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DURDUSTOOLS: CURRENT STATE AND USE IN DUS TESTING

Document prepared by an expert from Austria

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The annex to this document contains a copy of a presentation “DurdusTools: Current state and use in DUS testing”, to be made by an expert from Austria, at the third session of the TWM.

[Annex follows]

DurdusTools:

CURRENT STATE AND USE IN DUS-TESTING

Department of DUS Testing and Plant Variety Protection

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TOWARDS DurdusTools

Within five years, we have built a workable and useful tool!

- Objectives of **DURDUS** (Jan 2018 – Dec 2020)
  - To investigate the potential of using a commercial chip to identify varieties to be grown in the field as references and to enable **pre-selection**
  - **Efficient management** of variety collections
  - Participating EOs*: France, Hungary, Italy, Spain and Austria (project lead)
- Objectives of follow-up project **DURDUStools** (Jan 2021 – Jun 2023)
  - To provide an **easily accessible tool** to be used by DUS experts
  - Integration of molecular data into **DUS testing** in durum wheat
  - Participating EOs*: Hungary, Italy, Spain and Austria (project lead)
- Objective **after the end of the project** (since 2024)
  - Routine use to support DUS testing
  - Participating EOs*: France, Hungary, Italy, Spain and Austria (coordinator)

*EOs: CPVO entrusted Examination Offices

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PRINCIPLES OF DurdusTools

Harmonized and easy use of molecular data

- DurdusTools fosters **harmonization** and **collaboration** between EOs
- Use of a **commercially available DNA SNP micro array ("chip")**
  - Data generated by the service provider and uploaded by the coordinator
- Easily accessible **online** genetic distance calculation **tool**
  - Easy use and maintenance of the tool, automatization
- Tool uses information from two collections to create the output
  - **Molecular data** stored in a secured **database**
    - Encrypted information, limited access and defined use
    - 1074 individuals (status as of March 2025)
  - **Selected variety information** stored in another database
    - Elements selected by the DUS experts
    - Regularly updated by the DUS experts

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DurdusTools IN ROUTINE USE

Partnership Agreement as foundation for effective collaboration

- DurdusTools is currently in the **second year of routine use**
  - Partnership agreement gives framework conditions
    - Will be evaluated throughout the second year
    - Prolongation foreseen every two years
- The most important **elements** within the **partnership agreement**
  - Responsibilities of coordinator, administrator and participating EOs
  - Clear timeline for every task
  - Limited access: two accounts per entrusted EO for durum wheat
  - Data curation: each EO needs to regularly update the variety information
  - Data security
  - The breeders were informed about the implementation of DurdusTools

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WORKFLOW DurdusTools

Timeline and tasks of the coordinator, EOs, and the service provider

Each EO Jan	• Update status of candidate varieties (C) • Upload C information by using template Excel file
Each EO End Feb	• Encode seeds according to the automatically assigned code • Send 20 seeds per C to the coordinator
Coordinator Mar	• Collect seeds of encoded Cs from all EOs • Send seeds of encoded Cs to the service provider
Service provider Mar - May	• Grow (encoded) Cs, extract DNA, genotyping • Prepare raw genotypic data • Send raw genotypic data to the coordinator
Coordinator/ Tool End May	• Upload of raw genotypic data in tool • Calculate GD between each pair of genotypes present in the molecular database (GD Output) • Create GD Output table: Merging of GD Output and variety list
Each EO Jun - Feb	• Select Cs to be compared and GD range of interest • Download GD Output table (Excel file with separate sheets for each C) • Select comparators to grow in the field based on information in the GD Output tables

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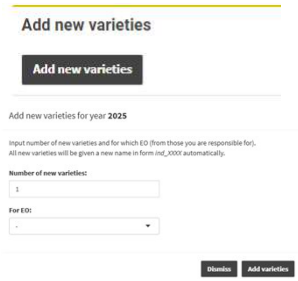
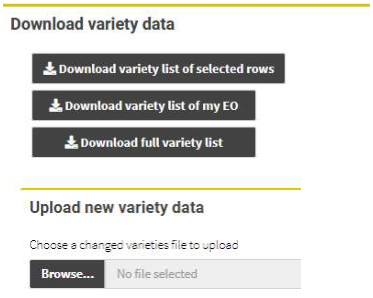
VARIETY FILE

Responsibilities of DUS experts: Adding new varieties and updating data

Add



Update



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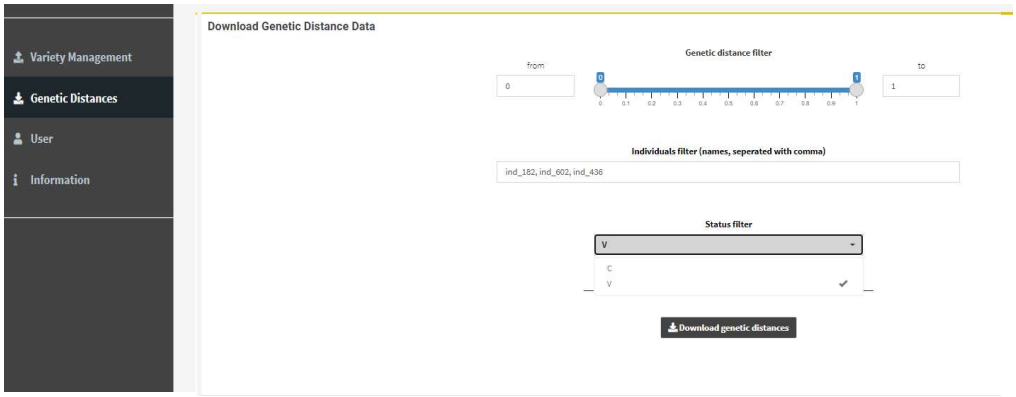
KEY FEATURE: DOWNLOAD GENETIC DISTANCES

