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ABSTRACT (FOR DISSEMINATION)	The ECOBREED wheat network (WP2) established diversity panels in durum and common wheat which were evaluated in organic multi-environment trials for a broad range of agronomic and quality traits. Thereby, genotypes were identified which are directly suitable for organic farming and/or may be used as parental genotypes in crosses specifically devoted to developing organic wheat varieties.



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	<p>Sub-sets of the diversity panels were used to study plant-plant (allelopathy) and plant-microbe (mycorrhiza) interactions. Moreover, molecular markers were applied to develop common bunt resistant breeding lines and to characterise the diversity panels with respect to rust resistance, adaptation and end-use quality traits. Knowledge gained from the different research studies was finally used to create new germplasm which is used by project partners for further selection and breeding work under organic conditions.</p>
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Executive summary

ECOBREED work package 2 (Wheat) established various diversity panels in durum (*Triticum durum*) and bread wheat (*T. aestivum*) to be studied in the various tasks. Their agronomic performance and quality were evaluated in organic multi-environment trials and identified some varieties from both conventional and organic breeding programmes with good grain yield and traits e.g. weed suppression suitable for organic farming. A major challenge in organic wheat production remains to reach a high enough protein content for the baking industry. Molecular selection for favourable alleles of NAM genes, for example the high grain protein gene *Gpc-B1*, may help in the remobilisation of nitrogen and micronutrients from vegetative tissues into the grain and, thus, realise higher grain protein content under organic farming. However, further introgression and exploitation of these genes in European wheat genetic backgrounds is necessary to develop germplasm with both satisfying grain yields and grain protein content. Marker-assisted selection (MAS) was successfully applied also in the characterisation of the bread wheat diversity panels regarding resistance genes against wheat rusts and some important adaptation traits. Moreover, MAS helped to accumulate resistance QTL/genes against common bunt, a devastating seed-borne fungal disease in organic wheat production, in European winter wheat germplasm. As shown in artificially inoculated field experiments, breeding lines including a major QTL and/or several resistance loci were highly resistant. This resistant germplasm will help to develop bunt resistant organic wheat varieties in the near future and, therefore, increase the opportunities to manage this disease under organic management. Further research is necessary to unravel the interaction between the wheat crop and its microbiome and neighbouring plant communities, respectively. Studies carried out with respect to the mycorrhiza compatibility and allelopathic effects revealed differences in genotypes' responses in pot experiments and *in-vitro* bioassays, respectively; however, in the field these effects could not be confirmed. Definitely it was shown that organic soils contain a high diversity of arbuscular mycorrhizal fungi (AMF) and that colonisation of wheat roots by AMF was higher under organic compared to conventional management. Further research is also necessary with respect to advanced phenotyping methods. Applied sensing techniques showed potential to be successfully applied in the selection of genotypes tolerant to abiotic stresses such as drought and salinity, especially if not only above-ground but also below ground biomass development can be evaluated. Finally, besides hundreds of crosses carried out by the involved partners two multi-parent advanced generation inter-cross (MAGIC) populations were developed and distributed for further selection of pure lines or the development of organic heterogeneous material (OHM). This germplasm shall be used in further research on site specific and evolutionary adaptation and its genetic drivers.



1 Screening of genetic resources and breeding material

At start of the ECOBREED project, five wheat diversity panels were established for field testing in multi-environment trials. The specific panel with Cretan landraces was later merged with appropriate other panels. The selection of germplasm was based on (i) previous experiences of organic farmers and extension services (e.g. organic VCU tests, organic variety trials and other on-farm variety evaluation), (ii) previous results of project partners, (iii) published results (e.g. project publications or recommended lists of cereals for organic farming), (iv) interaction between project partners and national stakeholders and (v) evaluations carried out during multiplication of the material before start of the project.

