures

In this section the indexes WAAS and WAASY (considering a fixed-effect model), and the indexes WAASB and WAASBY (considering a mixed-effect model) were compared in terms of genotypes' ranking with the following five AMMI derived stability indexes, namely: (i) absolute values of the first principal component axis, $IPCA1_i = \sum_{k=1}^{1} \left| \lambda_k^{0.5} a_{ik} \right|$; (ii) AMMI stability value (Purchase et al., 2000), $ASV_i = \left[\left[b\lambda_1^2 / b\lambda_2^2 \times (\lambda_1^{0.5} a_{i1}) \right]^2 + (\lambda_2^{0.5} a_{i2})^2 \right]^{0.5}$, where *b* is the number of blocks; (iii) sums

of the absolute value of the IPCA scores, $SIPC_i = \sum_{k=1}^{P} \left| \lambda_k^{0.5} a_{ik} \right|$, and (iv) averages of the squared eigenvector values, $EV_i = \sum_{k=1}^{P} a_{ik}^2 / P$, described by Sneller et al. (1997), where P is the number of IPCA retained via F-tests; and (v) the absolute value of the relative contribution of IPCAs to the interaction (Zali et al., 2012), $Za_i = \sum_{k=1}^{P} \theta_k a_{ik}$, where θ_k is the percentage sum of squares explained by the kth IPCA. We also considered the simultaneous selection indexes (ssi) computed by summation of the ranks of the ASV, SIPC, EV and Za indexes with the ranks of the mean yields (Farshadfar, 2008) which resulted in ssiASV, ssiSIPC, ssiEV, and ssiZa, respectively. The genotypes' rankings were calculated according to the concept of each index and for GY for the *Oat* and *Maize* datasets. The ranks obtained for the *Maize* dataset were submitted to a PCA analysis; then, a loading plot was used to explore the relationships among the studied indexes. It should be emphasized that we used the *Maize* data in this procedure due to the larger number of subjects (55 genotypes).

2.4 RESULTS

2.4.1 Predictive success

BLUP was the most predictively accurate model in all evaluated datasets (Fig. 1). For the AMMI model, the number of IPCAs retained depended on the evaluated experiment. For *Maize*, *Oat*, *Soybean*, and *Wheat* datasets, the most predictively accurate AMMI models were AMM0, AMMI5, AMMI3, and AMMI5, respectively. This result confirms the hypothesis of different GEI patterns in the analyzed trials.

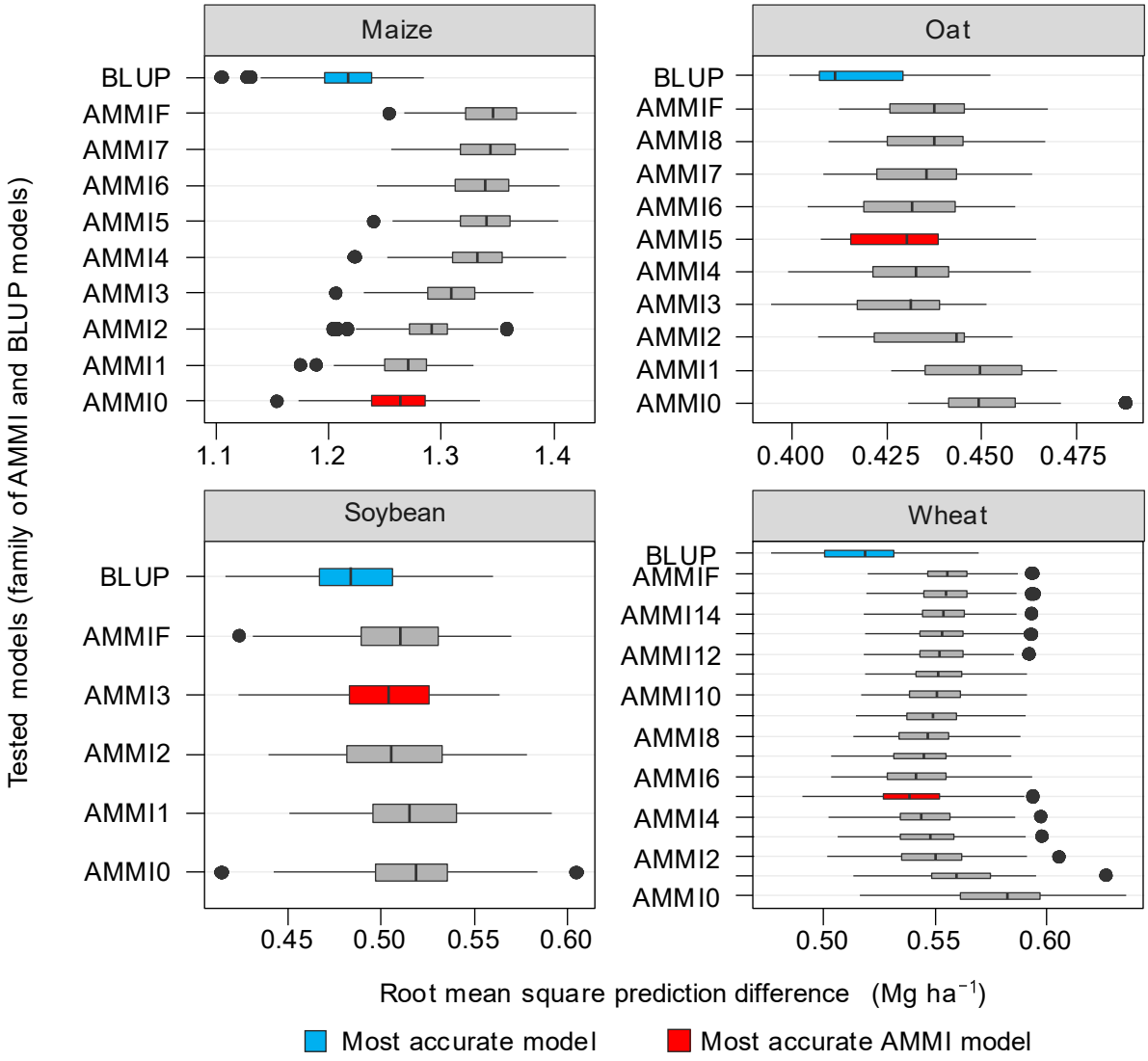

Figure 1. Predictive accuracy of the AMMI family and BLUP for trials with four different crops. The boxplots show the distribution of the 1000 RMSPD estimates.

2.4.2 Overall performance, variance components, and predicted means

The LR test indicated highly significant effects ($p < 0.001$) for both genotype and interaction effects in the *Oat* trial (Table 1). The Fig S1 shows that the interaction was qualitative (i.e., crossover type), since the rank order for the genotypes changed across environments (Fig. S1). The grand mean of GY was 2.69 Mg ha^{-1}, where the lowest mean was 1.37 Mg ha^{-1} (2014 NF) and the highest mean was 4.06 Mg ha^{-1} (2012 WF). It was noticed that, except for 2010, the use of fungicide seemed to provide higher GY. This was most evident in the years 2013 and 2015 (Figs. S2 and S3). The

individual analysis revealed that nine of 16 environments (~56.3%) showed significant differences for genotype effects. The block effect was significant for only six (37.5%) environments (Table S2).

Table 1. Deviance analysis, estimated variance components and genetic parameters for grain yield of 10 oat genotypes evaluated in 16 environments

	Likelihood ratio test†	
Statistics	G	GEI
χ^2	19.23	61.10
p-value	1.15×10^{-5}	5.42×10^{-15}
	Variance components	
REML‡	Estimates	
$\hat{\sigma}^2_{\alpha}$	0.0241 (12.6%)§	
$\hat{\sigma}^2_{\alpha\tau}$	0.0637 (33.4%)	
σ^2_{ε}	0.1032 (54.0%)	
σ^2_{p}	0.191	
h^2_{g}	0.126	
R^2_{gei}	0.333	
h^2_{mg}	0.531	
As	0.728	
r_{ge}	0.381	
CV_g (%)	5.8	
CV_e (%)	11.9	
CV_g/CV_r ratio	0.483	
$\hat{\sigma}^2_{\alpha\tau} / \hat{\sigma}^2_{\alpha}$ ratio	2.643	

† G, genotype; GEI, genotype-vs-environment interaction.

‡ $\hat{\sigma}^2_{\alpha}$, genotypic variance; $\hat{\sigma}^2_{\alpha\tau}$, variance of G × E interaction; σ^2_{ε}, residual variance; σ^2_{p}, phenotypic variance; h^2_{g}, broad-sense heritability; R^2_{gei}, coefficient of determination for the GEI effects; h^2_{mg}, heritability of the genotypic mean; As, accuracy of genotype selection; r_{ge}, correlation between genotypic values across environments; CV_g (%), genotypic CV; CV_e (%), residual CV.

§ Parenthetical values indicate the percentage of the observed phenotypic variance (σ^2_{p}).

Proximally 54% of the phenotypic variance ($\hat{\sigma}^2_{p}$) was due to the residual variance ($\hat{\sigma}^2_{\varepsilon}$) (Table 1). The contribution of the genotypic variance ($\hat{\sigma}^2_{\alpha}$) was 12.6% only. Consequently, low estimates of broad-sense heritability were observed. The genotypic accuracy of selection (As), which measures the correlation between predicted and observed values was of 0.89. The genotypic CV (5.8%) was found to be

less than half of the residual CV (11.9%). In addition, the high $\hat{\sigma}^2_{\alpha\tau} / \hat{\sigma}^2_{\alpha}$ ratio (2.64) resulted in a low correlation between genotypic values across environments (0.38).

The G8 and G3 genotypes stood out for having the highest predicted means among the tested genotypes (Fig. 2). The genotypes G7, G2, and G4 were the other genotypes that performed well (GY above the grand mean), however, with a very small difference among them.

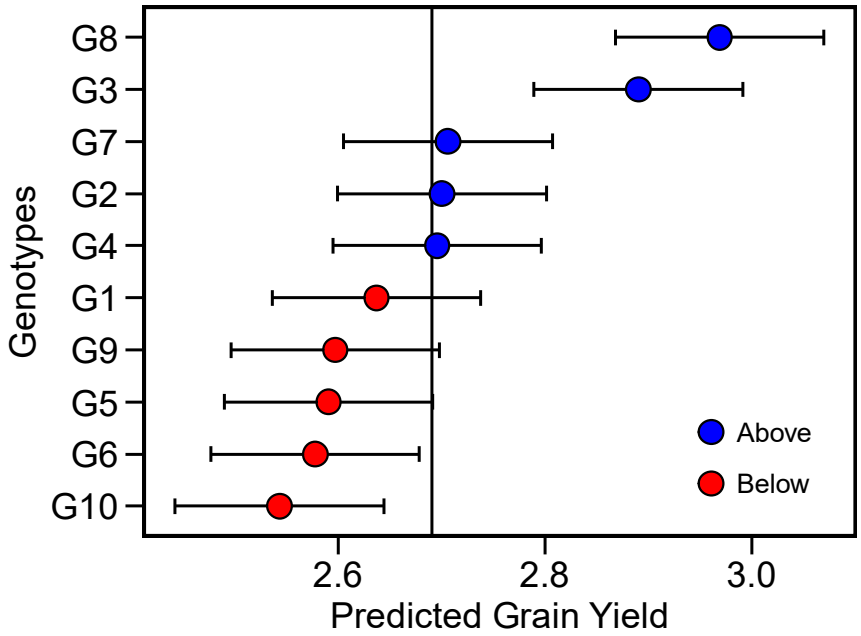

Figure 2. Predicted grain yield (BLUP) for 10 oat genotypes. Blue and red circles represent the genotypes that had BLUP above and below of BLUP means, respectively. Horizontal error bars represent the 95% confidence interval of prediction considering a two-tailed *t*-test. G01: BARBARASUL; G02: BRISASUL; G03: FAEM CARLASUL; G04: FAEM CHIARASUL; G05: UPFA GAUDÉRIA; G06: URS 21; G07: URS CHARRUA; G08: URS CORONA; G09: URS TARIMBA; G10: URS TAURA.

2.4.3 Understanding the genotype-by-environment interaction

2.4.3.1 Biplot interpretation

The cumulative variance in the first two IPCA of the oat trial was 59.3% (Table S3). From the eight cultivation years, three (2011, 2015 and 2016) had a positive correlation – since the angle among them was <90°. This suggests that the magnitude

of the interaction effects tended to be the same independently on the fungicide application (Fig. 3). Negative correlations –indicated by vector angles >90°– were observed in the years 2012, 2013, 2014, and 2017.

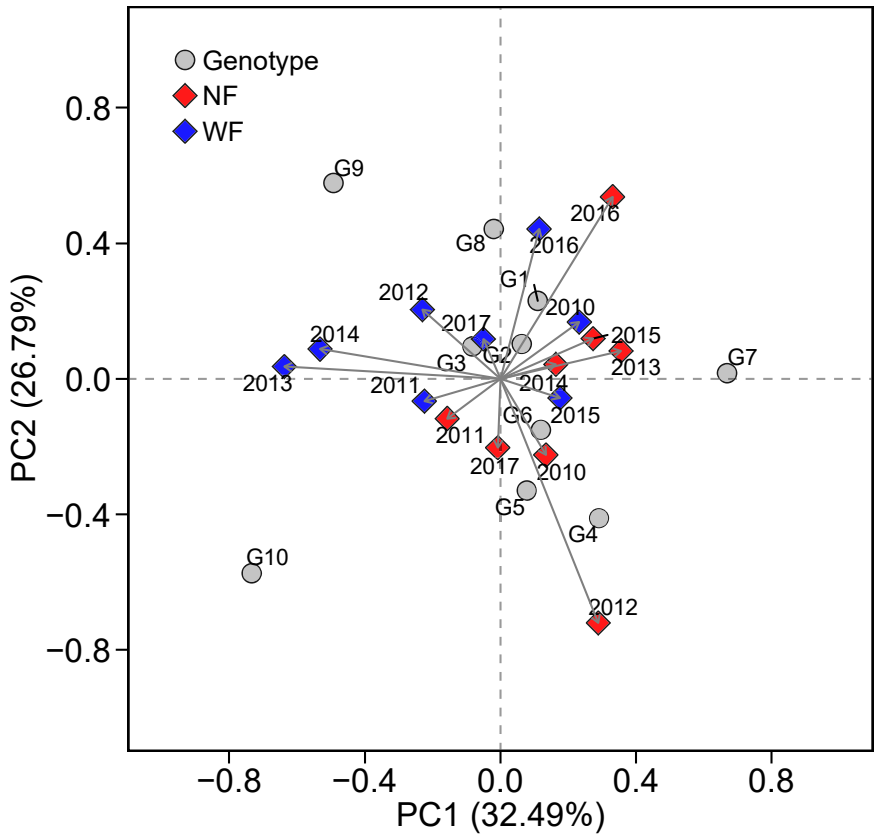

Figure 3. Biplot of 10 oat genotypes evaluated in 16 environments [combinations of 8 cultivation years with application (WF) and with no application of fungicide (NF)]. The scores were obtained from fitting the singular value decomposition of the double-centered BLUP interaction effects matrix obtained in a linear mixed model with symmetric singular value partitioning (α = 1/2). The axes are equally scaled. G01: BARBARASUL; G02: BRISASUL; G03: FAEM CARLASUL; G04: FAEM CHIARASUL; G05: UPFA GAUDÉRIA; G06: URS 21; G07: URS CHARRUA; G08: URS CORONA; G09: URS TARIMBA; G10: URS TAURA.

Fig. 4 allows an easy interpretation of the “which-won-where” pattern. In our example, G8 won in all studied environments. This genotype is depicted by a line with the equation $y = 3.039 + (-0.020x)$, where x is the environmental IPCA1 score. The left-most score of −0.637 implies a yield of 3.051 Mg ha^{-1}, whereas the right-most score of 0.356 implies a yield of 3.046 Mg ha^{-1}. These two coordinate pairs give two points that define the line for G8. Considering the first IPCA, G8 won in all

environments because of its highest yield (3.039 Mg ha^{-1}) and the smallest IPCA1 score (which defines the slope of the line) among the tested genotypes (Table S4).

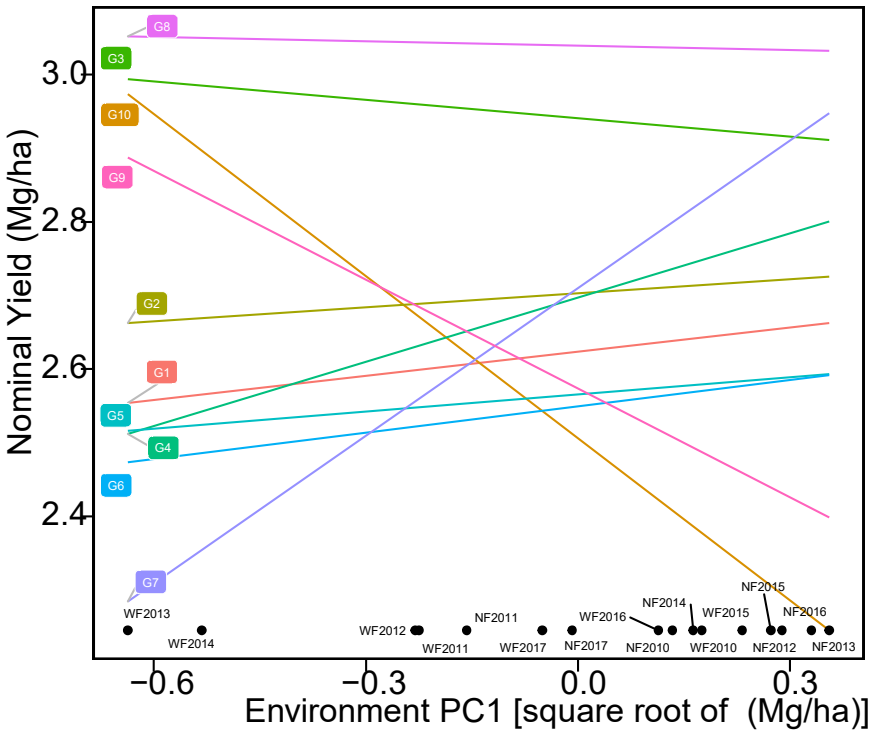

Figure 4. Nominal grain yield for 10 oat genotypes as a function of the environment scores of the first interaction IPCA (IPCA1). G01: BARBARASUL; G02: BRISASUL; G03: FAEM CARLASUL; G04: FAEM CHIARASUL; G05: UPFA GAUDÉRIA; G06: URS 21; G07: URS CHARRUA; G08: URS CORONA; G09: URS TARIMBA; G10: URS TAURA.

At this point, we may make a relationship between figure 2 and 4. For example, G8 had the highest predicted mean (Fig 2) and due to the smallest IPCA1 score, this genotype was classified as the 'universal winner' (Fig. 4). On the other hand, G2 and G7 had very similar predicted means (Fig. 2) but completely distinct lines in Fig. 4. This has occurred because while G2 had an IPCA1 score of 0.063, G7 had a score of 0.669 (Table S4). It will be discussed later in this article that these genotypes compose different groups when considering both stability and productivity for the genotypes' ranking.

The quadrants in Fig. 5 represent the four classes of genotypes/environment for a joint interpretation of performance and stability. In the 1st quadrant, the most unstable

genotypes -the ones that contribute much to GEI- and environments with high discrimination ability are included. The magnitude of the response variable (i.e., GY), however, is below the grand mean. Oat genotypes G10 and G9 were included in this quadrant. Although they presented GY close to the grand mean, they presented the highest WAASB values. Thus, specific adaptation (Figs. 3 and 4) should be investigated for genotypes within this quadrant. We may see here how the low percentage of GEI pattern explained in IPCA1 may mask the interpretation of a biplot. Genotype G7 had a higher score (in absolute values) in IPCA1 than genotype G9 (Table S4). However, when all IPCAs were included, G9 had a higher WAASB value than G7 (Fig. 5).

