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Exploring molecular markers in conjunction with traditional DUS traits for managing reference collections in European rye varieties

Introduction

Registration of new varieties by examination offices requires successful testing for **D**istinctness, **U**niformity and **S**tability (DUS-testing). Traditional DUS-testing is performed on plants' phenotype in large field trials, which is time consuming and laborious. The rapid development of genotyping and sequencing technology allows to complement traditional DUS-testing by a molecular marker based approach.

Material and Methods

Genetic data:

We genotyped 82 rye varieties (42 hybrid- (HYB), 38 population- (POP), 2 synthetic- (SYN) varieties) with the Rye5K genotyping array (Haseneyer et al. 2011). For genetic analysis we examined 2,131 SNPs.

We calculated Roger's Distance (RD) $d_R = \frac{1}{m}\sum_{i=1}^{m}\sqrt{\frac{1}{2}\sum_{j=1}^{n_i}\left(p_{ij}-q_{ij}\right)^2}$, because it is a non- Euclidean distance which can be calculated without prior knowledge about evolutionary forces forming the considered variety.

Phenotypic data:

Phenotypic analysis comprises 71 out of the 82 examined varieties (40 HYB-, 29 POP-, 2 SYN- varieties) and 21 DUS traits, which are well-defined quantitative and qualitative characteristics that meet basic requirements like consistency and repeatability in particular environments. In rye, DUS traits are for example plant, stem and ear length, as well as ear density and thousand kernel weight. Data received from field examinations of the German Plant Variety Office (Bundessortenamt = BSA) consists of notes and measurements, therefore, it was scaled and centered (mean = 0, sd = 1).

We calculated **M**anhattan **D**istance $d_M = \sum |x_i - y_i|$, as it reflects the decision making process in DUS testing best (Jones et al., 2013).

Additionally, we calculated **E**uclidean **D**istance $d(p,q) = d(q,p) = \sqrt{\sum_{n}^{i=1}\left(q_i - p_i\right)^2}$

and **G**ower's **D**istance as range normalized Manhattan Distance.

Aim and Objectives

We analyzed 82 released rye varieties for distinctness by calculating genetic distances, focusing on Roger's Distance. We used the inter-varietal RDs to define molecular threshold levels to manage reference collections as proposed in UPOV BMT Model II (UPOV, 2011). Additionally, we analyzed phenotypic data from 71 out of 82 released rye varieties for 21 DUS traits calculating phenotypic distances, e.g. Manhattan and Euclidean Distance. To evaluate the ability to predict phenotypic characteristics by molecular markers, we combined both genetic and phenotypic distances as suggested by UPOV BMT model I and II (UPOV, 2011).

Furthermore, we investigated the structure as well as the relationship of the examined varieties by multivariate clustering methods, e.g. **P**rincipal **C**omponent **A**nalyses.

Management of reference collections

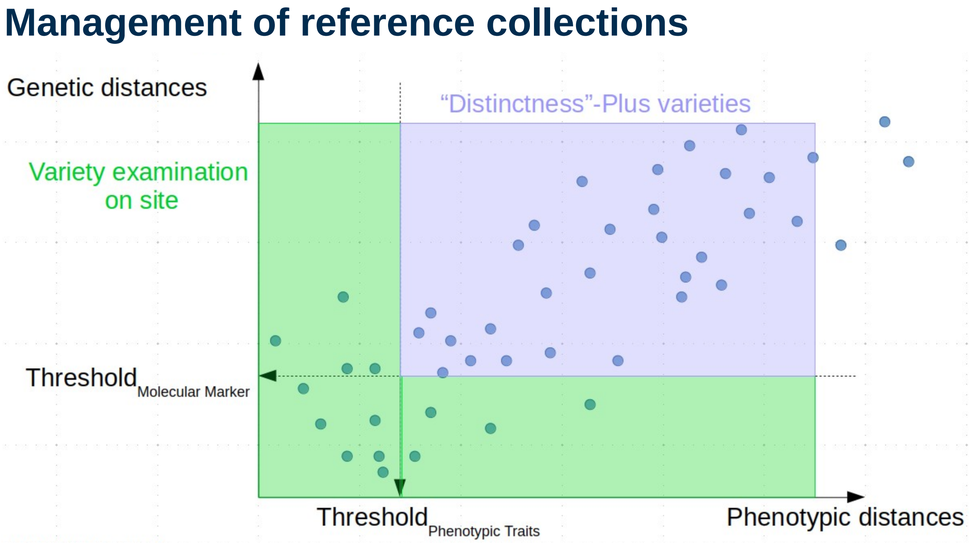


Fig. 1: To manage the increasing number of reference collections, genetic distances are correlated with phenotypic distances and threshold levels for each set are defined. Varieties above the threshold are co-called "Distinctness"-Plus varieties and they can be safely excluded from the phenotypic assessment. Varieties below the threshold need to be examined in the field. This approach would reduce the number of candidate varieties for field trials considerably. Details see UPOV/INF/18/1, 2011.