1.1 Methodology

Plant material

Two diversity panels of winter common wheat (*Triticum aestivum*) were established in 2019 with a total number of 136 genotypes, i.e. released varieties and advanced breeding lines. The two nurseries were assembled according to maturity. The **EARLY** panel included 80 genotypes from Austria, Croatia, France, Hungary, Romania, Serbia, Slovenia and Slovakia. The **LATE** panel included 60 genotypes from Austria, Czechia, Germany and Switzerland. Four varieties were included in both nurseries as controls. The **SPRING** panel of spring common wheat included up to 40 genotypes with ≈75% coming from European programmes and the remaining 25% from overseas.

The **DURUM** wheat (*T. durum*) panel was established originally with >70 genotypes from Central European and Mediterranean breeding programmes, however, was reduced after the first test year to ≈30 genotypes by removing genotypes with significantly inferior performance. The **CRETE** panel consisted originally of 27 old landraces (15 common wheat, 12 durum wheat) originally collected before 1945 in Crete, Greece.

Field trials

The EARLY panel was tested in field trials in Slovakia, Hungary, Slovenia, Serbia and Romania from 2020 to 2022. Three trials out of the 15 were affected by abiotic and biotic stress factors, severe drought in Slovenia and Romania in 2020 and infection with common bunt (*Tilletia* sp.) in Hungary 2022. Trials in Serbia were carried out on a conventional but untreated (no use of pesticides and fertilisers) field as long-time organic fields were not available. The LATE panel was tested in field trials in Germany, Czechia and Slovakia from 2020 to 2022. The trial in Germany 2022 was destroyed by hail a few weeks before harvest. The DURUM panel was tested in field trials in Italy, Austria and Hungary from 2019 to 2022. From the total 12 trials, the trial in Austria in 2020 was affected by severe damage caused by wheat dwarf virus (WDV). The SPRING panel was tested from 2019 to 2022 in Austria and Czechia, and from 2020 to 2022 in Germany. As for winter wheat the trial in Germany 2022 was destroyed by hail. The CRETE panel was tested in both 2019 to 2020 in Greece. Thereafter, the durum landraces were tested for another two years in Italy, while in Austria the whole panel was tested for another two years. In total 53 field trials were carried out between 2019 and 2022 in 10 European



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countries. About 11% of the experiments were affected by abiotic or biotic stresses to a level that grain yields were either significantly reduced or destroyed.

A specific field trial was established in Slovenia in order to determine the weed competitiveness of selected common wheat varieties including naturally weedy plots as well as plots inter-sown with barley to mimic a narrow-leaved weed.

Greenhouse experiments

Specific experiments were carried out to test for abiotic and biotic stresses. Frost tolerance of the LATE panel was tested by applying temperatures of -12°C to -16°C for 24 h in a growth chamber. After plant regeneration at 15-20°C in the greenhouse the number of surviving plants was counted.

Drought tolerance of 34 common wheat genotypes was evaluated in a greenhouse pot experiment using a high-throughput phenotyping platform. The stress treatment (no watering for 16 days) started at early stem elongation. Thereafter, a rewatering step to 50% soil relative water capacity took place for one week, followed by a second stress treatment (no watering). The stress treatment was applied to the genotypes according to their phenology.

Tolerance to drought and salt stress in durum wheat was investigated in a hydroponic experiment including control varieties and selected genotypes from the DURUM panel. Salt stress was applied in two concentrations and compared to an untreated control. To study the effect of salt stress on the root system architecture, the automated phenotyping platform GrowScreen-Rhizo 1 of the Institute of Plant Sciences (Forschungszentrum Jülich, Germany) was used. Automatic plant phenotyping was performed until roots reached the bottom of the rhizotrons. Additionally, a greenhouse pot experiment was performed to evaluate the response of some durum wheat varieties to the combination of salt and drought stress applied at the three-leaf stage, booting and flowering stages.

Observations

A descriptor including abiotic and biotic stresses, agronomic and morphological traits was developed describing in detail the respective traits and the appropriate scoring or measuring scheme for evaluation of the field experiments. The descriptor includes 32 different traits; 17 of them were mandatory to be reported in each field trial. The evaluation of traits in the field was done on a plot level. Grain yield was determined by combine harvest of the plots; protein content, falling number, grain and test weight were determined after cleaning.