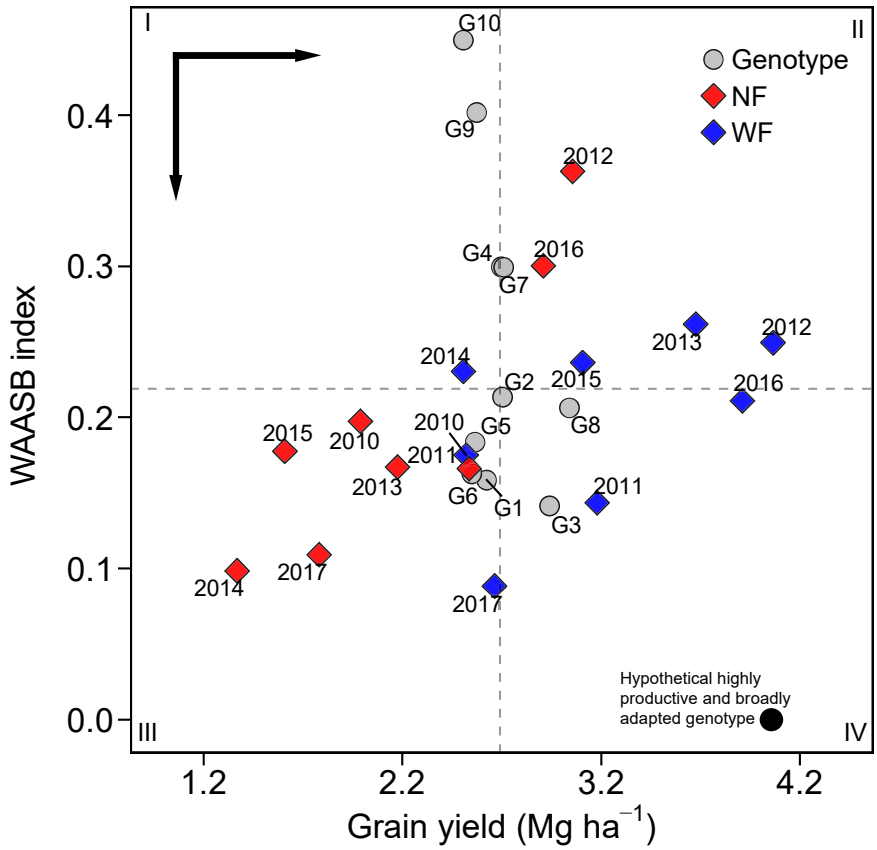

Figure 5. Biplot of the Grain yield vs WAASB of 10 oat genotypes evaluated in 16 environments [combinations of 8 cultivation years with application (WF), and with no application of fungicide (NF)]. A hypothetical highly productive and broadly adapted genotype is depicted by a black circle. Horizontal and vertical black arrows indicate the direction of the increase in yielding and stability, respectively. G01: BARBARASUL; G02: BRISASUL; G03: FAEM CARLASUL; G04: FAEM CHIARASUL; G05: UPFA GAUDÉRIA; G06: URS 21; G07: URS CHARRUA; G08: URS CORONA; G09: URS TARIMBA; G10: URS TAURA.

In the 2nd quadrant, highly-productive but unstable genotypes are included. The environments included in this quadrant deserve attention since, in addition to providing high magnitudes of the response variable, they present a good discrimination ability of the genotypes. In 2012 and 2016, regardless of the fungicide management, the GY was higher than the grand mean; however, the discrimination ability of the genotypes was higher in environments without fungicide application (Fig. 5).

In the 3rd quadrant low-productive and wide-adapted genotypes are included due to the lower values of WAASB. The lower this value, the more stable is the performance of a genotype across the environments. The environments included in this quadrant can be considered poorly productive and with low discrimination abilities.

The genotypes within 4th quadrant have above-mean productivity and lower values of WAASB (broadly adapted). The environments included in this quadrant, however, can be considered productive but with low discrimination abilities. We have shown previously that G8 had the smallest IPCA1, being thus the most stable when only the first IPCA is used. In our example, 67.5% of the GEI variance was not explained by IPCA1. When this information was considered (WAASB), we may see that G3 was, in fact, the most stable (smaller WAASB value).

2.4.3.2 Genotype stability ranking depending on the number retained IPCA

Figure 6 shows the ranks of the genotypes in relation to stability depending on the number of IPCA used in WAASB estimation. For *Oat* data, nine axes were considered [min(10 − 1; 16 − 1)]. It is observed that the genotype ranking was altered by the extent to which IPCAs are included in the WAASB estimation. This was most evident up to three IPCA (Fig. 6). Groups of genotypes with similar stability

performance may be easily identified by the dendrogram on the left side of Fig. 6. For example, the G3, G1, and G6 genotypes showed the lowest WAASB values considering four or more IPCA and were, therefore, the 1st, 2nd, and 3rd most stable, respectively (as it can be seen also in Fig. 5). The most evident change was those of the G2. When using the first and second IPCA in the WAASB estimation, that genotype was considered the 2nd and 1st most stable, respectively; when more than three IPCAs were used, G2 was the 6th most stable (Fig. 6). This reinforces the benefits of using the WAASB index since it captures the variations of all IPCAs to compute the stability. If the ASV would be considered, the G2, in this case, would be ranked as most stable (Supplementary Table S5), when in fact it is not.

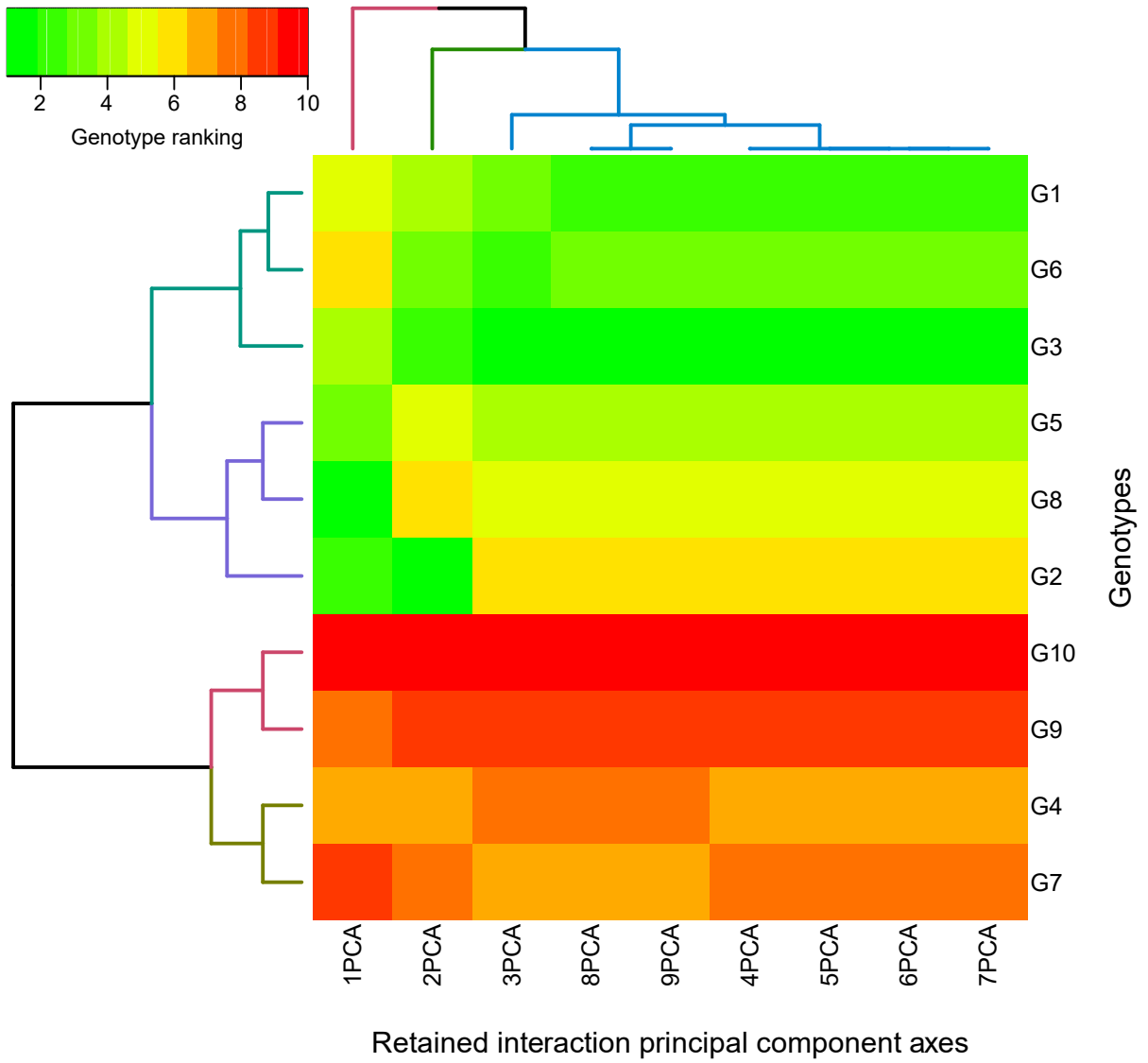

Figure 6. Heatmap showing the ranks of 10 oat genotypes in relation to the number of interaction principal component axes (IPCA) used in the WAASB estimation. G01: BARBARASUL; G02: BRISASUL; G03: FAEM CARLASUL; G04: FAEM CHIARASUL; G05: UPFA GAUDÉRIA; G06: URS 21; G07: URS CHARRUA; G08: URS CORONA; G09: URS TARIMBA; G10: URS TAURA.

2.4.3.3 Genotype ranking depending on the weights for stability and performance

Fig. 7 shows the WAASBY values considering the weights for GY and WAASB equal to 65 and 35, respectively. The genotypes that had the highest values of WAASBY were G8 (92.63) and G3 (88.01). It was previously discussed that these genotypes deserved prominence because they were within the quadrant IV of Fig. 5.

Unlike Fig. 7, which shows the WAASBY values considering a fixed WAASB/GY ratio, Fig. 8 allows identifying how the ranks of the genotypes are changed depending

on the weights assigned. The ranks in the left-most side are those obtained when only the stability was considered; from left to right, the weight for the response variable increase 5% each scenario. The ranks highlighted by a black rectangle are the same from those shown in Fig. 7. The ranks shown in the right-most side matches perfectly with the genotype's ranking for GY.

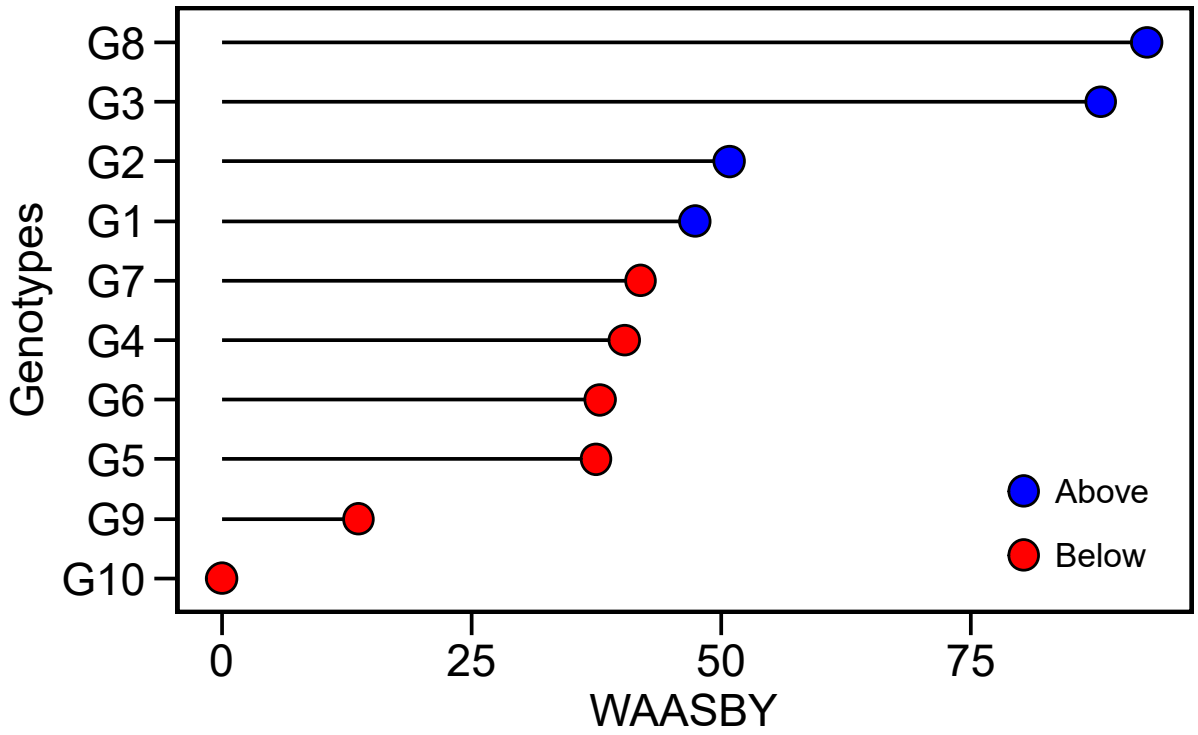

Figure 7. Estimated values of WAASBY for 10 oat genotypes considering the weights of 65 and 35 for yielding and stability, respectively. G01: BARBARASUL; G02: BRISASUL; G03: FAEM CARLASUL; G04: FAEM CHIARASUL; G05: UPFA GAUDÉRIA; G06: URS 21; G07: URS CHARRUA; G08: URS CORONA; G09: URS TARIMBA; G10: URS TAURA.

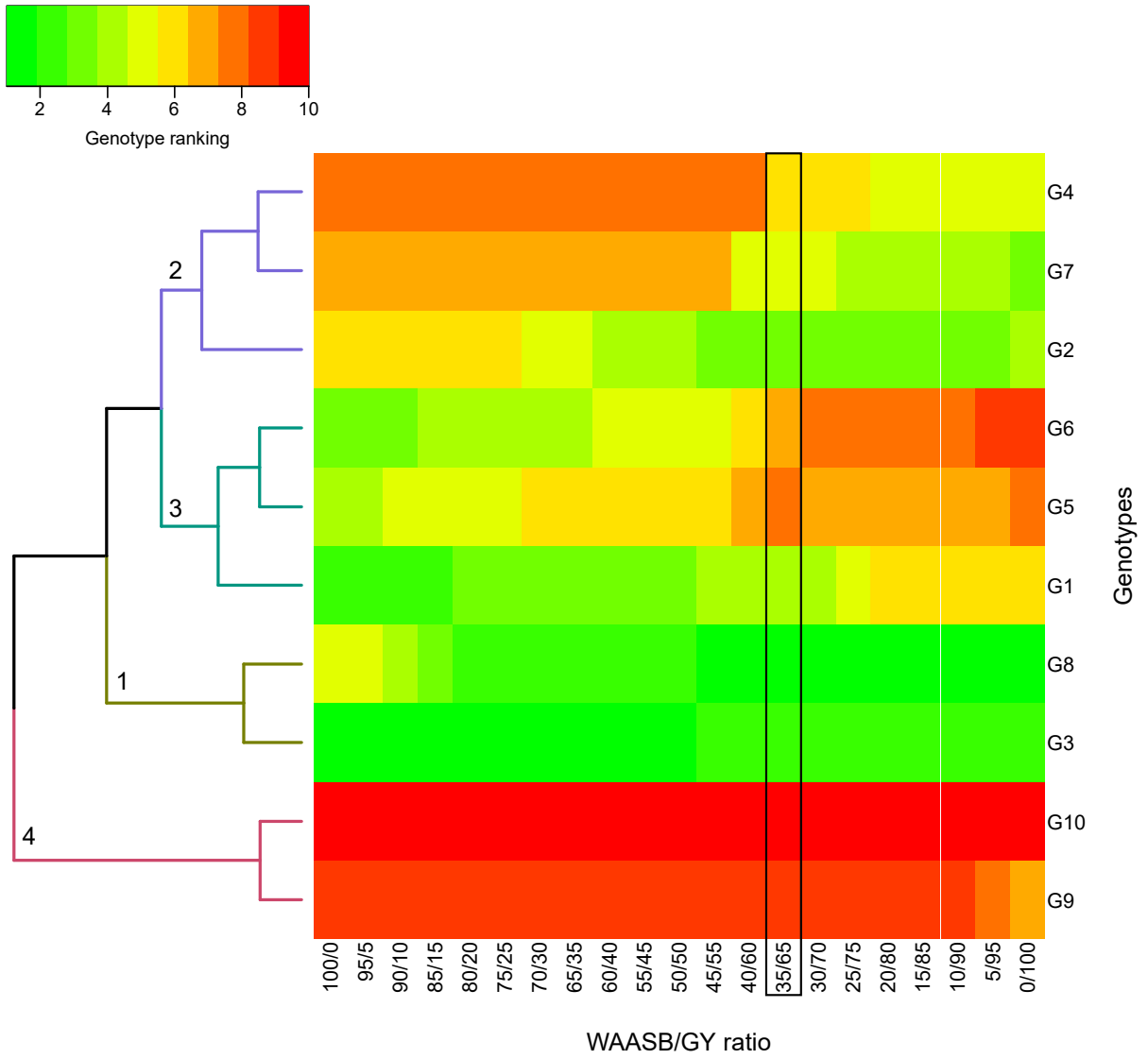

Figure 8. Ranks of 10 oat genotypes considering different weights for stability and yielding. The most-left ranks were obtained considering the stability only. The most right-ranks were obtained considering the grain yield only. Between the extremes, the ranks were obtained different weights for stability and yielding. The four clusters represent four classes of genotypes: (1) Poorly productive and unstable genotypes; (2) productive but unstable genotypes; (3) stable but poorly productive genotypes; and (4), highly productive and stable genotypes. G01: BARBARASUL; G02: BRISASUL; G03: FAEM CARLASUL; G04: FAEM CHIARASUL; G05: UPFA GAUDÉRIA; G06: URS 21; G07: URS CHARRUA; G08: URS CORONA; G09: URS TARIMBA; G10: URS TAURA.

The clusters shown on the left side of Fig. 8 may be also used to identify groups of genotypes with similar performance regarding stability and productivity. Cluster 1 included the genotypes G3 and G8, which as previously discussed are highly productive and broadly adapted genotypes. Note that these genotypes remained the firsts-ranked regardless of the WAASB/GY ratio (Fig. 8). Cluster 2 included genotypes G2, G4, and G7, that can be considered productive, but unstable, as they were well

ranked when the WAASB/GY ratio was low (greater weight for productivity). Cluster 3, conversely, included G1, G5, and G6, stable but low-productive genotypes because they were well ranked when the WAASB/GY ratio was high (greater weight for stability). Cluster 4 included G9 and G10, which we have shown in Fig 5 are poorly productive and unstable genotypes.