Results and Discussion

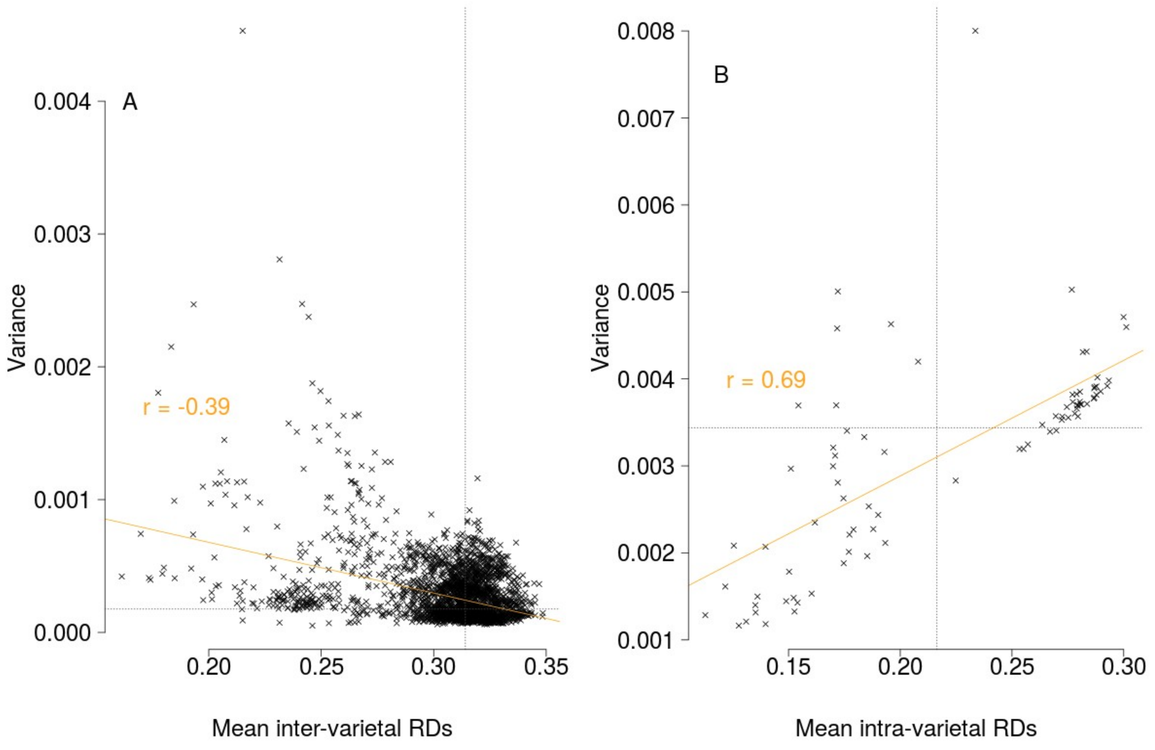
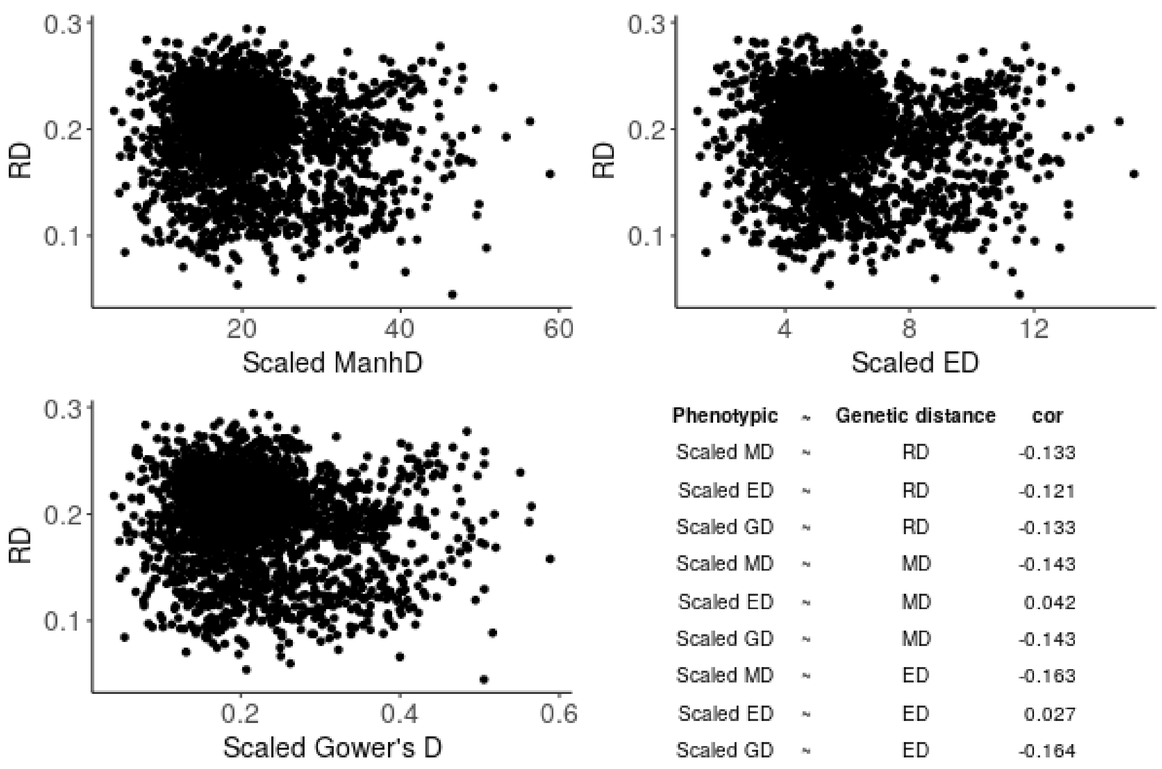