The weed competitiveness field trial was scored, measured and sampled using classical phenotyping at various growth stages for ground cover and growth type, fresh and dry above-ground biomass of wheat, weed mimic and weed plants, plant height, number of plants, etc. Additionally, imaging of the field plots was done at three different growth stages with an unmanned aerial vehicle equipped with a multi-spectral camera.

For individual pots in the greenhouse experiment with common wheat the following traits were determined: plant height, above-ground biomass, number of fertile and infertile tillers, number of green tillers, spike length and weight, number of infertile spikelets per



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spike, grain yield per spike and/or plant, thousand grain weight. Additionally, various parameters were extracted from RGB imaging.

In the durum stress experiments the measured traits were: root length of seminal and lateral roots, root system depth and width, convex hull area, leaf length, and SPAD. At harvest, shoot fresh weight, root dry weight and leaf area were measured. Roots were analysed using WhinRhizo software to obtain root traits such as total root length, surface area, total root volume, number of tips, forks and crossings. The root angle was measured based on images using ImageJ software. The shoot and root dry weight were weighed after plants were separated into roots and shoots and dried in an oven.

Statistical analyses

Individual field trials were analysed by linear mixed models. Best linear unbiased estimators (BLUEs) for genotypes were subsequently used for an analysis across environments. Analysis across environments (predefined as location by year combinations), was done with genotypes as fixed effect and environments as random term. Stability analysis and dissection of the genotype by environment interaction was done by considering univariate and multivariate statistics following both the static and dynamic concept of stability. In the greenhouse pot experiments pairwise comparisons between control and stressed pots were calculated and submitted to analysis of variance. Used software were SAS 9.4 and Genstat 23rd ed.

Images from Advanced Phenotyping experiments were processed to reflectance maps using Pix4dmapper software. Individual plot polygons were delineated using QGIS software. Various vegetation indices were calculated using R software. The NDVI (Normalised Difference Vegetation Index) represents the presence and quantification of vegetation, with higher values indicating a greener vegetation cover. Similarly, the PSSRa (Pigment Specific Simple Ratio - Chl-a) quantifies vegetation and chlorophyll-a content.

1.2 Results

Disease resistance

Generally, severity of diseases was low in the organic field trials from 2020 to 2022. However, some locations experienced a higher frequency of diseases. For example, a high incidence of powdery mildew (*Blumeria graminis* f. sp. *tritici*) was observed in the two tetraploid wheat panels DURUM and CRETE in Austria (Fig. 1).

The leaf blotch complex caused by *Septoria tritici*, *Stagonospora nodorum*, *Parastagonospora avenae* f. sp. *triticea* and *Drechslera tritici-repentis* was predominant in Slovakia. The most resistant genotypes across all environments in the LATE panel were 'Axioma', 'Asory', BTX428, 'Illusion', 'Spontan', 'Tengri', 'Rübezahl', 'Thomaro', 'Viki', 'Wendelin' and 'Wiwa'. On the other hand, the level of resistance in the EARLY panel was lower compared to the LATE panel which is most probably due to the fact that the leaf blotch complex usually plays no role in dry and warm environments where the EARLY panel is usually cultivated.



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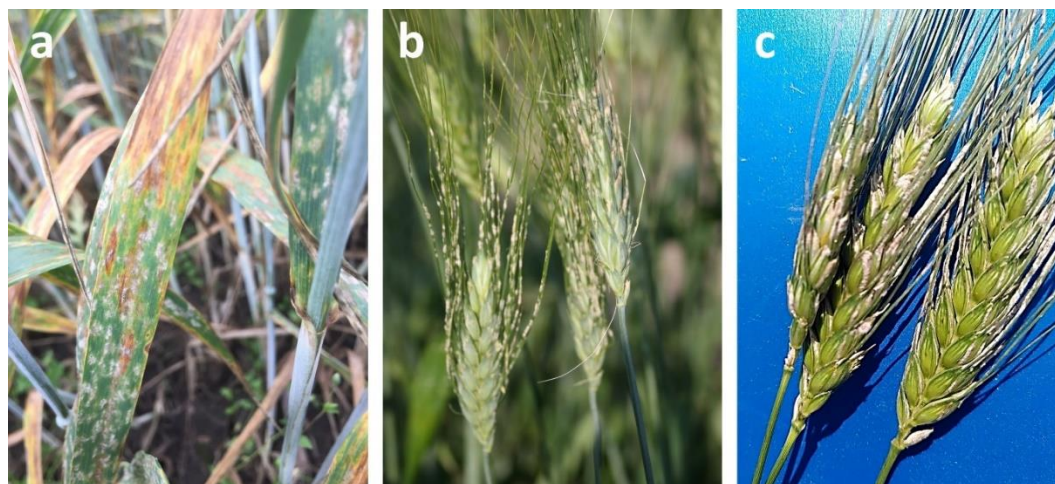