2.4.3.4 Correspondence among the stability and simultaneous selection indexes

The ranks obtained for each index for both Oat and Maize data are shown in Supplemental Tables S5 and S6, respectively. Figure 9 shows the loading plot obtained in the PCA analysis considering the correlation matrix among the methods obtained from with Maize data. The explained variance in the first two axes was 87.7%. Except for the IPCA1, ASV, and ssiASV, all of the other indexes were very near the edge of the circle, indicating that they were well represented by the plane of factors. All indexes had positive loadings on PCA1, and a clear separation between stability and simultaneous selection indexes was observed. Stability indexes included in the shading ellipse 1 were positively correlated with each other—the correlation between two indexes may be approximated by the cosine of the angle between its vectors—and they had positive loadings on PCA2. Our stability indexes WAAS and WAASB were highly correlated with Za, and in lower magnitude with ASV, EV, and SPIC indexes. The absence of perfect association between WAAS and WAASB suggests that beyond the relative differences, the rank order of some genotypes regarding the stability was changed when using a fixed- or a mixed-effect model (Supplemental Table S5, Fig. 9). Superiority indexes had negative loadings on PCA2 and were visually grouped into two clusters. The “ssis” indexes in shading ellipse 2 are based on AMMI principles, while

the WAASY and WAASBY that inherit principles from both AMMI and BLUP formed group 3. WAASY and WAASBY were highly positively correlated and provided ranks more similar to the GY compared to the simultaneous selection indexes within shading ellipse 2. This makes sense since in this study we attributed a high weight to productivity (65%). This freedom of attributing weights for stability and mean performance in genotype ranking may facilitate the genotype selection in cases when the research wants to prioritize one of these characteristics.

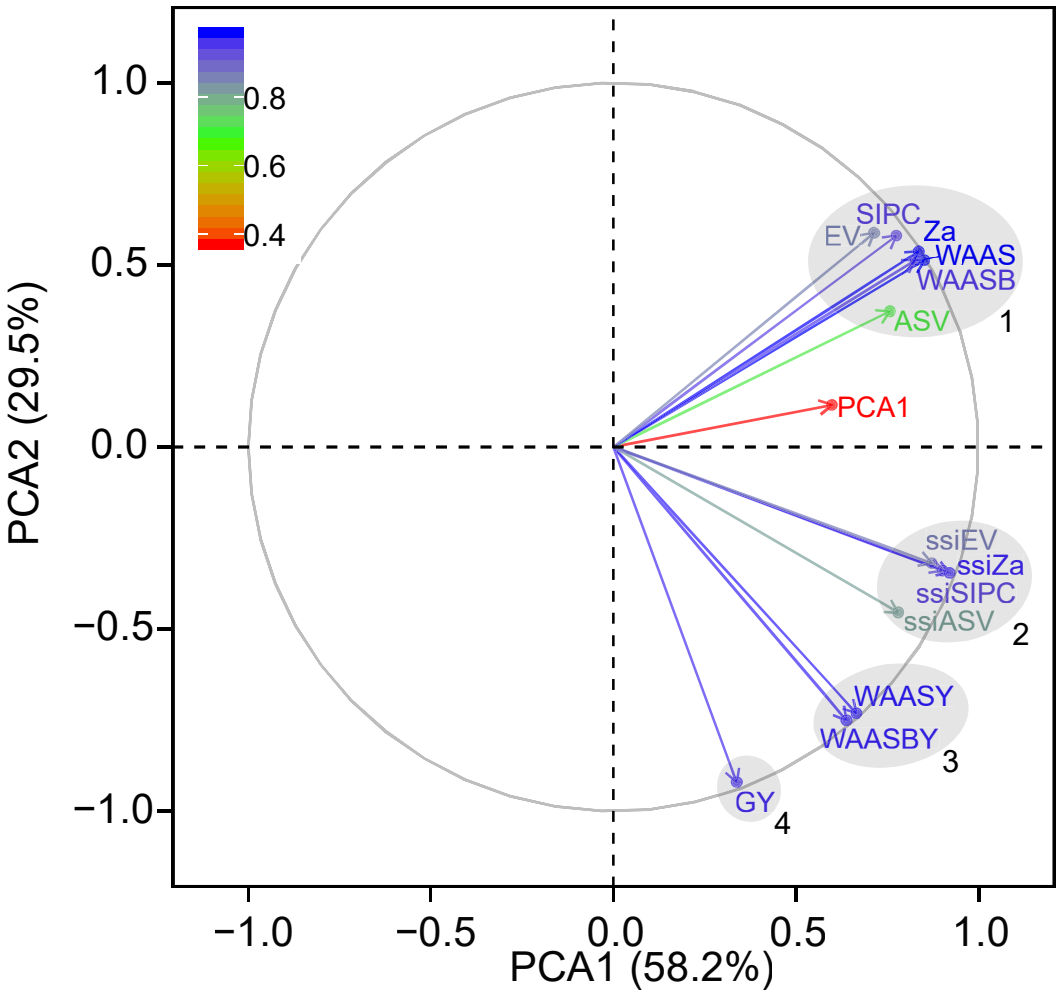

Figure 9. Loading plot obtained in the principal component analysis with the ranks for genotypes obtained for the WAAS and WAASB, weighted average of absolute scores; IPCA1, absolute values of the first principal component axis; ASV, additive main effects and multiplicative interaction stability value; SIPC, sums of the absolute value of the interaction principal component axis scores; EV, averages of the squared eigenvector values; Za, absolute value of the relative contribution of interaction principal component axes to the interaction. WAASY and WAASBY are the simultaneous selection indexes using WAAS and WAASB, respectively. The “ssi” are the simultaneous selection indexes using additive main effects and multiplicative interaction-derived stability indexes. The shaded ellipses highlight four visually formed groups. Color key represents the quality of the representation for variables on the factor map.

2.5 DISCUSSION

2.5.1 Successful statistical analysis of multi-environment trials

Multi-environment trials constitute the major efforts in plant breeding programs. Thus, the accuracy of prediction, that is, how close the predicted value is to the observed value is crucial for a successful selection, recommendation of cultivars, and delineation of mega-environments. According to Gauch and Zobel (1988), there are three main options to increase the accuracy of prediction in MET: The first, is to improve the experimental techniques, including the use of plots of ideal size and shape, the correct arrangement of plots in the experimental area, and uniform application of cultural management; The second, is to increase the number of replications, using sophisticated experimental designs; The third and last is to use statistical models with better prediction abilities. A special focus on the latter option was given in this article.

Studies comparing AMMI, BLUP, and GGE methods have been conducted (Balestre et al. 2009; Sa'diyah and Hadi 2016). In the present study, we have shown how the main advantages of AMMI and BLUP may be combined to increase the reliability of MET analysis. From the point of view of an agronomist, the biplot interpretation of the shrunken GEI effects (Fig. 3) and the "which-won-where" view (Fig. 4) may facilitate the recommendation of genotypes targeted to specific environments, thus exploiting narrow adaptations. This is important because in most cases no one genotype wins everywhere and always. On the other hand, the biplot WAASB × GY (Fig. 5) may be used for a joint interpretation of stability and productivity, thus exploiting broad adaptations. The main advantage of this biplot over the well-known AMMI1 biplot is that all IPCA axes are used, thus allowing that GEI patterns not retained in IPCA1 be considered in the genotypes' ranking.

From the point of view of a breeder, beyond the aforementioned advantages, the mixed model approach also allows the estimation of important parameters in quantitative genetics, such as the genotypic and interaction variances, broad-sense heritability, heritability on the mean basis and genetic correlations (Table 1). These pieces of information are essential in a plant breeding program and should be also exploited in MET's evaluation.

2.5.2 BLUP or AMMI? The assessment will show which model is better in a given situation

From four datasets with different GEI patterns, it is concluded that BLUP was the most predictively accurate model (Fig. 1). Our findings are according to those by Piepho (1994), who concluded that the BLUP outperforms any member of the AMMI family in predicting Faba bean (*Vicia faba*, L.) yield in MET. This viewpoint, however, is not unanimous. A study evaluating rice (*Oryza sativa*, L.) has shown that the estimates using the AMMI10 model was closer to the “true” value of yielding than the prediction by BLUP (Sa’diyah and Hadi 2016). Although it is not explicit, a cross-validation procedure similar to that used in this study and by Piepho (1994) was not used in such study.

Gauch (2013) pointed out that predictive accuracy merits special attention for model diagnosis in MET analysis. Due to the great data processing power of the current computers, it is reasonable to affirm that the choice of the best method to predict yield (or other response variables) should be based on the predictive ability assessment in each situation. This is due to the fact that both BLUP and AMMI have their efficacy increased depending on factors intrinsic to each trial. For example, the

larger the sample size −both regarding the number of levels of each factor and the number of observations at each of these levels− the more accurate the estimates of variance components are (Smith 1978). Unbiasedness in variance component estimation results in biased repeatability coefficients. In other words, the shrinkage effect associated with random effects can be over- or underestimated. Likewise, the efficiency of the AMMI model increases with the size of trials and the increase of noisiness present in the data (Gauch and Zobel 1988).

Provided that replicated data are available, the `cv_blup()` and `cv_ammif()` functions available in the `metan` package (S3.1) may be used to evaluate the predictive ability of BLUP and AMMI methods, respectively. Thus, for each specific situation, it is easy to identify the model with the smallest bias (in relative terms) and to use it in the prediction.

2.5.3 Identifying highly productive and broadly adapted genotypes with WAASB

The quantitative stability measure proposed in this study (WAASB) was found to be an important statistical tool for identifying highly productive and broadly adapted genotypes. WAASB may be seen as a mixed-effect model version of the AMMI-based stability indexes discussed here but have some advantages: (i) similar to ASV, the WAASB is a function of both cultivar and environment GEI pattern components but it is based on a mixed-effect model –shown here as to outperform fixed-effect models in terms of predictive accuracy– or even a random model; (ii) WAASB is based on absolute deviations instead squared deviations as ASV, thus, some robustness is gained due to less sensitivity to outliers; (iii) WAASB is more realistic in quantifying the stability in complex GEI structures since it is computed considering all the estimated

IPCA; (iv) differently from the SIPC, which considers the sum of absolute values of the IPCA scores, the WAASB considers the weighted average of the IPCA scores; thus, more reliable results should be the obtained since high scores in the last axes will have a smaller contribution to the estimation; (v) the WAASB × GY biplot (Fig. 5) allows the joint interpretation of stability and productivity in a bi-dimensional plot considering all the IPCAs of the model.

The AMMI1 biplot is used worldwide, and some AMMI-based stability indexes, such as ASV, have recently met with some success in quantifying the stability (Adjebeng-Danquah et al., 2017; Shahriari et al. 2018). But would it be a good decision quantifying the stability using one −or even two− IPCAs? If we look back to Fig. 6 the response will probably be: No. We have shown that G2 was considered the most stable genotype when the WAASB was estimated with two IPCA (Fig. 6). It would be reasonable since the scores for this genotype in the first two IPCAs were very low (Table S4). Considering the explained variance in such axes (Table S3), the WAASB for G2 would be then $WAASB_{G2} = [(0.063 \times 32.49) + (0.103 \times 26.79)] / 59.28 = 0.081.$ This genotype was also classified as the most stable by the ASV (Table S5). Considering two IPCA, WAASB and ASV's ranks matched perfectly (data not shown). We need to be cautious since in our case only 59% of the GEI pattern was explained by the first two IPCA. The extent to which the trial networks and the complexity of GEI increase, the GEI pattern is retained in a larger number of axes, tending to be captured in the last IPCA axes. The G2 still remains a good example here. The score for this genotype on IPCA3 was 0.84, and even though IPCA3 explained only 16.9% of GEI pattern, we need to take it into account; WAASB does that. Based on this difference, for example, we may explain why the association between WAASB and ASV was lower than the WAASB and Za (Fig. 9).

Studies with a relatively low percentage of explanation in IPCA1 were observed in trials with *Brassica* spp. [38.6-54.6% (Bocianowski et al., 2019)], sugarcane [32.9−35% (Ramburan et al. 2011)], maize [26.4% (Balestre et al. 2009); 40.8% (Oyekunle et al. 2017)], and wheat [43.5%, (Bornhofen et al. 2017); 32.1%, (Tigabu et al. 2017); 33.1−38.2% (Veenstra et al., 2019)]; In this sense, biplots with low GEI pattern recovery must be interpreted cautiously in trial networks with prevalence of crossover interaction, since only the simple part of the GEI can be represented in the first main components and the complex part of the GEI may be discarded. For example, Shahriari et al. (2018) identified genotypes of *Plantago* spp. with high mucilage content and high stability by using an AMMI1 biplot with only 36.6% of the GEI pattern explained by that axis. Thus, the use of WAASB x GY (Fig. 5) may be promising in identifying highly productive and broadly adapted genotypes in future studies. Assuming that a specific member of the AMMI family (say AMMI5) is the most predictively accurate model, the Weighted Average of Absolute Scores (WAAS) can also be obtained based on traditional AMMI usage. The estimates are also based on Eq. [10] and may be obtained with the function `waas()` (Supplemental R codes S3.3.1 and S3.3.2).

2.5.4 Weighting between mean performance and stability with WAASBY

Yan and Kang (2003, p 91) have said that "…stability has rarely been used by plant breeders for various reasons. One reason is that it is difficult to weigh between mean performance and stability…". There have been earlier studies that used ranking ASV and mean performance to compute a nonparametric simultaneous selection index (ssiASV) to identify genotypes that combine high performance and stability

(Farshadfar, 2008; Farshadfar et al., 2011; Adjebeng-Danquah et al., 2017; Bocianowski et al., 2019). Simultaneous selection using ssiASV would be promising, provided that the rank for stability −in such studies computed by the ASV− is reliable. We have shown that the ASV's ranking may be misleading if the explanation of GEI pattern in the first two IPCA is low. Thus, we recommend careful when using this index. In a fixed-effects model framework, future studies should then consider using simultaneous selection indexes that are based on significant IPCAs, such as WAASY, ssiZa, ssiEV, and ssiSIPC.

The WAASBY was found to be a useful simultaneous selection index in future analysis of MET under a mixed-effects model framework. This index differs from the "ssis" shown in Fig. 9 in two main ways: The first and logical is that the WAASBY is based on a mixed-effects model −or even on a random-effects− model framework, and may have more reliable results than the ssiASV, for example, since the stability (WAASB) is quantified considering all estimated IPCA. The second −and perhaps the more interesting− difference is that different weights may be assigned to the performance and stability. This is important because depending on the goal of a breeding program or a cultivar recommendation trial, the researcher may want to prioritize the productivity of a genotype rather than its stability (and vice-versa). Thus, Fig. 7 and Fig. 8 should help breeders and agronomists make selection and cultivar recommendation decisions in addition to identifying groups of genotypes with similar mean performance and stability.

2.6 CONCLUSIONS

Based on a cross-validation procedure using four real multi-environment trials (MET) datasets we have shown that the predictive accuracy was higher using BLUP than any member of the AMMI family. We also have shown how nice graphical tools may be obtained to model a random genotype-vs-environment interaction (GEI) effect in the analysis of MET using a linear mixed-effect model (LMM). In our study, we considered a genotypic random effect, but for future studies, the same procedures may be used considering an LMM with random effect for environment or even a completely random-effect model. The genotypic stability index introduced in this article, WAASB, has the potential to provide reliable estimates of stability in future studies allowing a joint interpretation of performance and stability in a bidimensional plot considering two or more IPCA. This was important because the ranking of some genotypes can be mistakenly calculated when only the first IPCA is considered. In addition, our simultaneous selection index, WAASBY, may be useful when different weights should be assigned for performance and stability. Finally, the complete support provided in the **metan** R package will be useful for the reproduction and possible adaptation of all the procedures shown in this article.

2.7 ACKNOWLEDGMENTS

We thank the National Council for Scientific and Technological Development (CNPq) and Coordination for the Improvement of Higher Education Personnel (CAPES) for fellowships and grants to the authors. The first author also thanks Prof. Dr. Maicon Nardino from Fed. University of Viçosa for your numerous helpful suggestions in the planning step of the manuscript. The authors are also thankful to

the anonymous reviewers and the *Agronomy Journal* editorial board for their constructive criticism and valuable suggestions during the peer-review process.

2.8 SUPPLEMENTAL MATERIAL

Supplemental material for this article is available online. It contains six tables, three figures, and a brief introduction to the **metan** R package with the codes and data used to illustrate the method.

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3 ARTIGO II - MEAN PERFORMANCE AND STABILITY IN MULTI-ENVIRONMENT TRIALS II: SELECTION BASED ON MULTIPLE TRAITS

Submetido para o periódico: Agronomy Journal

ISSN: 0002-1962

Qualis Capes na data da publicação: A1

Fator de impacto na data da publicação: 1,897

Situação: Publicado

DOI: 10.2134/agronj2019.03.0221

Material suplementar disponível online em:

https://dl.sciencesocieties.org/publications/aj/supplements/0/agronj2019.03.0221-supplement1.pdf

Páginas: 20

Número de Figuras: 4

Número de Tabelas: 8

Core ideas

- The genotypic stability was quantified in multi-environment trials (MET) using mixed models.
- A superiority index that allows weighting between mean performance and stability was used.
- A multi-trait stability index (MTSI) for identifying superior genotypes in MET was proposed.
- Using a real dataset from a MET, stable and high-performance genotypes were identified.
- The MTSI should facilitate the genotype selection in a multi-trait framework.

Mean performance and stability in multi-environment trials II: Selection based on multiple traits

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Abbreviations: AMMI, Additive Main effects and Multiplicative Interaction; ASV, AMMI stability value; BLUP, Best Linear Unbiased Prediction; GEI, genotype-by-environment interaction; IPCA, interaction principal component axis; LMM, linear mixed-effect

model; MET, multi-environment trials; MPE, mean performance and stability; MTSI, multi-trait stability index; SD, selection differential; SVD, singular value decomposition; WAASB, weighted average of absolute scores from the SVD of the matrix of BLUPs for the GEI effects generated by an LMM; WAASBY, weighted average of WAASB and response variable.

3.1 ABSTRACT

Modeling the genotype-vs-environment interaction (GEI) and quantifying genotypic stability are crucial steps for selecting/recommending genotypes in multi-environment trials (MET). The efficiency in selection/recommendation could be greater if based on multiple traits, but identifying genotypes that combine high performance and stability across many traits has been a difficult task so far. In this study, we propose a multi-trait stability index (MTSI) for simultaneous selection considering mean performance and stability (MPE) in the analysis of MET using both fixed- and mixed-effect models. Data from a MET where 14 traits were assessed in 22 genotypes of *Avena sativa*, L. were used to illustrate the application of the method. The genotypic stability was quantified for each trait using the WAASB index (lower is better). A superiority index, WAASBY (higher is better) was calculated to consider the MPE. The selection differential (SD) for the WAASBY index ranged from 9.7 to 44.6%. Positive SDs (1.75% ≤ SD ≤ 17.8%) were observed for trait means that wanted to increase and negative (−11.7%) for one variable that wanted to reduce. The negative SDs observed for WAASB (−63% ≤ SD ≤ −12%) suggested that the selected genotypes were more stable. The MTSI should be useful for breeders and agronomists who desire a selection for MPE based on multiple traits since it provides a robust and easy-to-handle selection process, accounting for the correlation structure of the traits. The application of the

MTSI in future studies is facilitated by a step-by-step guide and an R package containing useful functions.

3.2 INTRODUCTION

Multi-environment trials (MET) are experiment networks where a set of genotypes are evaluated in a series of environments, which may have a spatial separation (e.g., locations), a temporal separation (e.g., cultivation years) or a combination of these factors (e.g., combination of location and years), aiming at the recommendation of genotypes to specific environments or delineation of mega-environments. It is very common that a relatively large number of genotypes are conducted in each environment, usually in a complete randomized block design (RCBD) with 2−4 replicates (Piepho, 1994). In this context, MET allows identifying genotypes that exhibit a small temporal variability or that are consistent from location-to-location (Yan and Kang, 2003).

When several genotypes are evaluated in multiple environments, in addition to the additive effect of genotype and environment, a third effect arises from the interaction between these factors. This effect is the so-called genotype-environment interaction (GEI). The GEI is related with discrepancy of the genotype's response in each environment. The same way that the response of some genotypes is not the same in all environments, to others the response does not vary. Change in rank order of genotypes across environments is one form of qualitative GEI, but GEI also responds quantitatively from just expansion or contraction of scale over the range of environments without a change in rank order. Therefore, a genotype is regarded as

stable if its response to environments is parallel to the mean response of all genotypes under test (Yan and Kang, 2003).