Most relevant feature for DUS experts



- Options to insert in search field: ID, Denomination or Breeder's reference

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DOWNLOAD RESULTS AS EXCEL FILE

Familiar format for DUS experts

- Separate sheets for each ID

- Most similar varieties are at the top
  - Individuals are sorted by their genetic distances

Individual	Distance	SNPs used	Year of genotyping	Denomination	Breeder's reference / Synonym	Responsible EO	Status	Year of registration	Name of Breeder	Comment
ind_189	0.20	4342	2018	Variety 1	abc	ES	V		Breeder 1	
ind_59	0.22	4793	2018	Variety 2		ES	V		Breeder 2	Deletion from EU plant variety database 10.07.2020
ind_177	0.22	4796	2018	Variety 3		ES	V		Breeder 3	Deletion from EU plant variety database 22.11.2018
ind_51	0.24	4777	2018	Variety 4		IT	V		Breeder 4	Deletion from EU plant variety database 15.03.2017
ind_284	0.25	4225	2018	Variety 5		FR	V	1999	Breeder 5	Deletion from EU plant variety database 07.05.2020
ind_366	0.25	4178	2018	Variety 6		IT	V		Breeder 6	
ind_417	0.26	4539	2018	Variety 7		ES	V		Breeder 7	Deletion from EU plant variety database 22.11.2018
ind_396	0.26	4797	2018	Variety 8		ES	V		Breeder 8	

- Basic information of the individuals is stored in the variety file

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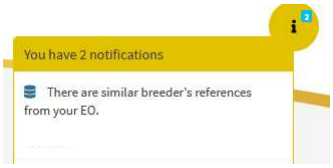
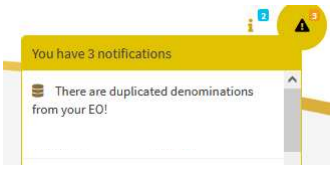
COLLABORATION BETWEEN EOs IS NEEDED

Automated notifications for the users ensure currentness and harmonization

- Only **one reference entry** per variety
  - Information when two breeder's references are similar
  - Exchange between the participating EOs
    - EOs need to decide which entry will be used for the genetic distance calculation



- Additional **warnings** when
  - No variety data was uploaded in current calendar year
  - Duplicated or similar denominations are found in the variety list
  - Status and information of candidate varieties need to be updated



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KEY ELEMENTS OF USING A COMMERCIAL SNP CHIP

Specific needs in DUS testing

- High quality of **SNP selection**
  - **Consistency** over years
  - 3,928 high quality SNPs
- Calculating the **genetic distance** in DurdusTools
  - Modified Roger's distance
  - Pairwise deletion
    - only SNPs that have no missing values between the two varieties are used for genetic distance calculation
- Data curation
  - Technical and plausibility checks to ensure data consistency

Individual	Distance	SNPs used	Year of genotyping
ind_2074	0,01785942	3910	2024
ind_682	0,1330592	3923	2020
ind_2073	0,13370739	3903	2024
ind_164	0,32013877	3907	2018
ind_167	0,33534604	3915	2018
ind_185	0,35868298	3907	2018
ind_399	0,37949146	3915	2018

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USE OF GENETIC DISTANCE FOR TRIAL DESIGN

Selection process for side-by-side comparison

Candidate 1

Variety Description: no similar varieties

GD: to all other varieties >0,43

Individual	Distance	SNPs used	Year of genotyping
ind_835	0,43062566	3530	2022
ind_810	0,43140679	3906	2022
ind_811	0,43148942	3903	2022
ind_752	0,43768476	3923	2021
ind_227	0,44049252	3916	2018
ind_226	0,44212535	3917	2018

No side-by-side trial needed

Note: the DUS experts decide if comparison with parental varieties is needed

Candidate 2

Variety Description: one similar variety

GD: 0,13 to one variety of common knowledge

Individual	Distance	SNPs used	Year of genotyping
ind_2073	0,13370739	3903	2024
ind_447	0,40712848	3921	2018
ind_835	0,43062566	3530	2022
ind_810	0,43140679	3906	2022
ind_811	0,43148942	3903	2022
ind_752	0,43768476	3923	2021

Side-by-side trial needed

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FURTHER READING

Scientific article and technical report

- Ribarits, A., Bomers, S., Zerak, T., Alber, O., Seereiter, J., Escolano García, A., Lázaro Somoza, A., Giulini, APM., Somogyi ,F., Kőrösi, S., Taferner-Kriegl, J., 2024. DurdusTools – An Online Genetic Distance Calculation Tool for Efficient Variety Testing in Durum Wheat (*Triticum turgidum* L. subsp. *durum* (Desf.) Husn.). Crops. 2024; 4(4):584-601. **doi.org/10.3390/crops4040041**

- https://cpvo.europa.eu/sites/default/files/documents/2024-02/durdustools_final-technical-report.pdf

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Austrian Agency for Health
and Food Safety

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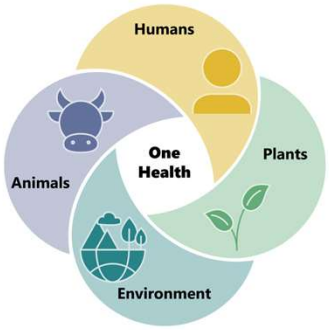
Thank you!



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[End of Annex and of document]