Fig 1 Powdery mildew in durum wheat: **a** mixed infection of leaves with powdery mildew and leaf blotch; **b** powdery mildew pustules on awns; **c** pustules on glumes and awns.

Natural infections of common bunt caused by *Tilletia caries* or *T. foetida* was observed in some field plots of the LATE panel in Czechia 2020 and the EARLY panel in Hungary 2022. These natural infections revealed that common bunt infection can occur not only by contaminated seeds but also by contaminated soil. However, the natural infections did not allow a reliable evaluation of the resistance level. It is known from other experiments that most of the tested germplasm is susceptible against common bunt and only 'Aristaro' has an excellent resistance, while 'Graziaro' and 'Tilliko' show resistance against specific isolates, and 'Genius', 'Spontan' and 'Unitar' show an acceptable resistance against isolates with a low aggressiveness.

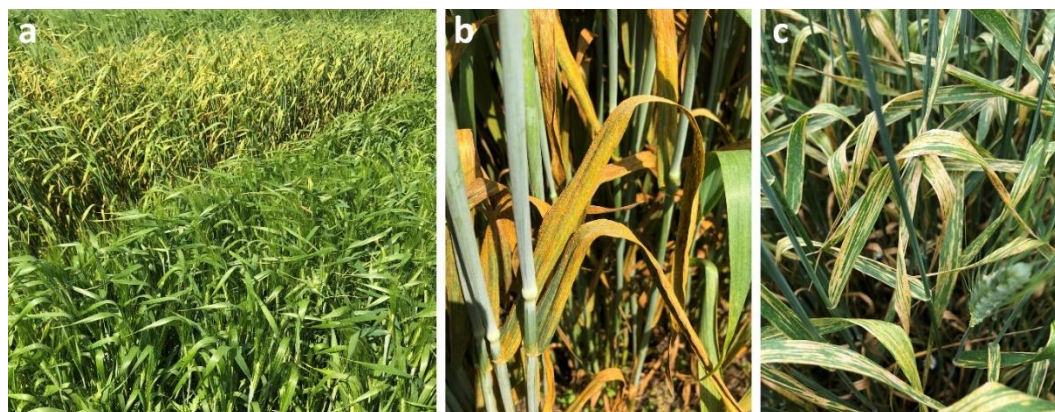


Fig 2 Yellow rust susceptibility in Cretan durum landraces in Raasdorf, Austria, May 2023: **a** highly susceptible landrace 374 besides low susceptible landrace 80; **b** typical yellow rust symptoms: small, bright yellow, elongated uredial pustules arranged in stripes; **c** typical symptoms after heavy rainfalls and ablation of spores: remaining bleached stripes on the leaves.

Incidence of yellow rust was low during the main evaluation years 2020 to 2022, while a severe outbreak affected large parts of Europe in 2023. A generally high susceptibility was observed in Austria 2023 for the Cretan durum landraces (Fig. 2), with 'Rovaki' being already completely diseased at flowering. In modern durum varieties the infection was less severe. The best genotypes with a low disease susceptibility were 'Mv Magnadur', 'Mv Pelsodur', 'Lunadur', 'Lupidur', 'Sambadur', 'Sebatel 2', 'Simeto', 'Tempodur', and the



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two landraces 'Carlantino' and 'Verdial'. In common wheat the highest resistance was observed for 'KWS Emerick', 'Aurelius', 'Wendelin' and the purple grained breeding line BTX273. From Serbia it was reported that 'NS Mila' was showing only very limited symptoms in 2023.