The desire to properly model the GEI has led to the development of procedures called stability analyzes, whose concepts precede even analysis of variance (Mooers, 1921). Yates and Cochran (1938) introduced the theoretical bases of the joint regression analysis, popularized years later by Finlay and Wilkinson (1963) and Eberhart and Russell (1966). During many years, stability analysis were limited to the regression-based method. Because of the ease of access to personal computers from the late 1970s, more sophisticated methods, which had not been used in practice due to the complexity of matrix operations, were proposed (Gollob, 1968).

Nowadays, the Additive Main Effect and Multiplicative Interaction AMMI (Gauch, 2013) has been widely used in the MET analysis, since it provides more accurate estimates when compared to the traditional ANOVA, besides presenting nice graphical tools for an easy interpretation of the GEI. In the near future, however, methods for MET analysis will depend less on linear-bilinear models –such as AMMI– and more on linear mixed-effects model, LMM (van Eeuwijk et al., 2016). This is because the estimates of genotypic responses obtained by Best Linear Unbiased Prediction (BLUP) are generally more accurate than those obtained by fixed-effect models (Piepho, 1994).

It is very common that stability analysis in MET is performed for a single variable, often the grain yield (e.g., Nowosad et al., 2016, Bornhofen et al., 2018, Mohammadi et al., 2018). Reliability in recommending genotypes, however, could be increased if the mean performance and stability (MPE) of several traits were considered. Recent studies that assessed several variables in MET have been conducted (eg, Adjebeng-Danquah et al., 2017; Nduwumuremyi et al., 2017; Shahriari et al., 2018; Bocianowski

et al., 2019; Koundinya et al., 2019; Veenstra et al., 2019), and the simultaneous selection for stability and mean performance has met with some success when each variable was analyzed individually.

As far as we know, there is no method for MET analysis that combines the simultaneous selection for MPE of several traits into a single and easy-to-interpret index, especially in an LMM framework. Thus, our efforts in this study were focused on to: (i) introduce the theoretical foundations of an index for selecting high-performance and stable genotypes based on multi-trait; (ii) evaluate the applicability of this index in a real dataset from a MET with the white oats (*Avena sativa*, L.) crop; (iii) introduce an R package that facilitates the application of the index by breeders and agronomists in future studies.

3.3 MATERIAL AND METHODS

3.3.1 Plant material, site description, and experimental design

Twenty-two white oat (*Avena sativa* L.) genotypes released by Brazilian breeding programs between 2001-2015 were used as plant material (Supplementary Table S1). The phenology of these cultivars range between 53 to 79 days from sowing to flowering (mean ~67 days), and 94 to 128 days from sowing to maturation (mean ~110 days). The trials were carried out in the experimental area of the Regional Institute of Rural Development, in Augusto Pestana, RS, Brazil (28˚26’30”S, 54˚00’58”W, at 250 masl) during three cultivation years (2015-2017). According to the Köppen’s classification, the climate is *Cfa* (humid subtropical cli- mate), with hot summers and well-distributed rainfall amounts, between 100 and 170 mm monthly (Alvares et al., 2013).

For each year, a randomized complete block design with three replicates was used, totaling 198 plots. Each plot had five 5-m-long cropping rows spaced at 0.18 m. The sowing was carried out in the first week of June using a tractor-seeder and a seeding rate of 300 seeds m^{-2}. 10 Kg ha^{-1} of N, 45 Kg ha^{-1} of P_2O_5, and 30 Kg ha^{-1} of K_2O were applied in basal fertilization. The remainder of the nitrogen was applied in topdressing at GS14 Zadoks' scale (Zadoks et al., 1974)], with a rate for an expected yield of 4 Mg ha^{-1}. Weed control was performed using the herbicide metsulfuron-methyl (2.4 g ha^{-1} AI). Three applications of propiconazole 250 g L^{-1} (0.75 L ha^{-1} commercial product) were performed at 60, 75 and 90 days after sowing to prevent foliar diseases.

3.3.2 Assessed traits

Biweekly evaluations were performed to monitor the progress of the necrotic leaf area in each plot. The first evaluation was performed at 60 days after sowing (DAS) and the last one at 105 DAS, totaling four measurements. Three plants of each plot were randomly collected and taken to the laboratory for analysis. The top three leaves of each plant were scanned and the leaf area necrosed (in percentage) obtained using the ImageJ software. The area under the disease progress curve (AUDPC) was calculated to combine the multiple measurements into a single value, according to the formula described by Jeger and Viljanen-Rollinson (2001).

At the harvest, the average value of 10 panicles randomly selected in each plot was obtained for the following traits: panicle length (PL, cm), panicle mass (PM, g), number of spikelets per panicle (NEP, n); number of grains per panicle (NGP, n), grain weight per panicle (GWP, g). By using the grains harvested in the three central cropping rows of each plot the following traits were assessed: grain yield (GY, kg ha^{-1}),

estimated by adjusting the GY obtained in each plot to GY per hectare; thousand-grain weight (TGW, g), obtained by counting and weighing 250 grains in a precision scale and multiplying the result by four; hectoliter weight (HW, kg hL^{-1}): estimated by the weight ratio of grains in a volume of 250 cm^{-3}; number of grains greater than 2 mm (NG2, n), the number of grains of a sample of 100 grains remaining in a 2 mm sieve; grain weight (GW, g), weight of 50 grains greater than 2 mm; caryopses weight (CW, g), obtained by weighing the caryopses of the 50 hulled grains; hulling index (HI, g g^{-1}), obtained by the equation HI = WC / GW; industrial grain yield (IGY, kg ha^{-1}), obtained by the equation IGY = GY × NG2 × HI.

3.3.3 Statistical analysis

3.3.3.1 BLUP-based genotypic stability

In this study, we used the singular value decomposition (SVD) of the matrix of BLUPs for the GEI effects generated by a linear mixed model (LMM) to quantify the genotypic stability. Briefly, each variable was analyzed using LMM where the GEI effects were assumed to be random and the effects of cultivation year (environment) and of block-within-environment were assumed to be fixed effects, so that

$$\mathbf{y = Xb + Zu + e} \quad [1]$$

where $\mathbf{y}$ is an $n[=\sum_{j=1}^{e}(gb)]\times 1$ vector of observations in the *k*th block of the *i*th genotype in the *j*th year (*i* = 1, 2, ..., *g*; *j* = 1, 2, ..., *e*; *k* = 1, 2, ..., *b*); $\mathbf{b}$ is an $eb\times 1$ vector of fixed effects; $\mathbf{u}$ is an $m[=g+ge]\times 1$ vector of random effects; $\mathbf{X}$ is an $n\times eb$ design matrix relating $\mathbf{y}$ to $\mathbf{b}$; $\mathbf{Z}$ is an $n\times m$ design matrix relating $\mathbf{y}$ to $\mathbf{u}$; and $\mathbf{e}$ is an $n\times 1$ vector of within-group errors.

The vectors **b** and **u** were estimated using the well-known mixed model equation (Henderson, 1975).

$$\begin{bmatrix} \hat{\mathbf{b}} \\ \hat{\mathbf{u}} \end{bmatrix} = \begin{bmatrix} \mathbf{X}'\mathbf{R}^{-1}\mathbf{X} & \mathbf{X}'\mathbf{R}^{-1}\mathbf{Z} \\ \mathbf{Z}'\mathbf{R}^{-1}\mathbf{X} & \mathbf{Z}'\mathbf{R}^{-1}\mathbf{Z} + \mathbf{G}^{-1} \end{bmatrix}^{-} \begin{bmatrix} \mathbf{X}'\mathbf{R}^{-1}\mathbf{y} \\ \mathbf{Z}'\mathbf{R}^{-1}\mathbf{y} \end{bmatrix} \quad [2]$$

The variance component estimates in $\mathbf{G}$ and $\mathbf{R}$ were obtained by Restricted Maximum Likelihood (REML) using the expectation-maximization algorithm (Dempster et al., 1977).

The matrix $\hat{\mathbf{u}}_{ge}$, containing the shrunken effects for the GEI was decomposed as follows

$$\hat{\mathbf{u}}_{ge} = \mathbf{U}_{gp}\mathbf{\Lambda}_{pp}\mathbf{V}_{pe}^{T} \quad [3]$$

where $\mathbf{\Lambda}_{pp}$ is a diagonal matrix with *p* singular values, in decreasing order [$p \leq \min(g - 1; e - 1)$]. The matrices $\mathbf{U}_{gp}$ and $\mathbf{V}_{ep}$ are orthonormal matrices with genotype singular vectors from $\hat{\mathbf{u}}\hat{\mathbf{u}}^{T}$ and environment singular vectors from $\hat{\mathbf{u}}^{T}\hat{\mathbf{u}}$, respectively. The genotypic stability of each genotype was quantified by the WAASB index, acronym for **W**eighted **A**verage of **A**bsolute **S**cores from the SDV of the matrix of **B**LUPs for the GEI effects, estimated as follows:

$$WAASB_i = \sum_{k=1}^{p} \left| IPCA_{ik} \times EP_k \right| \Big/ \sum_{k=1}^{p} EP_k \quad [4]$$

where $WAASB_i$ is the weighted average of absolute scores of the *i*th genotype; $IPCA_{ik}$ is the score of the *i*th genotype in the *k*th IPCA, and EP_k is the amount of the variance explained by the *k*th IPCA. The genotype with the lowest WAASB value is considered the most stable (Companion paper, 2019)

3.3.3.2 Simultaneous selection for performance and stability

The simultaneous selection for MPE was performed by using the WAASBY index, which allows weighting between mean performance (Y) and stability (WAASB), as follows:

$$WAASBY_i = \frac{(rY_i \times \theta_Y) + (rW_i \times \theta_S)}{\theta_Y + \theta_S} \quad [5]$$

Where $WAASBY_i$ is the simultaneous selection index for the *i*th genotype that weights between MPE; θ_Y e θ_S are the weights for the response variable and the WAASB, assumed to be 65% e 35%, respectively. We chose these weights to illustrate the selection prioritizing the mean performance rather than the stability of the genotypes. [To see how the ranks of genotypes changes regarding the weights, see the companion paper (Olivoto et al., 2019)]. rY_i and rW_i are the rescaled values (0−100) for the response variable and WAASB, respectively, estimated as follows:

$$rY_i = rW_i = \frac{nma - nmi}{oma - omi} \times (o_i - oma) + nma \quad [6]$$

where *nma* and *nmi* are the new maximum and minimum values after rescaling; *oma* and *omi* are the original maximum and minimum values, and o_i is the original value for the response variable or the WAASB index of the *i*th genotype. The values for *nma* and *nmi* were chosen according to the variable. Except for AUDPC, higher values are desired; thus *nma* = 100 and *nmi* = 0. In other words, the genotype with the higher mean had rY_i = 100 after rescaling. For AUDPC −and WAASB− where lower values are desired, we used *nma* = 0 e *nmi* = 100. Thus, a two-way table containing the WAASBY values for each genotype and trait was obtained. The codes for this procedure are in Supplementary material S1.2.

3.3.3.3 Multi-trait stability index based on factor analysis

The selection for MPE considering multi-trait was based on the genotype-ideotype distance (Euclidian) using the scores obtained in an exploratory factor analysis as follows:

$$\mathbf{X} = \boldsymbol{\mu} + \mathbf{L}\mathbf{f} + \boldsymbol{\varepsilon} \quad [7]$$

where **X** is a *p*×1 vector of observations; **μ** is a *p*×1 vector of means; **L** is a *p*×*f* matrix of factorial loadings; **f** is a *p*×1 vector of common factors; and **ε** is a *p*×1 vector of residuals, being *p* and *f* the number of traits and common factors retained, respectively. The eigenvalues and eigenvectors were obtained from the correlation matrix of the two-way table described above. The initial loadings were obtained considering only factors with eigenvalues higher than one. The *varimax* rotation criteria was used for the analytic rotation and estimation of final loadings. The scores for the genotypes were obtained according to the following equation.

$$\mathbf{F} = \mathbf{Z}(\mathbf{A}^{\mathbf{T}}\mathbf{R}^{-1})^{\mathbf{T}} \quad [8]$$

where **F** is a *g*×*f* matrix with the factorial scores being *g* the number of genotypes and *f* the number of factors; **Z** is a *g*×*p* matrix with the standardized means (WAASBY means); **A** is a *p*×*f* matrix of canonical loadings, and **R** is a *p*×*p* correlation matrix between the variables.

The second step was the ideotype planning. By definition (Eq. [5]), the ideotype has the highest WAASBY (100) for all analyzed variables. Thus, the ideotype was defined by a 1×*p* vector **I** such that **I** = [100, 100, ..., 100]. The scores of the ideotype were also estimated according to Eq. [8]. The third and last step was the estimation of the multi-trait stability index (MTSI), according to the following equation.

$$MTSI_i = \left[\sum_{j=1}^{f}\left(F_{ij} - F_j\right)^2\right]^{0.5} \quad [9]$$

where the MTSI is the multi-trait stability index for the *i*th genotype; F_{ij} is the *j*th score of the *i*th genotype, and F_j is the *j*th score of ideotype. The genotype with the lowest MTSI is then closer to the ideotype and therefore presents high MPE for all analyzed variables.

The selection differential (SD) for mean performance and both WAASB and WAASBY index was calculated for each trait considering a selection intensity of 15%. To assist with an intuitive interpretation, graphics showing the mean and biplots for response variable *vs* WAASB were created for GY, AUDPC, and IGY. The codes in S1.3 may be used to reproduce the examples of this section.

3.4 RESULTS

3.4.1 Overall performance, likelihood ratio tests and variance components

According to the likelihood ratio test, the genotype effect was significant for the TGW only. On the other hand, the GEI effect was significant for all traits except for TGW. The environment effect was highly significant for all variables except for HI (Supplementary Table S2). The overall mean of GY was 3349±113.4 Kg ha^{-1} whereas the grand mean for IGY was 1402±55.63 Kg ha^{-1}. In 2016 was observed the higher oat yield (4331 Kg ha^{-1}), while 2017 was the less productive year (2310 Kg ha^{-1}), mainly due to the high disease incidence in this year (Supplementary Table S2, and Fig. S1). For all traits (except for TGW), the GEI variance ($\hat{\sigma}^2_{ge}$) was higher than genotypic ($\hat{\sigma}^2_g$) and residual ($\hat{\sigma}^2_e$) variance and, therefore, GEI variance is the more important component of the phenotypic variance (Fig. 1).

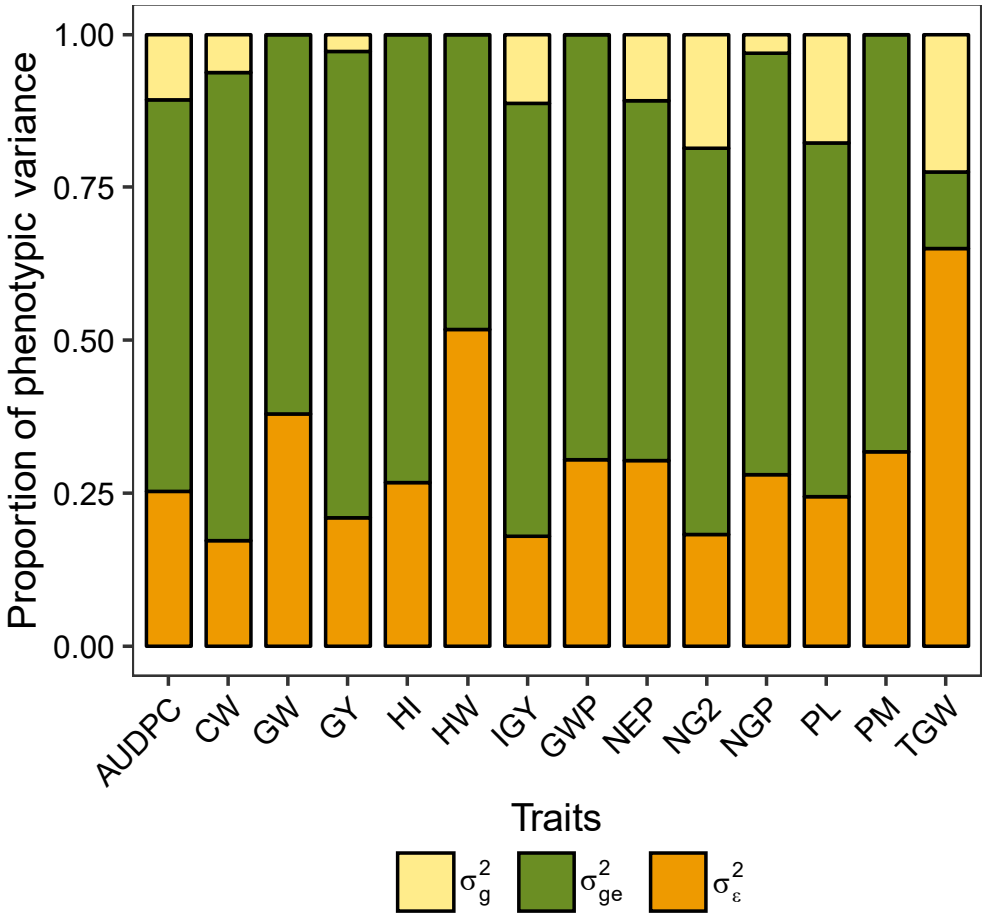

Figure 1. Proportion of the phenotypic variance for fourteen oat traits evaluated during three cultivation years. AUDPC, area under the disease progress curve; index; CW, caryopses weight; GW, grain weight; GY, grain yield; HI, hulling; HW, hectoliter weight; IGY, industrial grain yield; GWP, grain weight per panicle; NEP, number of spikelets per panicle; NG2, number of grains greater than 2 mm; NGP, number of grains per panicle; PL, panicle length; PM; panicle mass; TGW, thousand-grain weight.

3.4.2 Linear relationships

Pearson's correlation matrix for the WAASBY values is presented in Supplementary Fig. S2. High-magnitude correlations were observed among PL, PM, NEP, NGP, and GMP, suggesting that these traits may be grouped in a common factor. A positive association between GY and ADUPC was observed, which is explained due to *nma* and *nmi* value used for rescaling AUDPC. For this variable, genotypes with higher WAASBY present, in fact, lowest AUDPC and consequently higher GY.

3.4.3 Exploratory factor analysis

3.4.3.1 Loadings and factor delineation

Five principal components were retained, and the accumulated variance in these components was 83.1% (Table 1). After *varimax* rotation the average communality (*h*) was 0.83 (TGW 0.55 ≤ *h* ≤ 0.95 GWP. The values of WAASBY in each one of the 14 traits were grouped in the five factors (FA) as follows: FA1, PM, NEP, NGP, and GWP; FA2, the traits related to grain density TGW, HW, and GW; FA3, the traits related to industrial yield NG2 and IGY; FA4, the traits CW and HI; and FA5, the traits GY, PL and AUDPC (Table 1).