Fig. 2: Correlation of (A) inter-varietal variance and inter-varietal means and (B) intra-varietal variance and intra-varietal means. Grey dashed lines represent median.
Inter-varietal variance reduces with increasing mean inter-varietal RD, whereas intra-varietal variance increases with increasing mean intra-varietal RD. This indicates that varieties can be clearly differentiated.



Phenotypic	~	Genetic distance	cor
Scaled MD	~	RD	-0.133
Scaled ED	~	RD	-0.121
Scaled GD	~	RD	-0.133
Scaled MD	~	MD	-0.143
Scaled ED	~	MD	0.042
Scaled GD	~	MD	-0.143
Scaled MD	~	ED	-0.163
Scaled ED	~	ED	0.027
Scaled GD	~	ED	-0.164

Fig. 3: Scaled phenotypic distances in relation to genetic RD. Pearson's correlation between phenotypic and genetic distances are low. A permutation based Mantel's test compared phenotypic and genetic distances, but results were not significant.

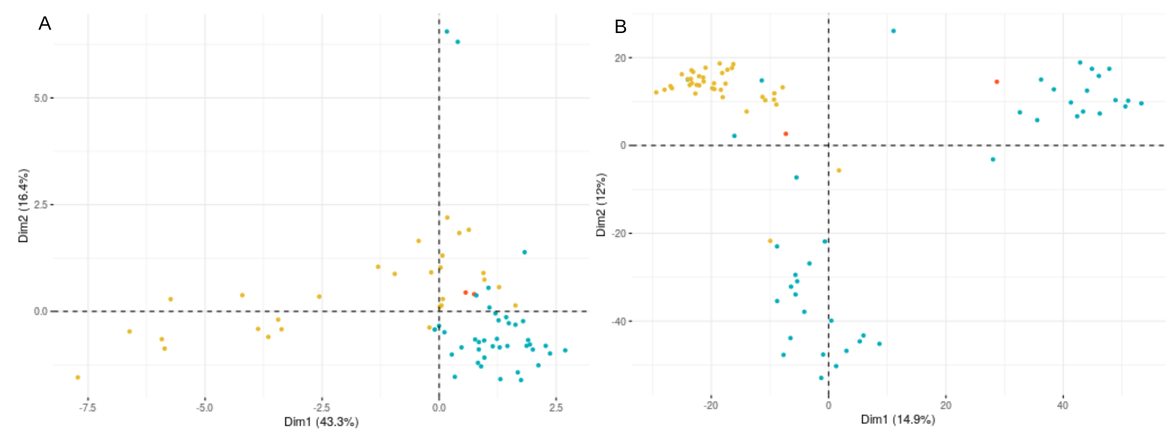


Fig. 4: **P**rincipal **C**omponent **A**nalysis based on (A) phenotypic (71 individuals) and (B) genetic (82 individuals) data. ● = HYB ● = POP ● = SYN
Phenotypic PCA shows that HYB and POP varieties tend to group together, respectively. Genetic PCA shows three clusters, separated in HYB and POP varieties.

Conclusions

Our analysis show that molecular markers have a great power to differentiate varieties. Most varieties are differentiated by an inter-varietal RDs of approximately 0.33. Differentiation is guaranteed independent of the number of markers. However, a clear threshold level cannot be defined. Analyzing genetic distances in conjunction with phenotypic distances show that they are not correlated. Therefore, phenotypic distances cannot be predicted by genetic distances in our study.

Multivariate methods show that HYB and POP varieties tend to cluster together, respectively. However, multivariate methods do not have the power to differentiate varieties solely.

References:
Haseneyer G, Schmutzer T, Seidel M, et al (2011) From RNA-seq to large-scale genotyping - genomics resources for rye (Secale cereale L.). BMC Plant Biol 11:1–13.
Jones, H., C. Norris, D. Smith, J. Cockram, D. Lee, et al. 2013. Evaluation of the use of high-density SNP genotyping to implement UPOV Model 2 for DUS testing in barley. Theoretical and Applied Genetics 126(4): 901–911.
UPOV. 2011. UPOV - Possible use of Molecular Markers in the Examination of DUS – UPOV/INF/18/1.: 1-26.