Weed competitiveness

Weed suppression is a complex characteristic and cannot be attributed to a single plant trait. Besides potential allelopathic effects, weed competitiveness of a wheat variety is mainly an interaction of various plant physiological characteristics such as early vigour, rapid juvenile growth, crop ground cover at early tillering, growth habit, leaf characteristics and plant height. Considering plant height, flag leaf area, flag leaf inclination, ground cover and growth habit a weed suppression index was calculated for each genotype of the EARLY and LATE diversity panel based on the results of the multi-environment field trials. The highest suppression index in the EARLY panel was calculated for 'Radosinska Karola', 'Slovenska 200', 'A15', 'Bankuti 1201', 'Ehogold', 'Capo', 'PS Dobromila', 'PS Puqa' and 'IS Gordius'. In the LATE nursery, the highest suppression index was calculated for 'Stupicka Bastard', 'Brandex Population', 'Saludo', BTX279, 'Philaro', 'Tengri', 'Liocharls Population', 'Aristaro', 'Butaro', 'Arminius', 'Capo', BTX428, 'Curier', 'Jularo', 'Graziaro' and 'Tobias'. The suitability of this weed suppression index is demonstrated in the LATE nursery by the fact that almost all varieties with a high index were developed by organic breeding programmes and/or are known by practical experience for their excellent weed suppression. On the other hand it has to be considered that the very tall landraces 'Radosinska Karola', 'Slovenska 200', 'A15', 'Bankuti 1201' and 'Stupicka Bastard' are significantly inferior to modern varieties with respect to grain yield and lodging tolerance and, hence, are of no practical relevance.

In the weed competitiveness trial in Slovenia, visual scoring and digital imaging of ground cover were highly and significantly correlated ($R^2=0.9$), however, the digital technique also exhibited a limitation. Specifically, when examining weeded and weed mimicry plots, the tool used was unable to differentiate between wheat and barley crops, whereas a trained expert could easily distinguish between the two crops. The analysis of the dry biomass of competitor barley and naturally occurring weeds revealed genotypic differences in weed suppression, particularly in the wheat-barley trial. 'Marinka' exhibited the highest suppression of barley plants, while plots of 'Tata Mata' contained the highest barley biomass. 'Marinka' also had the lowest percentage of barley seeds in harvested plots, while 'Tata Mata' had the highest percentage. Correlations between the dry weed mimic biomass and the percentage of barley grains in the sample, however, were found to be weak ($R^2<0.4$). The results of this trial were in good agreement with the weed suppression index calculated from the multi-environment trials with the exception of 'Vulkan' which had a low calculated suppression index but a medium good weed suppression in the weed mimic trial. Analysis of the imaging data revealed that sPLS regression and sPLS-SVM classification might be good models, however, still need major improvements as the significant year effect allowed a variety classification only with an overall accuracy of 0.45.



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Phenology

Flowering time of wheat is an important trait with respect to adaptation to specific natural environments. Wheat plants able to adjust flowering to seasonal conditions are better in adaptation to extreme climates (e.g. frost, heat, drought). Flowering time is highly correlated with heading date (i.e. emergence of the wheat spike from the leaf sheath of the flag leaf) and easier to score for. Heading dates of the two common wheat panels EARLY and LATE were recorded in 24 environments and expressed as days after sowing. The four control varieties present in both diversity panels were used to standardise the heading date in each environment and finally classify the tested winter wheat germplasm into seven phenological groups (Table 1).

Table 1 Classification of the ECOBREED common wheat germplasm according to heading date. Underlined genotypes are deviant from their original grouping in the EARLY and LATE nurseries.