Table 1. Eigenvalues, explained variance, factorial loadings after varimax rotation and communalities obtained in the factor analysis

Trait†	**FA1‡**	**FA2**	**FA3**	**FA4**	**FA5**	***h*§**
GY	−0.162	−0.332	−0.009	−0.138	**0.878**	0.926
TGW	−0.212	**−0.630**	−0.270	−0.010	0.194	0.553
HW	0.091	**−0.777**	0.247	0.145	0.207	0.736
PL	0.453	−0.124	0.197	−0.096	**−0.595**	0.623
PM	**0.948¶**	−0.039	0.000	0.177	−0.069	0.936
NEP	**0.901**	0.128	0.145	0.021	−0.260	0.918
NGP	**0.936**	0.139	0.107	0.064	−0.082	0.917
GWP	**0.945**	−0.019	0.021	0.203	−0.130	0.952
AUDPC	−0.132	0.145	−0.332	−0.489	**0.522**	0.660
NG2	−0.029	−0.110	**−0.955**	0.084	−0.021	0.933
GW	−0.027	**−0.835**	−0.165	−0.209	−0.164	0.796
CW	−0.077	−0.592	−0.123	**−0.745**	−0.104	0.938
HI	−0.212	0.024	0.015	**−0.868**	0.126	0.815
IGY	−0.180	−0.065	**−0.839**	−0.292	0.321	0.929
Eigenvalues††	5.03	2.37	1.73	1.34	1.16	−
Variance††	35.95	16.90	12.35	9.56	8.32	−
Accumulated (%)††	35.95	52.85	65.20	74.77	83.09	−

† GY, grain yield; TGW, thousand-grain weight; HW, hectoliter weight; PL, panicle length; PM; panicle mass; NEP, number of spiklets per panicle; NGP, number of grains per panicle; GWP, grain weight per panicle; AUDPC, area under the disease progress curve; NG2, number of grains greater than 2 mm; GW, grain weight; CW, caryopses weight; HI, hulling index; IGY, industrial grain yield.
‡ FA, Factor retained
§ *h*, Communality
¶ Bold values indicate the variables grouped within each factor.
†† The values for all factors are in Supplementary table S3.

3.4.3.2 Multi-trait stability index and genotype selection

Figure 2 shows the genotype ranking for the MTSI. Considering the selection intensity of 15%, the three genotypes selected were G16, G1, and G15 (MTSI = 3.61, 3.99, and 4.27, respectively). The MTSI of 4.27 shows the cut point (red circle) considering the selection intensity. The genotype G11 was near to this circle and could present interesting features. Thus, in future studies it would be interesting to investigate the performance of the genotypes that are very close to the cutpoint.

The contribution of each factor in the MTSI indicated that ~54% of the distance from G1 to the ideotype was related to FA2 (Supplementary tables S4 and S5). In other words, G1 had lower WAASBY for the traits TGW, HW, and GW. Regarding the G16, most of the MTSI was due to the FA1 (~52%) and FA4 (~31%). In practice, this means that a breeding program should aim at improving the ear-related traits PM, NEP, NGP, and GWP (FA1) as well as the traits CW and HI (FA4), which are related to the industrial quality. For the genotype G15, on the other hand, the FA3 had the lowest contribution to the MTSI (0.017%). This means that G5 is close to the ideotype regarding the traits NG2 and IGY, important traits for industrial oat yields.

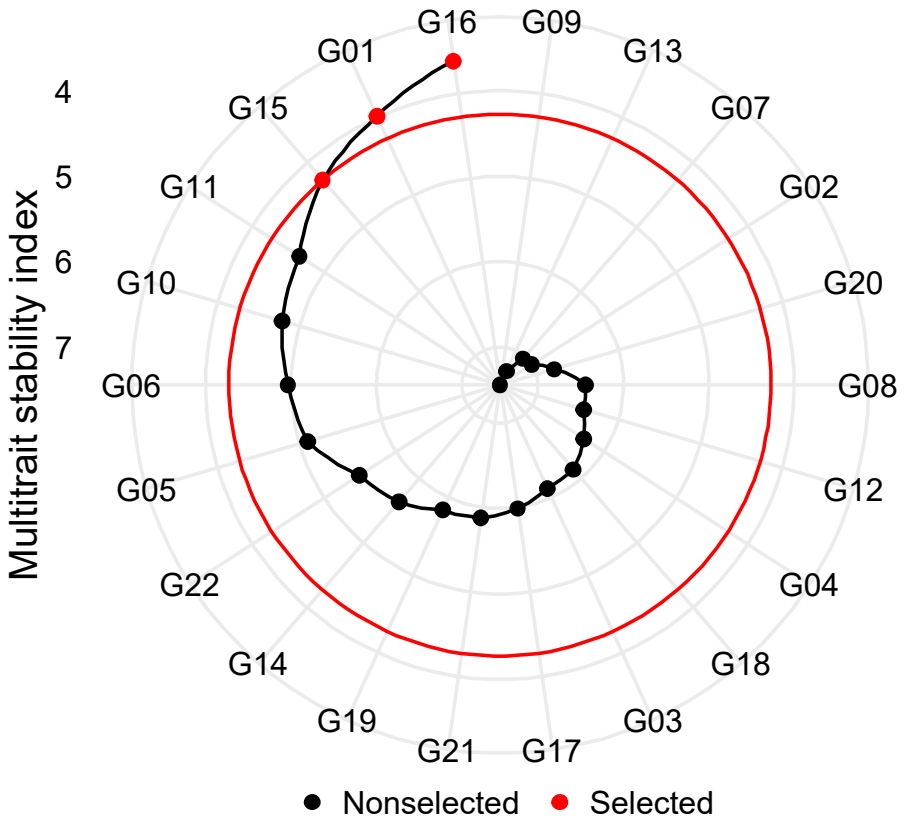

Figure 2. Genotype ranking and selected genotypes for the multi-trait stability index considering a selection intensity of 15%. G01, Barbarasul; G02, Brisasul; G03, FAEM 006; G04, FAEM 007; G05, FAEM Carlasul; G06, FAEM Chiarasul; G07, IPR Afrodite; G08, UPFA Gaudéria; G09, UPFA Ouro; G10, UPFPS Farroupilha; G11, URS 21; G12, URS Altiva; G13, URS Brava; G14, URS Charrua; G15, URS Corona; G16, URS Estampa; G17, URS Fapa Slava; G18, URS Guará; G19, URS Guria; G20, URS Tarimba; G21, URS Taura; G22, URS Torena.

The SD for the WAASBY index was positive for all traits, suggesting that the method was efficient in selecting the best performing and stable genotypes. The mean SD for the WAASBY index was 25.1%, being the lowest one (9.68%) for the GY and the highest one (44.6%) for the CW (Table 2).

Table 2. Selection differential of the WAASBY index for fourteen oat traits

Factor	Trait†	Xo‡	Xs§	Selection differential (%)
FA1	PM	59.46	74.89	15.44 (25.96)
	NEP	55.10	65.61	10.51 (19.08)
	NGP	64.46	77.48	13.02 (20.20)
	GWP	59.88	74.00	14.12 (23.58)
FA2	TGW	56.93	70.18	13.25 (23.27)
	HW	55.03	61.12	6.08 (11.05)
	GW	57.49	72.87	15.38 (26.75)
FA3	NG2	58.66	77.14	18.48 (31.50)
	IGY	47.80	64.30	16.49 (34.50)
FA4	CW	44.54	64.43	19.89 (44.66)
	HI	51.64	68.15	16.51 (31.97)
FA5	GY	49.53	54.33	4.80 (9.68)
	PL	51.22	59.69	8.47 (16.53)
	AUDPC	57.45	76.28	18.83 (32.77)
Mean	-	54.94	68.61	13.66 (25.11)

† PM; panicle mass; NEP, number of spikelets per panicle; NGP, number of grains per panicle; GWP, grain weight per panicle; TGW, thousand-grain weight; HW, hectoliter weight; GW, grain weight; NG2, number of grains greater than 2 mm; IGY, industrial grain yield; CW, caryopses weight; HI, hulling; GY, grain yield; PL, panicle length; AUDPC, area under the disease progress curve; index.
‡ Xo, Mean for WAASBY index of the original population
§ Xs, Mean for WAASBY index of the selected genotypes (G01, G15 and G16).

3.4.3.3 Mean performance and stability of selected genotypes

The joint interpretation for MPE regarding the GY, AUDPC, and IGY is presented in Fig. 3. Unlike the well-known AMMI1 biplot, the ordinates (WAASB) quantify the stability considering all possible IPCA (Eq. [4]). Genotypes within quadrants I and II are assumed to be unstable. Genotypes within quadrant IV −for GY and IGY−, and within quadrant III −for AUDPC− are assumed to be desirable because they present desirable mean and lesser variation from environment-to-environment, which is explained by the WAASB values. The same plot for all variables is shown in Fig. S3.

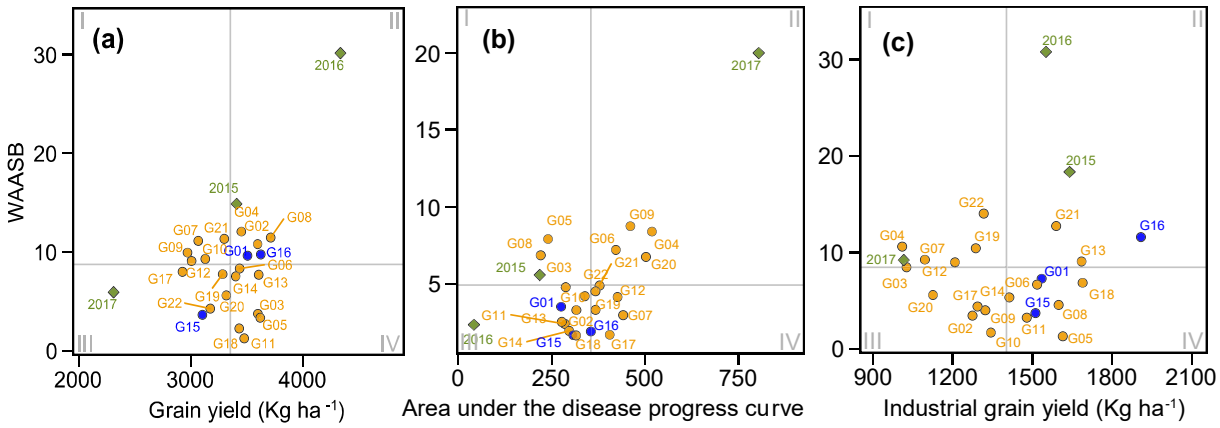

Figure 3. Joint interpretation for mean performance and stability for grain yield (a), area under the disease progress curve (b) and industrial grain yield (c). X axis shows the arithmetic mean for each genotype/environment. In the online version of the manuscript, environments are depicted by dark green squares and selected genotypes by blue circles.

The IGY of the selected genotypes was 1652.1 kg ha^{-1} (17.8% higher than the grand mean). In addition, an SD of −11.7% for AUDPC and 1.8% for GY was observed (Fig. 4). Thus, the selected genotypes such as G1 (third most resistant genotype) may serve as genitor in future breeding programs aiming at the development of oat cultivars tolerant to the main diseases. The lower SD for GY is compensated by the higher IGY, which was resultant from the higher NG2 and HI of the selected genotypes (Supplementary Fig. S4). For most of the other analyzed traits, positive SDs (1.75% ≤ SD ≤ 17.8%) were observed. Regarding the WAASB, negative SDs were observed for all traits (−63.9% ≤ SD ≤ −12.3%), which indicates that the selected genotypes were considerably more stable (Supplementary Table S6).

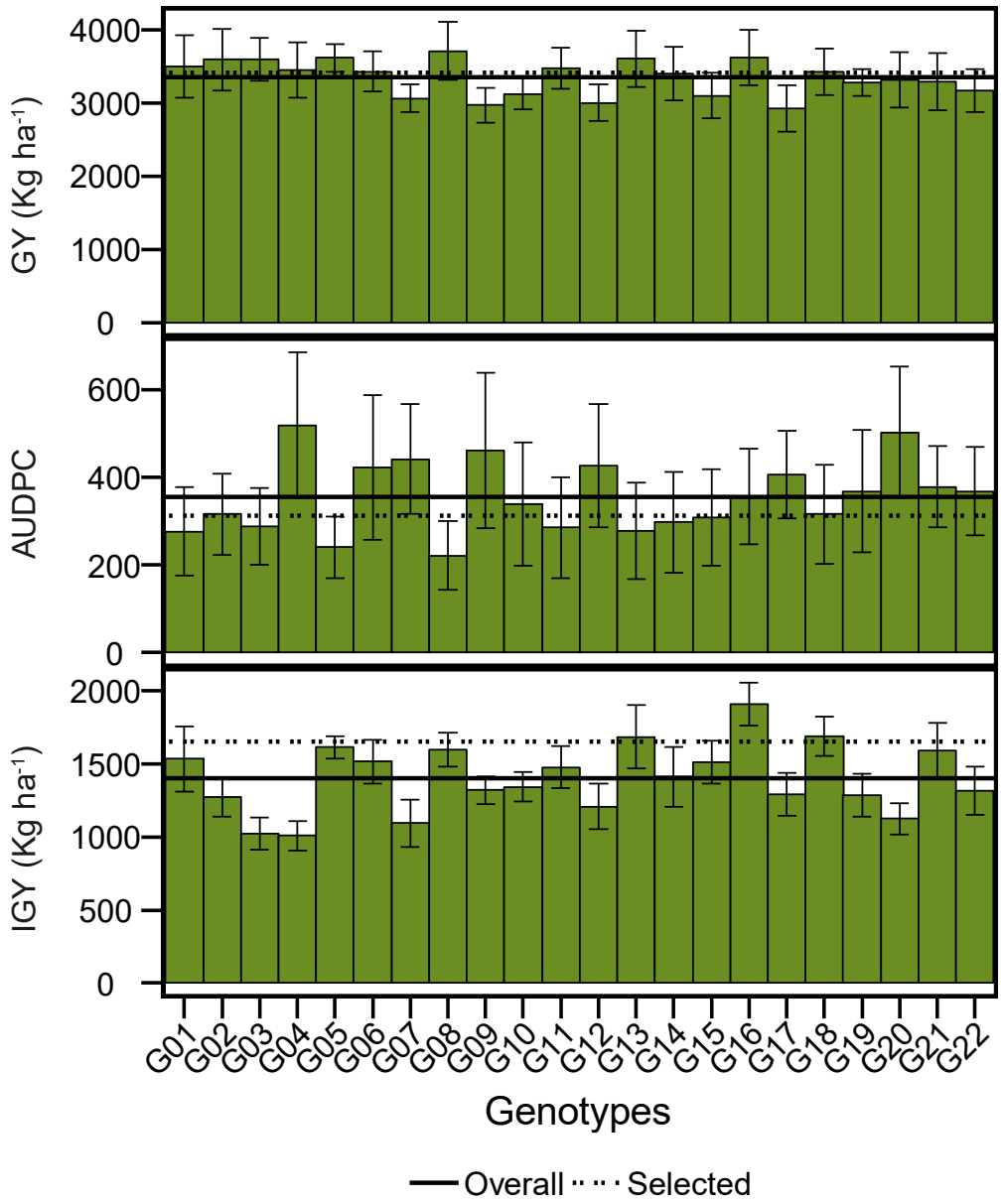

Figure 4. Observed values for grain yield, area under the disease progress curve, and industrial grain yield of 22 oat cultivars evaluated during three cultivation years. Horizontal solid lines represent the grand mean whereas dashed lines represent the mean of the selected genotypes. Bars represents means±SE with *n* = 9.

3.5 DISCUSSION

3.5.1 Quantifying the stability using linear mixed-effect models

Quantifying stability is fundamental to the development of genotype selection/recommendation strategies. This has become an increasingly common practice in MET analysis. In the context of models with multiplicative terms, the AMMI

stability value (ASV) has been used for this purpose (Purchase et al., 2000; Adjebeng-Danquah et al., 2017; Shahriari et al., 2018; Bocianowski et al., 2019, Koundinya et al., 2019). In this study, we have shown how genotypic stability can be quantified in the MET analysis using WAASB (Eq. [4]), which may be seen as a mixed model version of the ASV. In addition, different effects (environment as random or a random model) may be used for WAASB estimation as shown in Fig. 5.

In our study, two IPCA were used for WAASB estimation [min(22−1; 3−1)]; thus, the ranks for GY, AUDPC, and IGY obtained with the ASV and WAASB were highly and positively correlated (Supplementary Table S7). The extent to which the MET and the complexity of the GEI increase, the GEI pattern in AMMI analysis is retained in a larger number of IPCAs, tending to be captured in the last IPCAs. This results in a lower percentage of GEI explained in the first two IPCAs [(60-69% (Veenstra et al., 2019), 65.7% (Bocianowski et al., 2019) and 68% (Liang et al., 2015)], and may, for example, compromise the interpretation of the ASV. Thus, the WAASB index may be promising for quantifying genotypic stability in future studies.

3.5.2 Simultaneous selection for performance and stability

Successful selection of high-performance and stable varieties is fundamental to breeding programs. Non-parametric methods have been proposed (Kang, 1988; Lin and Binns, 1988) and used for this purpose, but no method developed so far in the context of mixed models has been universally adopted. More recently, the genotype stability index (GSI) proposed by Farshadfar, (2008) has been used in the simultaneous selection for MPE in AMMI analysis (Adjebeng-Danquah et al., 2017;

Bocianowski et al., 2019). Due to the increasing use of this index, a brief comparison with the WAASBY index is presented.

Briefly, the GSI is computed by summation of the ranks for ASV (rASV) and response variable (rY), GSI = rASV + rY. More details may be seen in Companion paper (2019). Lower GSI values are desirable. Although it is an index of easy interpretation, its ambiguity can lead to misunderstandings in genotype selection/recommendation. Let us consider two brief examples to make this concept a bit clearer.

The first example concerns the IGY of genotypes G4, G12, G19 and G22. The same value of GSI (36) was observed for these genotypes (Supplementary Table S8). These same genotypes were in quadrant I of Fig. 3c, characterizing them as poorly productive and highly unstable. Many researchers may not realize, but GSI = 36 may be the result of, for example, 36 = 14 + 22 or 36 = 22 + 14. In other words, genotypes with distinct patterns for MPE are assumed to be similar. Thus, we should keep in mind that the recommendation of a stable (but low-performance) genotype is completely different from the recommendation of a genotype that performs well in one environment but not in others. In the latter, the recommendation for specific environments should be explored. Considering the WAASBY index, the ranking of these genotypes ranged from 17th (G19) to 22th (G4), which is mainly explained by the difference in IGY of that genotypes (Supplementary table S8; Fig 3c).

The second example still remains concerning the IGY, but now of the G16. According to the GSI, the G16 would be the ninth-ranked (Supplementary Table S8). This genotype showed the third largest value of WAASB (in other words, the third less stable) but ranked second for the WAASBY index. Why? There are two main reasons for this: The first is clearly the highest IGY of G16 (Fig. 3c). The second one is the

highest weight assigned to the response variable (in our case 65%). It should be noted that this genotype was selected by the MTSI because it presented GY above the grand mean, AUDPC below the grand mean (Fig. 4) and performed well for important traits such as TWG, GW, CW and NG2 (Supplementary Fig. S4).

We have shown here how the simultaneous selection for MPE considering an LMM may be performed using the WAASBY index. Compared with already used indexes, the WAASBY is not ambiguous and weights can be used when the selection of genotypes should prioritize the mean performance over the stability or vice versa. In future studies, these weights should be chosen according to the purpose of the selection.

3.5.3 The theoretical basis and applicability of the MTSI index

3.5.3.1 Ideotyping procedure

It was shown that in the context of simultaneous selection for MPE considering several variables, the ideotype is assumed to have the maximum value of WAASBY (100) in all variables. In future studies, it will be up to the researcher to define the values of *nma* and *nmi* in Eq. [7] for rescaling the variables, as well as the weights for MPE. In our example, we considered a weight of 65% for mean performance in all variables. It should be emphasized that these weights could be changed across traits in the way the researcher wants. A brief code example is given in supplemental material S1.5.

3.5.3.2 Accounting for the correlation structure

The hypothesis is that in a multi-trait framework, the WAASBY values may be related in some way due to an underlying correlation structure that is unknown beforehand. Thus, the EFA was used to account for this correlation structure. Using the EFA, it was possible to determine how many factors exist −in other words, in how many latent variables the original set of variables could be reduced−, the relationship between the factors and how the variables were associated with these factors (Ullman, 2006). Finally, the estimation of final factor scores allowed dealing with the multicollinearity, a systemic issue in multivariate analyses (Olivoto et al., 2017), incorporating in the new first latent variables the original structure of the data, thus leading to dimensional reduction.

3.5.3.3 The genotype-ideotype distance as a selection criterion

The MTSI (Fig. 2) allowed a unique and easy-to-interpret selection process. In addition, the MTSI was found to have many practical applications by breeders and agronomists who desire the simultaneous selection for MPE when data of several traits are available. For example, it could have been useful for already published studies that evaluated the stability and mean performance of genotypes considering several traits [e.g., Koundinya et al., (2019), six traits evaluated in *Solanum melongena* genotypes; Nduwumuremyi et al. (2017), nine traits evaluated in *Manihot esculenta* genotypes; and Bocianowski et al. (2019), five traits evaluated in *Brassica* spp. lines].

3.5.3.4 A step-by-step guide for future studies

In the near future, methods for GEI analysis in MET will rely less on linear-bilinear models and more on mixed-effect models (van Eeuwijk et al., 2016). This is mainly due to the higher predictive ability of BLUP, which often outperforms known models such as AMMI (Piepho, 1994), and the rapid dissemination of statistical packages that include specialized routines for mixed-effect model procedures. In this context, the use of MTSI should become broad. To assist with easy and correct implementation of the MTSI in future studies we provide a workflow (Fig. 5) suggesting the steps to be followed in the context of both mixed- and fixed-effect models. The thicker line represents the steps we followed in this study. Note that depending upon the nature of the model the first step is to choose the proper function. If more than one trait is assessed in the experiment, the MTSI may be computed, considering the stability only, or the selection based on mean performance and stability. The R codes provided in the supplementary material S1 may be used and adapted for specific cases.

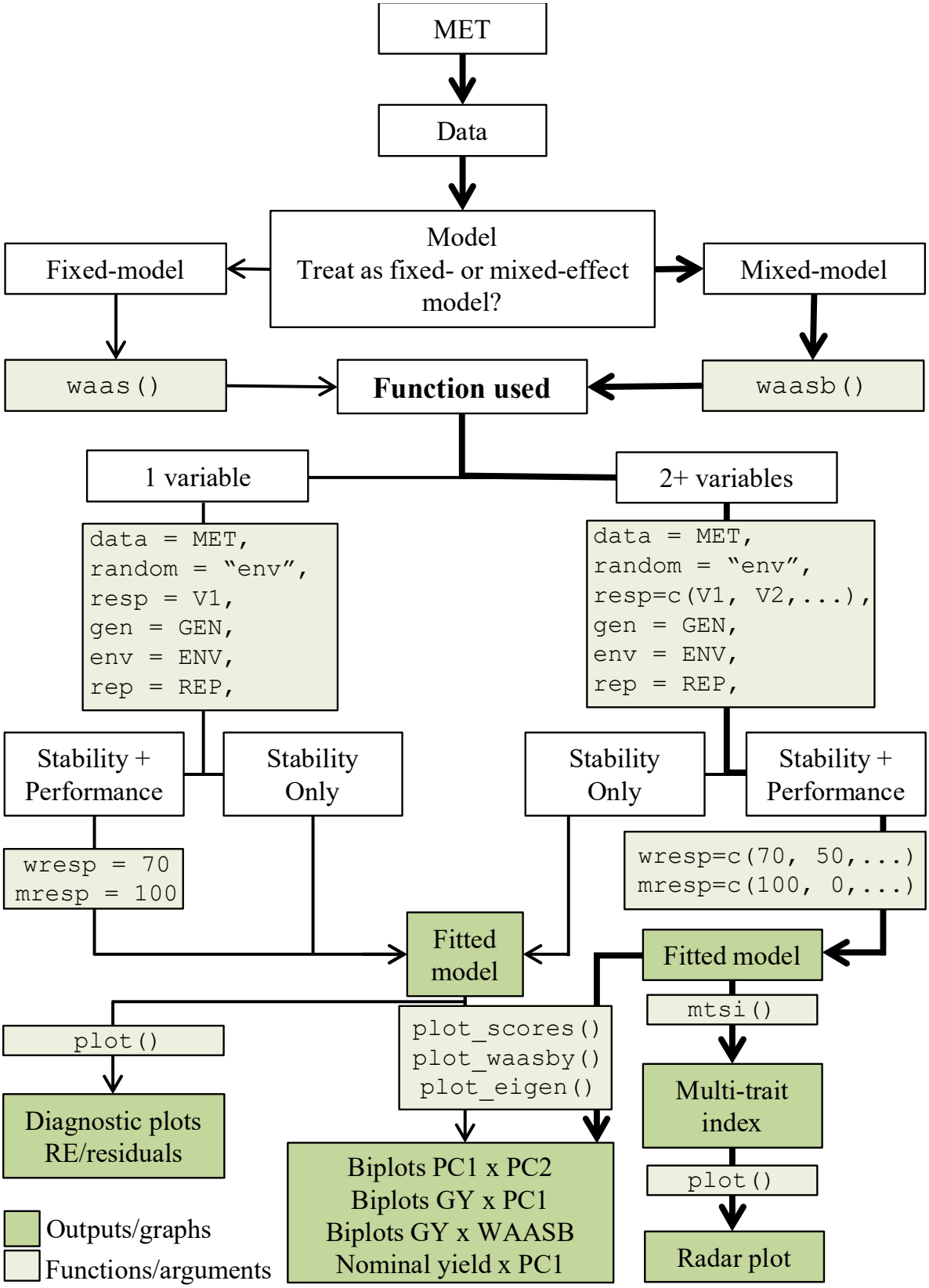

Figure 5. Suggested workflow for simultaneous selection for mean performance and stability in the analysis of multi-environment trials. The thicker line represents the steps that were followed in this article. The first option is choosing between fixed- and mixed-effect models. If more than one variable is available, the multi-trait stability index (MTSI) may be estimated. Shaded areas represent the functions/arguments that should be set to achieve the outputs, which are depicted by olive green rectangles in the online version of the manuscript.

3.6 CONCLUSIONS

In this study, we introduced the theoretical foundations of a multi-trait stability index (MTSI) for selecting high-performance and stable genotypes in multi-environment trials (MET) based on multiple traits considering both a fixed- or mixed-effect model framework. The MTSI is based on the genotype-ideotype distance estimated with scores of factor analysis. The application of the MTSI was demonstrated using real data from 14 traits assessed in a MET with 22 oat genotypes. The MTSI allowed the selection of stable genotypes, with positive selection differentials for traits that wanted to increase and negative selection differential for one trait that wanted to decrease. This suggests that the MTSI should be useful for breeders and agronomists who aim at the simultaneous selection for mean performance and stability considering several traits since it provides a unique selection process that is easy-to-interpret and considers the correlation structure among the traits. Finally, the application of the MTSI in future studies is facilitated by a step-by-step guide (Fig. 5) and the introduction of an R package containing all the required functions.

3.7 ACKNOWLEDGMENTS

We thank the National Council for Scientific and Technological Development (CNPq) and Coordination for the Improvement of Higher Education Personnel (CAPES) for fellowships and grants to the authors. The authors are also thankful to the anonymous reviewers and the *Agronomy Journal* editorial board for their constructive criticism and valuable suggestions during the peer-review process.

3.8 SUPPLEMENTAL MATERIAL

Supplemental material for this article is available online. It contains eight tables, four figures, and a brief introduction to the **metan** R package with the codes and data used to illustrate the method.

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4 ARTIGO III - METAN: AN R PACKAGE FOR MULTI-ENVIRONMENT TRIAL ANALYSIS

Submetido para o periódico: Methods in Ecology and Evolution

ISSN: 2041-210X

Qualis Capes na data de defesa da tese: A1

Fator de impacto na data de defesa da tese: 6,363

Situação: Em processo de revisão por pares

`metan`: an r package for multi-environment trial analysis

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Type: Application

Running headline: analysis of multi-environment trials

4.1 ABSTRACT

1. Multi-environment trials (MET) are crucial steps in plant breeding programs that aim increasing crop productivity to ensure global food security. The analysis of MET data requires the combination of several approaches including data manipulation, visualization, and modeling. As new methods are proposed, analyzing MET data correctly and completely remains a challenge, often intractable with existing tools.
2. Here we describe the `metan` R package, a collection of functions that implement a workflow-based approach to (a) check, manipulate and summarise typical MET data; (b) analyze individual environments using both fixed and mixed-effect models; (c) compute parametric and non-parametric stability statistics; (c) implement biometrical models widely used in MET analysis; and (d) plot typical MET data quickly.

3. In this paper, we present a summary of the functions implemented in `metan` and how they integrate into a workflow to explore and analyze MET data. We guide the user along a gentle learning curve and show how adding only a few commands or options at a time, powerfull analyzes can be implemented.
4. `metan` offers a flexible, intuitive, and richly documented working environment with tools that will facilitate the implementation of a complete analysis of MET data sets.

Key-words: AMMI, biometry, genotype--environment interaction, GGE biplot, multi-environment trials, R software, stability, statistics

4.2 INTRODUCTION

In 50 years (1967-2017) the world average of cereal yields has increased by 64%, from 1.68 to 2.76 t ha^{-1}. In the same period, the total production of cereals has raised from 1.305×10^9 to 3.6×10^9 t, an increase of 175%, while the cultivated area increased by only 7.9% in the same period (FAOSTAT, 2019). These unparallel increases have been possible due to the improved cultivation techniques in combination with superior cultivars. For maize, for example, 50% of the increase in yield was due to breeding (Duvick, 2005). Plant breeding programs have been developing new cultivars for adaptation to new locations, management practices, or growing conditions, in a clear and crucial example of exploitation of genotype-vs-environment interaction (GEI).

The breeders' desire to modeling the GEI appropriately has led to the development of the so-called stability analysis, which includes ANOVA-based methods (Yates & Cochran, 1938; Wricke, 1965; Shukla, 1972; Annicchiarico, 1992) regression-

based methods (Eberhart & Russell, 1966); non-parametric methods (Huehn, 1979; Lin & Binns, 1988; Fox, Skovmand, Thompson, Braun, & Cormier, 1990; Thennarasu, 1995) and some methods that combines different statistical techniques, such as the Additive Main Effect and Multiplicative Interaction, AMMI (Gauch, 2013), and Genotype plus Genotype-vs-Environment interaction, GGE (Yan & Kang, 2003). Then, it is no surprise that scientific production related to multi-environment trial analysis has been growing fast in the last decades. A bibliometric survey in the SCOPUS database revealed that in the last half-century (1969−2019) 6590 documents were published in 902 sources (Journals, books, etc.) by 19.351 authors. In this period, the number of publications has been increased on average by 11.22% $year^{-1}$ but were in the last ten years that the largest amount (~64%) of the documents were published (See Appendix S1, item 1 for more details).

Linear Mixed-effect Models (LMM) has been more frequently used to analyze MET data. For example, between 2013 and 2015, the larger number of papers proposing methods to deal with GEI were related to the Best Linear Unbiased Prediction (BLUP) in LMMs (van Eeuwijk, Bustos-Korts, & Malosetti, 2016). Recent advances in this field showed that BLUP is more predictively accurate than AMMI and that the main advantages of these methods can be combined to help researchers to select or recommend stable and high productive genotypes (Olivoto, Lúcio, da silva, Marchioro, et al., 2019). Thus, the rapid spread of these methods to users around the world can be facilitated if these procedures are implemented in specific software.

In most cases, analyzing MET data involves manual checking of the data subset(s) to identify possible outliers, using some biometrical model to explore the relationships between traits(or groups of traits), computing a within-environment ANOVA, computing a joint-ANOVA, and, in case of a significant GEI, applying some

stability method to explore it. While a spreadsheet program (e.g. Microsoft Excel) may be used to perform a visual check for outliers, an integrated development environment (IDE, e.g. R, SAS, or Matlab) is often required to process the complex matrix operations required in some stability methods. IDEs, however, require a certain degree of expertise to use and have steep learning curves, which sometimes prevents that a coding layman implements certain methods. In this sense, R (Team, 2019) packages have been making easier the life of hundreds of thousands of researchers by providing freely collections of functions developed by the community.

Some open-source R software packages that are designed –or are suitable– for analyzing MET data are available. The `stability` package (https://CRAN.R-project.org/package=stability) contains a collection of functions to perform stability analysis. The `ammistability` package (https://CRAN.R-project.org/package=ammistability) computes multiple AMMI-based stability parameters. The `gge` (https://CRAN.R-project.org/package=gge) and `GGEBiplots` (https://CRAN.R-project.org/package=GGEBiplots) packages may be used to perform a GGE model. The R packages `agricolae` (https://CRAN.R-project.org/package=agricolae) and `plantbreeding` (http://plantbreeding.r-forge.r-project.org/), while not specifically coded for MET analysis provides useful functions for computing parametric and nonparametric stability statistics. Although useful, these packages do not offer options to perform a complete analysis of MET data, i.e., to provide tools for all steps of the analysis (check, manipulation, analysis, and visualization of data). For example, `GGEBiplots` requires as input data a two-way table containing genotype by environment means with genotypes in rows and environments in columns, but doesn't provide any function to create quickly such table from data that often is in a *"long"* format in R. In addition, several studies often compare

different stability methods (e.g., Scapim et al., 2010; Bornhofen et al., 2017; Freiria et al., 2018; Woyann et al., 2018; Shahbazi, 2019; Teodoro et al., 2019). This requires a range of different packages to be used, making it the coding tedious and difficult to follow. Thus, it seems to be value the creation of an R package that presents an easy workflow, and incorporates the most used stability statistics, as well as recent introduced stability methods (Olivoto, Lúcio, da silva, Marchioro, et al., 2019; Olivoto, Lúcio, da silva, Sari, & Diel, 2019) in addition to options for computing cross-validation (Piepho, 1994) and BLUP-based stability statistics (Colombari Filho et al., 2013), features frequently used but not yet implemented in any other R package for MET analysis.

Here, we describe the `metan` (**m**ulti-**e**nvironment **t**rial **an**alysis) package, an open-source R package designed to provide an efficient and reproducible workflow for the analysis of MET data. Our main aim in this paper is to describe the features of `metan` and how this collection of functions can be useful for an intuitive and complete analysis of MET data.

4.3 THE METAN PACKAGE

The conceptual focus of `metan` is centered on five components (Fig. 1): (a) check, manipulate and summarise typical MET data; (b) performs within-environment analysis of variance; (c) compute parametric and non-parametric stability analysis; (d) compute biometrical models widely used in plant MET analysis of plant breeding trials; and (e) quickly create typical plots for two-way data considering any combination of qualitative and quantitative factors.

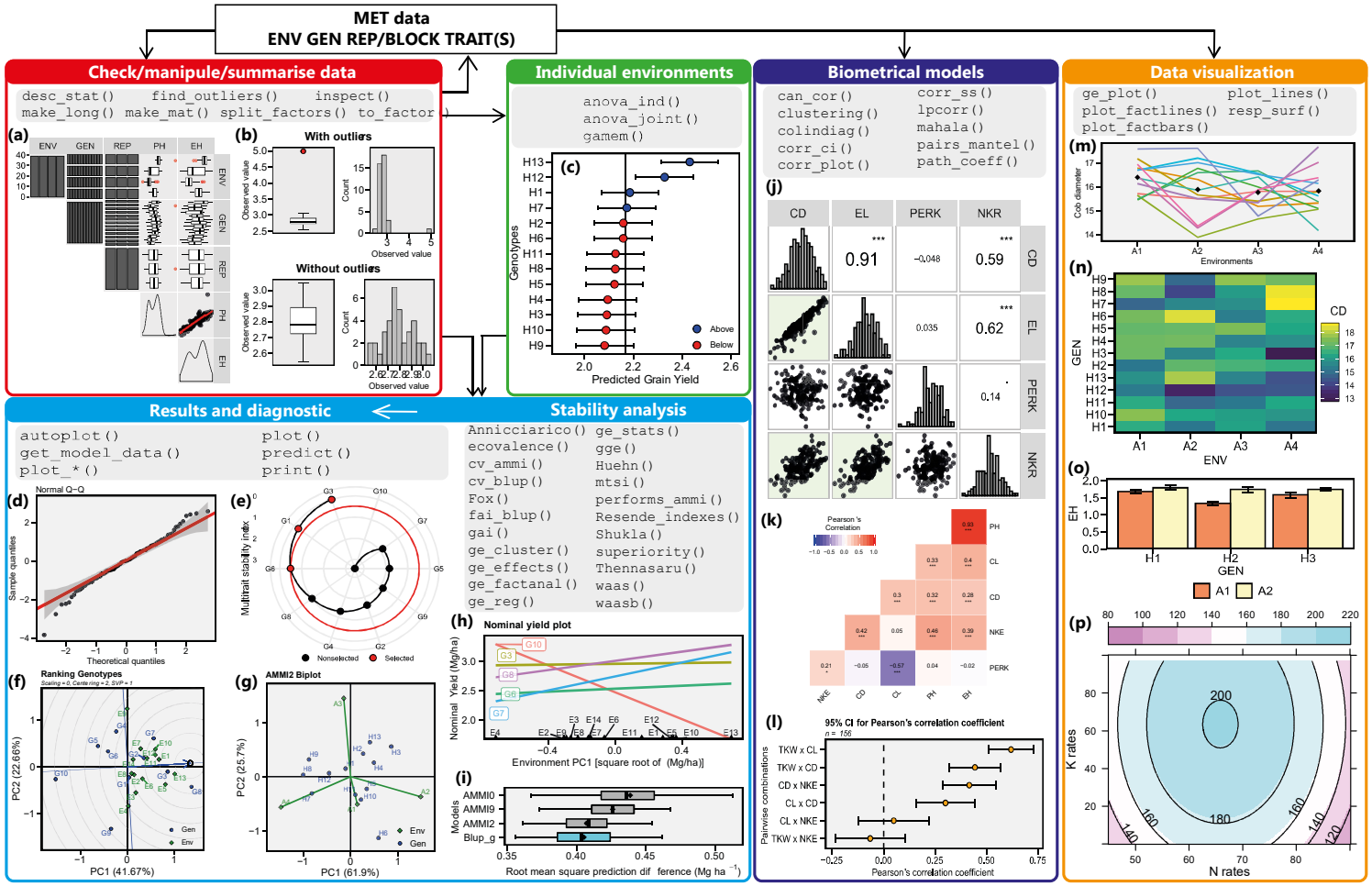

Figure 1. Diagram showing steps in a typical workflow in the analysis of multi-environment trial data using `metan`. (a) inspect plot made with `inspect()`; (b) outlier check plot made with `find_outliers()`; (c) blups for genotypes made with `plot_blup()`; (d) model diagnostic made with `plot.*()`; (e) radar plot showing the multi-trait stability index made with `plot.mtsi()`;(f) a gge biplot made with `plot.gge()`; (g-h) an AMMI2 biplot and a nominal yield plot, respectively, made with `plot_scores()`; (i) results for a cross validation procedure made with `plot.cv_ammif()`; (j-k) visualization of correlation matrices with `corr_plot()` and `plot.corr_coef()`, respectively; (l) nonparametric confidence intervals for correlation made with `plot.corr_ci()`; (m-n) genotype-vs-environment plot made with `ge_plot()`; (o) a barplot created with `plot_factbars()`; (p) a contour plot created with `plot.resp_surf()`.

The development version of `metan` is available on Github (https://github.com/TiagoOlivoto/metan) and can be installed directly via the R console using `devtools`:

```
# install.packages("devtools") uncomment to run
devtools::install_github("TiagoOlivoto/metan")
library(metan)
```

To illustrate the main features of the package, six example datasets (`data_alpha`, `data_g`, `data_ge`, `data_ge2`, `int.effects`, and `meansGxE`) are distributed with `metan`. Comprehensive details and examples of the functionality of `metan` are available in our online documentation (https://tiagoolivoto.github.io/metan/). Indeed, we strongly encourage readers to refer to the vignettes as the primary source for information on `metan`'s functionality since they are updated with every package release.

The `metan` package is constructed on an object–oriented approach, which allows for –among other things– the reliable use of S3 generic functions such as `plot()`, `predict()` and `print()`. These functions can be called any time to inspect and visualize a model. All functions in `metan` have a non–standard evaluation, where the expressions are evaluated in the specified data frame rather than in the current or global environments, thus avoiding ambiguity in input data. This makes it possible to evaluate code in non-standard ways. Basically, we can pass the argument as an expression rather than a value, reducing the amount of typing.

In `metan`, all functions have as first argument the input data. So, all of them work naturally with the forward-pipe operator `%>%` (Bache & Wickham, 2014), which makes the typing cleaner and more logical. Most of MET analyze more than one trait in each genotype. Thus, when possible, functions in `metan` analyze a vector of

variables and return the results into a list, saving a lot of time and code when several variables need to be analyzed. In `metan`, if we want to compute the AMMI stability value (Purchase, Hatting, & van Deventer, 2000) for several traits, we can combine the functions `performs_ammi()`, `AMMI_indexes()`, and `get_model_data()` with `%>%` to get a two-way table with the statistic for each genotype and traits (see an example in Appendix S1, item 8.5.4). To our current knowledge, no other package designed for MET analysis presents these features.

Sometimes in MET, a certain analysis needs to be run for each level of a factor, e.g., compute a path analysis or check outliers for each environment of the trial. The R base function `subset()` could be useful, but worryingly tedious if a large number of levels need to be evaluated. Users of `metan` can count with the function `split_factors()`, which split the original data into *n* subsets according to the grouping variable(s), where *n* is the total number of combinations of the factors used. The object of class `split_factors` can be passed on to several functions `%>%`. If a function recognizes such class of data them it will take care of details and compute what is required for each one of the *n* levels (See an example in Appendix S1, item 6.3).

4.3.1 Checking data

It is assumed that MET data has the following structure (columns): **ENV**, a factor with *e* levels, being *e* the number of environments; **GEN** a factor with *g* levels, being *g* the number of genotypes; **REP** a factor with *r* levels, being *r* the number of replicates within each environment; and at least one numeric variable, e.g., grain yield. The expected number of rows in a typical MET data is then $e \times g \times r$.

The function `inspect()` scans all columns of a data frame object for errors that may affect the use of functions in `metan` and return a warning if (i) the data has less than three columns as factor; (ii) the data has less than the expected number of rows based on the levels of factor variables; (iii) any variable has missing values; (iv) any possible outliers is detected. Running `inspect()` is an optional and exploratory step that flags potential issues before analysis. Error check results are summarized in the R console as warnings while a plot (Fig. 1a) can also be created by using the argument `plot = TRUE` in the function (See more details in Appendix S1, item 6.1).

Outliers may violate the assumption of identically distributed errors in ANOVA models. Anomalous values tend to increase the estimate of sample variance, thus lowering the chance of rejecting the null hypothesis. In this regard, we strongly recommend checking for outliers, especially if the function `inspect()` returned a warning about them. Users of `metan` can use the function `find_outliers()` to check for possible outliers in a numeric variable, returning a summary in the console (Appendix S1, item 6.2) and a plot (Fig. 1b) if `plots = TRUE` is used.

Descriptive statistics help researchers to describe and understand the structure of a MET data. The function `desc_stat()` computes a total of 30 statistics and when combined with `split_factors()` can be used to implement a descriptive analysis for each level of a factor, e.g., for each genotype (See more details in Appendix S1, item 6.3).

Frequently in MET analysis two-way tables (e.g., genotypes in rows and environments in columns) need to be created to serve as data input in some procedure, for example, in the R package `GGEBiplots`. The function `make_mat()` can be used to create such a table. You inform the data frame in the *"long"* format, the two variables to be mapped to rows and columns and one numeric variable from which the values

will fill the table and `make_mat()` take care of the details. Conversely, `make_long()` can be used to quickly convert a *"wide"* table to a *"long"* data frame (See an example in Appendix S1, item 6.4).

4.3.2 Analyzing individual environments

Individual analysis performed within each environment gives to researcher important information regarding the performance of genotypes in such environments. Provided that a typical MET data is available, the function `anova_ind()` can be used to compute, for each environment, a fixed-effect ANOVA considering a Randomized Complete Block design. The function returns the significance of factors, coefficient of variation, heritability, and accuracy of selection (See a numeric example in Appendix S1, item 7).

The function `gamem()` is used to specifically analyze genotypes using a mixed-effect model considering both a randomized complete block design or an alpha-lattice design (Patterson & Williams, 1976). The function `get_model_data()` can be used to extract the model information such as variance components, genetic parameters, and *P*-values for the Likelihood ratio test for random effects. By using the function `plot_blup()` with an object of class `gamem` the plot in Fig. 1c is produced.

4.3.3 Stability analysis

After inspecting data, checking for outliers and possibly analyzing individual environments, a quick visual inspection of the genotype−environment interaction can be performed with the function `ge_plot()`, which will generate the plots in Fig. 1m-n.

Statistically, GEI can be checked in a joint analysis of variance performed with the function `anova_joint()` (Appendix S1, item 7). If GEI is significant, then it is reasonable to proceed with some stability analysis to explore such interaction. `metan` provides a collection of functions to implement widely used methods for stability analysis in the evaluation of multi-environment trials (Table 1).

After fitting a model, users can obtain custom plots to interpret the GEI. By invoking `plot()` in an object of class `performs_ammi` residual plots (Fig. 1d) can be obtained. In AMMI analysis, biplots (Fig. 1f) are produced with the function `plot_scores()`, provided that an object of class `performs_ammi`, `waas` or `waasb` is available in the Global Environment (See Appendix S1, item 8.5.3 for more details). In GGE models, fitted with the function `gge()`, 10 types of biplots (Yan & Kang, 2003) can be created. Fig. 1g shows the biplot type 8, used for ranking genotypes. All plots are produced with package `ggplot2` (Wickham, 2016). So, users of `metan` can count on the high level of personalization provided by `ggplot2` to change any non-data elements of your plot (See an example in Appendix S1, item 7.5.3).

Table 1. Functions available in `metan` version 1.1.0 for computing stability analysis

Function	Method	Reference
Parametric		
`Annicchiarico()`	Genotypic confidence index	Annicchiarico (1992)
`ecovalence()`	Wricke's ecovalence	Wricke (1965)
`gai()`	Geometric adaptability index	Shahbazi (2019)
`ge_factanal()`	Environment stratification	Murakami & Cruz (2004)
`ge_reg()`	Joint Regression Analysis	Eberhart & Russell (1966)
`ge_stats()`	Wrapper function	NA
`gge()`	GGE biplot method	Yan & Kang (2003)
`mtsi()`	Multi-trait stability index	(Olivoto, Lúcio, da silva, Sari, et al., 2019)
`performs_ammi()`	AMMI method	Gauch (2013)
`Resende_indexes()`	BLUP-based stability statistics	Colombari Filho et al. (2013)
`Shukla()`	Shukla's stability variance	Shukla (1972)
`waas(), waasb()`	Weighted average of absolute scores	(Olivoto, Lúcio, da silva, Marchioro, et al., 2019)
`wsmp()`	Stability and mean performance	(Olivoto, Lúcio, da silva, Marchioro, et al., 2019)
Non-parametric		
`Fox()`	The 'top third' method	Fox et al. (1990)
`Huehn()`	Huehn's stability statistics	Huehn (1979)
`Superiority()`	Superiority()	Lin & Binns (1988)
`Thennarasu()`	Thennarasu's stability statistics	Thennarasu (1995)

Users who research the associations between stability indexes (e.g.., Bornhofen et al., 2017; Freiria et al., 2018; Woyann et al., 2018; Shahbazi, 2019) often find difficulties in computing the set of statistics and binding them into a *"ready-to-read"* file. `metan` provides an efficient solution for doing that. The function `ge_stats()` is a wrapper function and can be used to compute all the stability methods shown in Table 1 at once. Then, users can use `get_model_data()` to extract either the statistics or the ranks related to each genotype in each index and variable –if multiple variables are used in `ge_stat()`–, or `corr_stab_ind()`, to compute a Spearman's rank correlation matrix between the computed stability indexes (See Appendix S1, item 8.9 for more details).

4.3.4 Biometrical models

Multi-environment trials often generate data on several traits, and this data should be exploited. In breeding trials (as well as in many other areas), indirect selection helps geneticists and breeders to select superior genotypes (Meira et al., 2017; Olivoto, Nardino, et al., 2017; Olivoto, Souza, et al., 2017; Ferrari et al., 2018; Santos et al., 2018; Fonseca, Lima, Dardengo, Silva, & Xavier, 2019; Gediya et al., 2019; Lopes Costa, Melo, & Oliveira Mano, 2019); thus, any tool that facilitates this work is welcome. metan provides useful functions for implementing biometrical models easily. This includes the functions corr_coef() for computing Pearson product-moment correlation with *P*-values, `lpcor()` for computing partial correlation coefficients; `covcor_design()` for computing phenotypic, genotypic, and residual (co)variance/correlation matrices based on designed experiments; `can_cor()` for computing canonical correlation analysis; `path_coeff()` for computing path coefficients; `corr_ss()` for sample size planning; `corr_plot()` for a mixed (text and plot) visualization of a correlation matrix (Fig. 1j); `corr_ci()` for computing nonparametric confidence intervals of Pearson's correlation (Fig. 1k); and `clustering()` for clustering analysis (Fig. 1l).

Since `metan` was conceived for multi-environment trial analysis, the function `split_factors()` can be used to pass grouped data allowing, for example, that a path analysis or a canonical correlation be computed within each level of a factor, as shown in(Santos et al., 2018). For more details, please, refer to Appendix S1, item 7.

4.3.5 Data visualization

`metan` provides useful functions for creating quickly typical plots of two-way data, such as those observed in MET data. The function `ge_plot()` can be used for a visual inspection of the GEI (Fig. 1m-n). The function `plot_factbars()` is used to create bar plots with two factors (Fig. 1o). That plot like that shown in Fig. 1o has as mandatory arguments only the data, factors 1 and 2, and the response variable. Similarly, line plots with options for fitting different polynomial degrees can be made with the function `plot_factlines()`. In an experiment with two quantitative factors, the function `resp_surf()` can be used to fit a response surface model; Then a surface plot (Fig. 1p) can be created with `plot()` (See more details in Appendix S1, item 10).

4.4 CONCLUDING REMARKS AND FUTURE IMPROVMENTS

The package `metan` was designed to facilitate the analysis of multi-environment trials, allowing for more effective and less time-consuming handling and processing of MET datasets that have been increasing rapidly in the last years. Users will find in metan a complete framework to implement the most used parametric and non-parametric stability statistics for MET analysis. The package implements stability methods not available in any other R package, including the estimation of BLUP-based stability statistics (Colombari Filho et al., 2013), newer stability methods such as the weighted average of absolute scores from the (Olivoto, Lúcio, da silva, Marchioro, et al., 2019), the multi-trait stability index (Olivoto, Lúcio, da silva, Sari, et al., 2019), and the implementation of cross-validation procedures for AMMI and BLUP models

(Piepho, 1994). `metan` can also be useful for to a lot of other researchers since it provides options for implementing worldwide used multivariate statistics, e.g., path analysis, linear, partial and canonical correlations, thus allowing exploiting the maximum of (good or bad) information that a data set can offer. The estimation of stability indexes for several variables at once and the estimation of biometrical models for each level of a factor makes `metan` to outperform already published R packages for MET analysis. These features will reduce the amount of coding and save the precious time of the researchers when running their analyzes. The `metan` package is (and will always be) extensively documented online, with transparent and fully reproducible examples. `metan` is currently under active development; so, new functions will be implemented in the near future. Our next efforts will be focused on implementing cross-validation procedures for GGE models, allowing cross-validation to run in parallel, and increasing the number of stability methods available.

4.5 ACKNOWLEDGMENT

We thank the National Council for Scientific and Technological Development (CNPq) and Coordination for the Improvement of Higher Education Personnel (CAPES) for fellowships and grants to the authors. The authors have no conflicts of interest to declare.

4.6 AUTHOR'S CONTRIBUTIONS

T.O conceived the ideas, authored the software and manuscript; A.D.L assisted in the implementation of methods, and critically revised the manuscript; both authors gave final approval for publication.

4.7 DATA ACCESSIBILITY

The `metan` R package is open-source and available on GitHub (https://github.com/TiagoOlivoto/metan). Package vignettes are also open-source, accessible at https://tiagoolivoto.github.io/metan/. Installing and loading `metan` will automatically load all example data used in this paper. Since the package is updated regularly, all code, data, and documentation used in this manuscript have been archived at https://doi.org/10.5281/zenodo.3548917 as `metan` version 1.1.0.

4.8 SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

4.9 REFERENCES

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5 DISCUSSÃO GERAL

5.1 AMMI E BLUP COMBINADOS

As hipóteses da presente pesquisa foram comprovadas. Por meio da decomposição por valores singulares de uma matriz de dupla entrada contendo os BLUPs dos efeitos da interação obtidos em um modelo de efeitos mistos foi possível combinar as características do AMMI e BLUP para confecção de biplots com uma estrutura de IGA aleatória. Assim, futuros estudos poderão explorar de maneira conjunta os principais benefícios destas duas técnicas mundialmente conhecidas para a análise de ensaios multiambientes.

A determinação de se um efeito é fixo ou aleatório em estudos da IGA nem sempre é fácil e tem sido tema de extensa discussão na literatura (CULLIS et al., 1996; PIEPHO, 1998; SMITH; CULLIS; THOMPSON, 2001). Alguns pesquisadores sugerem que para estimar parâmetros de (co)variância de efeitos aleatórios com precisão suficiente, deve haver informações suficientes nos dados. Por exemplo, Stroup; Mulitze (1991) sugeriram que um fator (genótipo ou ambiente) deveria ter mais de 10 níveis antes de ser considerado como um efeito aleatório. Se considerarmos que tanto na fase inicial do melhoramento quanto na fase avançada de ensaios de valor de cultivo e uso, a seleção das melhores linhagens ou cultivares com base no ranqueamento –ao invés das comparações de médias– é o principal objetivo, é necessário que as classificações dos efeitos estimados das cultivares sejam o mais próximo possível das classificações dos verdadeiros efeitos das cultivares. Por definição, isso implica o uso do BLUP; então os efeitos de genótipos deveriam ser considerados aleatórios.

Melhoristas geralmente consideram que os anos e suas interações com os genótipos são aleatórios, mas debatem consideravelmente sobre como os locais devem ser considerados. Como o objetivo da maioria dos programas de melhoramento é inferir sobre o desempenho genotípico futuro em diversos locais não testados, é sugerido que os efeitos de locais também devem ser aleatórios. Logo, considerando um ensaio com uma série de genótipos conduzidos em diversos locais teríamos um modelo completamente aleatório (ao invés de misto), como observado em estudos prévios (BASFORD, 2001; BASFORD; FEDERER; DELACY, 2004; CROSSA et al., 2015; LADO et al., 2016). Apenas para fins didáticos, é apresentada

na Figura 1, o resultado de uma validação cruzada[5] realizada com os conjuntos de dados de aveia e trigo utilizados no primeiro artigo. Usando o argumento `random` da função `cv_blup()` do pacote `metan` três tipos de modelos foram ajustados: (i) com efeitos de genótipo, ambiente e IGA assumidos como aleatórios; (ii) efeitos de genótipo e IGA assumidos como aleatórios; e (iii) efeitos de ambiente e IGA como aleatório. Como observado, o modelo completamente aleatório apresentou a melhor capacidade de predição.

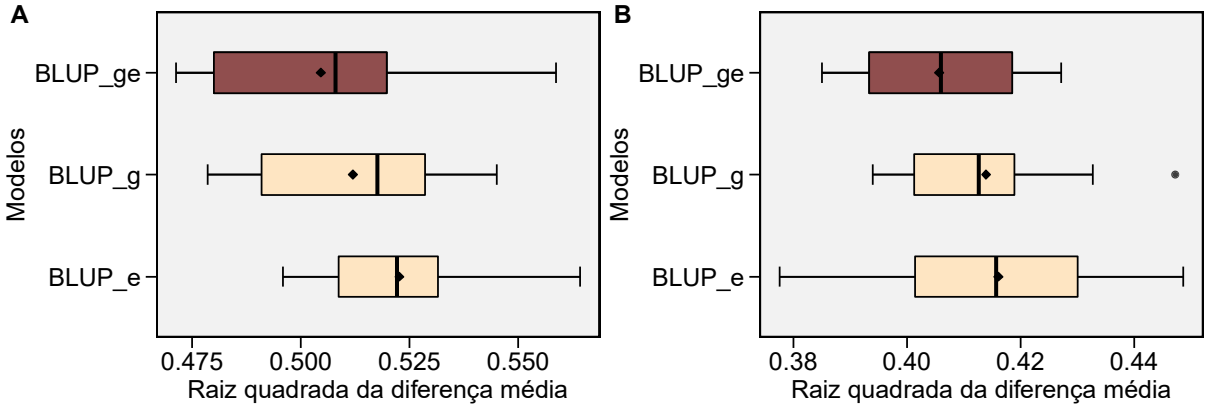

Figura 1. Raiz quadrada da diferença média de predição obtido em uma validação cruzada da cultura da aveia (A, 10 genótipos e 16 ambientes) e trigo (B, 40 genótipos e 17 ambientes). Blup_ge (modelo completamente aleatório); BLUP_g (genótipo e interação assumidos como fatores aleatórios); e BLUP_e (ambiente e interação assumidos como fatores aleatórios).

5.2 PROPRIEDADES DOS ÍNDICES PROPOSOS

Os índices WAASB e WAASBY (OLIVOTO et al., 2019a) podem ser estimadas independentemente das suposições para os efeitos aleatórios do modelo. Em outras palavras, tanto a estabilidade quanto a seleção simultânea para estabilidade e desempenho médio podem ser realizadas considerando os efeitos de genótipos ou ambientes como fixos ou aleatórios. Essa flexibilidade (de escolher a natureza dos efeitos) não existe, por exemplo, no modelo 54 do software Selegen (DE RESENDE, 2016), método utilizado para análise de adaptabilidade e estabilidade no melhoramento de plantas. Este modelo considera o efeito de ambiente como fixo para a estimação dos índices MHVG, PRVG e MHPRVG quando em alguns casos (ex.,

[5] Para maiores informações sobre o procedimento de validação cruzada adotado consulte a seção 2.3.3.1 “Cross-validation procedure”.

AZEVEDO PEIXOTO et al., 2018), a escolha para um efeito de ambiente aleatório seria a opção mais correta.

No `metan`, ficará a critério do pesquisador, então, definir se o efeito de genótipo ou ambiente será fixo ou aleatório. Ele fará isso utilizando o argumento `random` na função `waasb()`. Usando `random = "gen"` (padrão), o efeito de genótipo é considerado aleatório. Usando `random = "env"` o efeito de ambiente é considerado aleatório. Por outro lado, se `random = "all"` é utilizado, um modelo completamente aleatório é ajustado. A função `waasb()` facilitará a comparação dos padrões da IGA quando diferentes estruturas de modelos são utilizadas, estimulando o desenvolvimento de novas pesquisas nesta área de conhecimento.

Diferentemente de outros índices como o ASV, padrões de IGA explicados além do segundo IPCA (IPCA2) são também considerados na estimativa da estabilidade com os índices WAAS (modelo fixo) e WAASB (modelo misto). Um bom exemplo para esta discussão é o trabalho de Bocianowski; Niemann; Nowosad (2019). Os autores avaliaram cinco variáveis em 25 genótipos cultivados em 9 ambientes e utilizaram o ASV para quantificar a estabilidade destes genótipos. Logo, oito é o número de possíveis IPCAs. A percentagem da IGA explicada nos dois primeiros IPCAs variou entre 65,73 e 84,96%. Preocupantemente, todos os IPCA3 foram significativos ($P \leq$ 0,05) e para quatro das cinco variáveis, o residual também foi significativo a mesma probabilidade de erro. Isto significa que para todas as variáveis algum componente entre IPCA3 e IPCA8 deveria ter sido utilizado para quantificar a estabilidade. Inegavelmente, o índice WAAS seria mais preciso para a quantificação da estabilidade neste caso, pois consideraria todos os IPCAs significativos.

O MTSI (OLIVOTO et al., 2019b) apresenta propriedades até então não encontradas em nenhum outro índice na literatura, tais como: (i) pode ser estimado em uma estrutura de modelo tanto fixo quanto misto; (ii) permite a ponderação entre estabilidade e desempenho médio; (iii) considera a estrutura de correlação entre as variáveis; (Iv) não apresenta ambiguidade −o que resulta em uma classificação genotípica única−; (v) é de fácil interpretação, podendo incluir centenas de genótipos no gráfico conhecido como radar plot. O MTSI pode, também, ser estimado visando tanto a seleção para estabilidade multivariada – usando o argumento `index = "waasb"` na função `mtsi()` – quanto a seleção simultânea multivariada –atribuindo

os pesos para a estabilidade e desempenho médio na função `waasb()` e usando o argumento `index = "waasby"` na função `mtsi()`.

É fácil notar que dependendo do peso atribuído para a estabilidade e desempenho médio, o MTSI pode assumir três distintos papéis (um índice três em um). Por exemplo, se para todas as variáveis o peso para a estabilidade for 100, o MTSI será, então, um índice de estabilidade multivariado, pois classificará os genótipos de acordo com sua estabilidade, apenas. Por outro lado, se para todas as variáveis, o peso para o desempenho médio for 100, o MTSI será, por definição, um índice de seleção multivariado, pois classificará os genótipos de acordo com seu desempenho médio, apenas. Qualquer peso diferente de 100 para a estabilidade ou para o desempenho médio torna o MTSI um índice de seleção simultânea multivariada, pois classificará os genótipos de acordo com sua estabilidade e desempenho médio (considerando os respectivos pesos) em todas as variáveis em estudo.

5.3 APLICABILIDADE DOS ÍNDICES PROPOSTOS

É comum que em programas de melhoramento genético, a classificação/recomendação das cultivares seja baseada em seu desempenho médio em uma análise de variância conjunta. Por exemplo, o Ensaio Brasileiro de Cultivares de Aveia-Branca (*Avena sativa* L.), organizado pela Comissão Brasileira de Pesquisa de Aveia (CBPA) objetiva avaliar, em diferentes ambientes, o potencial de rendimento, qualidade de grãos e outras características agronômicas de cultivares de aveia-branca. Neste ensaio, diversas cultivares são avaliadas em uma série de locais, geralmente nos estados do RS, SC, PR e SP. Para cada variável em estudo, as cultivares são classificadas em superiores (S), aquelas cujas médias, na análise conjunta dos diferentes locais, foram iguais ou superiores à média geral das cultivares somada ao desvio padrão do caráter e inferiores (I), aquelas cujas médias, na análise conjunta dos diferentes locais, foram iguais ou inferiores ao valor da média geral das cultivares subtraída do desvio padrão da variável (REUNIÃO DA COMISSÃO BRASILEIRA DE PESQUISA DE AVEIA, 2019). Logo, nenhuma informação quanto a estabilidade é considerada para a classificação das cultivares. Considerando uma estrutura univariada, o índice WAASBY poderia ser utilizado para classificar as cultivares com base em sua estabilidade e desempenho médio. Assim, a utilização

deste índice como ferramenta para tomada de decisão é recomendada na avaliação de ensaios desta natureza.

Nos ensaios de linhagens[6], linhagens em avançado grau de homozigose são avaliadas e geralmente comparadas com cultivares comerciais (testemunhas) em diversos caracteres. Linhagens que atinjam ou superem em 105% o desempenho da melhor testemunha no ensaio brasileiro conduzidos por dois anos são consideradas para lançamento (RCBPA, 2019). Nestes ensaios, também, nenhuma informação quanto a estabilidade destas linhagens (e cultivares) é considerada. Como diversas variáveis são avaliadas em cada linhagem, o MTSI poderia ser utilizado como critério de seleção de linhagens, bem como comparação destas linhagens com as testemunhas em estudo. Por definição, o ideótipo utilizado na estimativa do MTSI apresenta o melhor desempenho e é o mais estável em todas as variáveis analisadas. Assim, informações importantes quanto a estabilidade e o desempenho de diversas variáveis estariam sendo levadas em consideração se o MTSI, não apenas o desempenho médio das cultivares, fosse utilizado como critério de seleção.

5.4 IMPLEMENTAÇÃO DOS ÍNDICES EM SOFTWARE ESTATÍSTICO

O pacote R `metan` apresenta uma coleção de funções para inspecionar, manipular, resumir e plotar dados típicos de ensaios multiambientes, analisar ensaios em ambientes individuais usando modelos de efeito fixo e misto, calcular estatísticas de estabilidade paramétricas e não paramétricas, bem como implementar análises multivariadas. Assim, os usuários encontrarão no `metan` uma plataforma completa, gratuita, ricamente documentada e designada especialmente para a análise de dados oriundos de ensaios multiambientes. Exemplos numéricos da utilização do pacote podem ser encontrados em <https://tiagoolivoto.github.io/metan/>.

Diferentemente de outros pacotes designados para análise de estabilidade, o pacote `metan` é construído utilizando programação orientada a objetos com avaliação não padrão (*non-standard evaluation*). Basicamente, o usuário pode informar os

[6] Ensaios de Linhagens são testes experimentais iniciais de VCU envolvendo linhagens destaques dos ensaios preliminares conduzidos pelos programas de melhoramento genético. Para a cultura da aveia branca, os resultados obtidos nestes ensaios irão definir se uma linhagem está ou não apta a ser promovida ao Ensaio Regional e Brasileiro de Linhagens de Aveia Branca, coordenados pela Comissão Brasileira de Pesquisa de Aveia e conduzidos em rede pelas instituições participantes.

argumentos como uma expressão e não como um valor, podendo reduzir drasticamente a quantidade de digitação necessária dependendo da análise realizada.

Para demostrar esta vantagem vamos, com um breve exemplo, comparar a sintaxe de código do pacote `metan` com o pacote `plantbreeding` <https://rdrr.io/rforge/plantbreeding/man/ammi.full.html> implementando uma análise AMMI para as variáveis disponíveis no arquivo de dados de ajuda do pacote `metan` "data_ge2". Mais detalhes sobre este conjunto de dados podem ser vistos em <https://tiagoolivoto.github.io/metan/reference/data_ge2.html>. Para realizar a análise das quinze variáveis numéricas deste conjunto de dados e obter os valores do IPCA1 utilizando o pacote `plantbreeding` (ou qualquer outro que com opção para análise AMMI), a função precisaria ser repetida quinze vezes. Uma maneira menos trabalhosa seria utilizar uma abordagem `for loop`[7] armazenando os resultados em uma lista para posteriormente acessar os objetos desta lista e concatenar as colunas do IPCA1.

```
# carregar os pacotes
library(metan)
library(plantbreeding)
# converter os dados para um data frame, formato aceito pelo
pacote plantbreeding
df <-as.data.frame(data_ge2)
# criar uma lista para armazenar os resultados
results_pb <- list()
# procedimento em looping
for(i in 4:ncol(data_ge2)){
  var <- names(data_ge2[i])
  ammi_results <- ammi.full(df, "ENV", "GEN", "REP", var)
  results_pb[[paste(names(df[i]))]] <- ammi_results
}
PC1 <-
  do.call(cbind,
          lapply(results_pb, function(x){
            x[["pc.scrs"]][["PC1"]]
          }))
rownames(PC1) <- rownames(results_pb$PH$pc.scrs)
PC1
```

Embora sendo a maneira menos trabalhosa, a implementação desta análise com o pacote `plantbreeding` está longe de ser amigável. Usuários iniciantes na linguagem R dificilmente memorizarão estes passos para aplicação em uma análise

[7] Conceitualmente, um *loop* é uma maneira de repetir uma sequência de instruções sob certas condições. Eles permitem automatizar partes de um código que precisam de repetição. No exemplo acima, o comando `for(i in 4:ncol(data_ge2))` faz com que o código dentro de `{}` seja repetido até que a variável indicadora `i` cumpra a sequência de 4 até o número de colunas dos dados. Esta sequência diz respeito a sequência das colunas das variáveis que se quer analisar.

futura. Vamos ver como esta mesma análise é realizada com o pacote `metan`. Para isto, serão utilizadas apenas duas funções: `performs_ammi()` para computar a análise AMMI e `get_model_data()` para extrair os valores do IPCA1.

```
library(metan)
performs_ammi(data_ge2, ENV, GEN, REP, resp = everything()) %>%
  get_model_data(what = "PC1")
```

Muito mais simples, não?! Cabe ressaltar aqui três diferenças básicas que fazem com que ao invés de 14 linhas de código no exemplo anterior, precisássemos apenas de duas linhas com o pacote `metan` para executar a mesma análise.

A primeira e mais sutil diz respeito a maneira com que declaramos os argumentos. Note que no pacote `plantbreeding` os argumentos `ENV`, `GEN` e `REP` precisam ser informados entre aspas (“”) pois este pacote utiliza avaliação padrão em sua sintaxe. Neste caso é preciso informar o valor, ou seja, o nome da variável que contém determinado fator. Devido a avaliação não padrão, no pacote `metan` isso não é necessário. Os argumentos são automaticamente avaliados em um contexto em que os nomes das colunas representam as posições de determinada coluna.

A segunda e mais impactante diferença está na declaração das variáveis a serem analisadas. Como no pacote `plantbreeding` apenas uma variável pode ser analisada cada vez, foi preciso montar a função em *loop*, armazenar os resultados em uma lista e extrair os valores posteriormente. No pacote `metan`, o argumento `resp` permite que múltiplas variáveis sejam analisadas em um único procedimento. Poderíamos, por exemplo, ter declarado o seguinte: `resp = c(PH, EH, EP, ...NKE)`, mas isso exigiria do usuário que os nomes das quinze variáveis fosse digitado. Há maneiras mais eficientes para isso. Novamente, aqui, as vantagens em se utilizar uma avaliação não padrão são notadas. Ao invés de informar os valores (nomes das variáveis), utilizamos a função `everything()` no argumento `resp` para informar que todas as variáveis numéricas do conjunto de dados devem ser analisadas. Consideremos que as variáveis estivessem nomeadas com algum sufixo ou prefixo para classifica-las em categorias. Variáveis relacionadas a espiga poderiam conter, por exemplo, o sufixo “`_ESP`”. Então, para analisar somente estas variáveis, poderíamos utilizar `resp = contains("_ESP")`. Essas funções são conhecidas como *select helpers*. Diversas estão implementadas no pacote `metan`. Para maiores detalhes sobre a utilização destas funções consulte <https://tiagoolivoto.github.io/metan/articles/vignettes_helper.html#select-helpers>.

A terceira é última diferença diz respeito a como os resultados são extraídos do modelo ajustado. No pacote `plantbreeding` foi necessário utilizar uma combinação de funções para concatenar os valores do IPCA1. No pacote `metan`, o resultado da função `performs_ammi()` é passado como argumento para a função `get_model_data()` com o operador `%>%`. A função reconhece a classe dos dados e extrai do modelo o que foi pedido no argumento `what`. Em nosso exemplo, `what = "PC1"` retorna os valores do IPCA1. Para obter outros resultados do modelo como, por exemplo, os *P*–valores para cada IPCA, basta utilizar `what = "ipca_pval"`. Para maiores detalhes sobre as classes de objetos suportados por esta função consulte <https://tiagoolivoto.github.io/metan/reference/get_model_data.html>.

O pacote `metan` está em desenvolvimento ativo e já foi acessado em mais de 45 países (Figura 2) e em 24 estados brasileiros (Figura 3), com *feedbacks* positivos por parte da comunidade científica. Assim, espera-se que este pacote atinja grande popularidade em um futuro próximo, facilitando a rotina de análise de dados e contribuindo para a melhoria contínua dos procedimentos aplicados na análise de ensaios multiambientes.

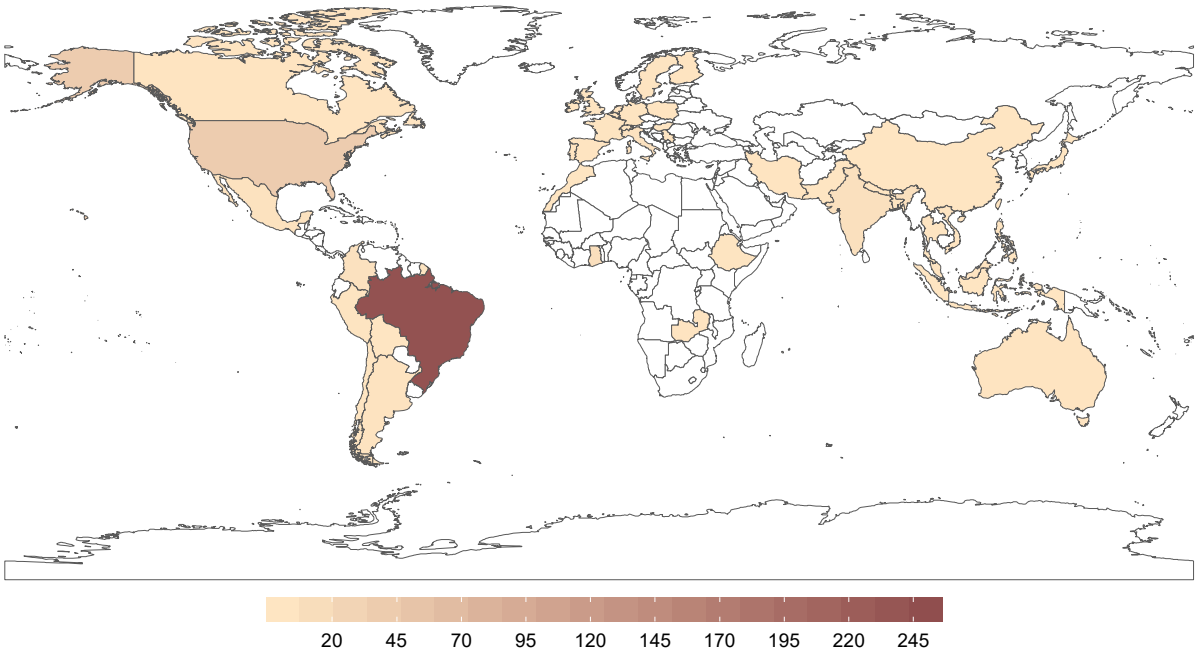

Figura 2. Países onde o pacote `metan` já foi acessado. A barra de legenda representa o número de usuários por país, atualizado em 17/01/2020.

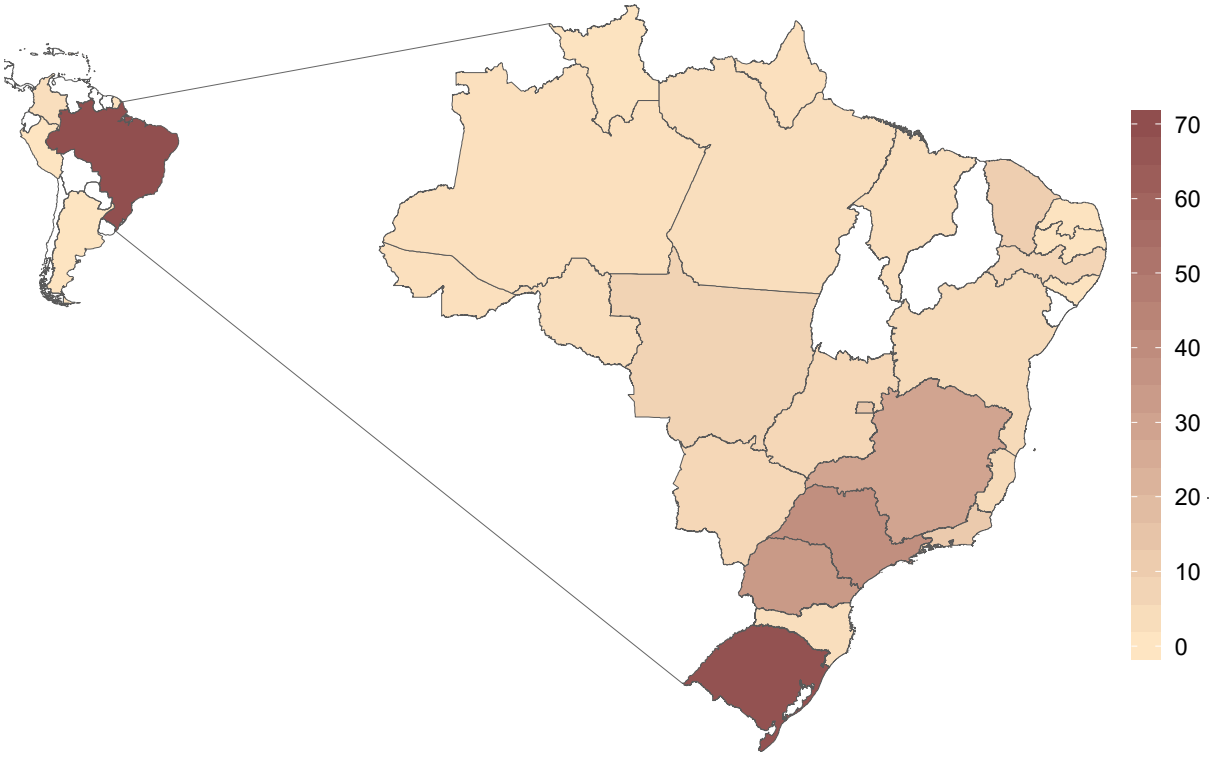

Figura 3. Estados brasileiros onde o pacote `metan` já foi acessado. A barra de legenda representa o número de usuários por estado, atualizado em 17/01/2020.

6 CONCLUSÃO GERAL

O índice WAASB se destaca como um índice quantitativo de estabilidade genotípica baseado em modelos mistos que também permite identificar por meio de gráficos classes de genótipos com diferentes padrões de estabilidade. O índice WAASBY permite atribuir pesos para a estabilidade (WAASB) e o desempenho médio (Y) e pode ajudar melhoristas a tomar decisões corretas ao selecionar ou recomendar genótipos estáveis e de alto desempenho, principalmente, quando a seleção considerar pesos diferentes para estas características. Quando diversas variáveis forem analisadas, o índice MTSI pode ser utilizado para realizar tanto a seleção para estabilidade multivariada quanto a seleção simultânea multivariada. Todos estes índices são facilmente estimados com o pacote `metan`, que oferece um ambiente de trabalho flexível, intuitivo e ricamente documentado com ferramentas que facilitarão a análise de dados de ensaios multiambientes no melhoramento de plantas.

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