Group	Genotypes
Very early	Mv Toborzo, Tata Mata, Glosa
Early	Pitar, IS Agilis, FDL Amurg, Litera, Izvor, FDLGPC1, Vulkan, Ursita, Unitar, FDL Miranda, NS Efrosinia, Zvezda, Mv Kaplar, Adelina, Semnal, Voinic, FDL Abund, Sofru, Bankuti 1201, Bona Vita, NS Mila, Izalco CS, Nexera 923, Simnic 60, NS Obala, NS 40S
Mid early	CCB Ingenio, Mv Uncia, Anapurna, Arnold, Nikol, Alex, Ilona, IS Escoria, Savinja, Solehio, Mv Menrot, Mv Karizma, Mv Elit CCP, Mv Karej, A15, Reska, Farinelli, <u>Dagmar</u> , Mv Lucilla, NS Frajla, <u>Vlasta</u> , Stanislava, Bertold, Slovenska 200, Mv Suba, PS Puqa, Juno, BC Lira, Aurelius,
Medium	Mv Pantlika, <u>Prim</u> , IS Gordius, IS Solaris, NS Ilina, <u>Pirueta</u> , <u>Arminius</u> , PS Kvalitas, PS Dobromila, Mv Magdalena, Gorolka, Illico, Ehogold, Viola, Mv Kolompos, Mv Kolo, Capo, IS Mandala, Mv Kepe, Mv Bojtar, Mv Mente, Alessio, <u>Annie</u> , <u>KM78-18</u> , IS Laudis, <u>KM72-18</u> , <u>Liseta</u>
Mid Late	Tengri, Blickfang, Wital, BTX279, Axioma, <u>Genoveva</u> , Graziaro, Royal, Jularo, Illusion, <u>Radosinska Karola</u> , Poesie, BTX428, <u>PS Jeldka</u> , Penelope, Thomaro, Naturastar, Brandex, Pizza, Ataro, Sultan, Liocharls, Genius, Edelman, Tobias, Spontan, Turandot, KWS Milaneco, Saludo
Late	Purino, Rubezahl, Roderik, Viki, Elixer, Butterfly, Aristaro, Wiwa, Moschus, Curier, SG-S269-09, Philaro, Wendelin, SG-S1004-18, SEC 121-11-2
Very late	Stupicka Bastard, Asory, Ibarra, Barranco, Argument, Butaro, SEC 261-05z-1-2

In the DURUM panel, the germplasm from Austria and Hungary was grouped mainly as mid-late or late, with the exception of 'Mv Magnadur' and 'MvTD16-19' classified as medium. On the other hand, the germplasm of Mediterranean origin was all classified as very early to medium.

Frost tolerance

The experiments revealed a broad genetic variation in the tested germplasm; however, no genotypes had a similar high survival index as the frost tolerant check 'Bohemia'. A high frost tolerance was observed for 'Philaro', 'Stupická Bastard', 'Viki', 'Vlasta', 'Wiwa',



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ST860/20 and 'Liseta'. Some varieties widely cultivated in organic farming in Central Europe showed a weak frost tolerance in the experiment despite no reports of winter damage recorded in the field experiments and the results were partly contradictory to previous experiments. These contradictory results could have been caused by different seed sources used. Seed-borne diseases of organic seeds may significantly affect germination and vigour of wheat seedlings which in turn may influence cold hardening and, hence, frost tolerance. Therefore, the use of healthy and vigorous seeds is essential for experiments targeted to evaluate the tolerance against abiotic stresses.

Drought tolerance

Results of digital biomass revealed that differences between control and drought treatment were lower in the very early and early maturity group, however, medium to late maturing genotypes are able to produce significantly higher biomass if not suffering from drought (Fig. 3). In the case of drought stress, varieties with mid late to very late maturity showed a significant reduction in digital biomass. Measurements of chlorophyll fluorescence revealed almost no difference in maximum quantum yield between control and drought treatment for varieties of the very early maturity group. In the early maturing varieties differences between treatments were visible but not before the second stress treatment, while for medium and late maturing varieties differences between treatments were already visible during/after the first stress treatment. A similar pattern over time was observed for the PSII operating efficiency. However, in this case, for a few late to very late varieties, e.g. 'Wiwa' and 'Tobias', regrowth of (infertile) tillers appeared after the second re-watering phase which caused an increase of PSII operating efficiency. This phenomenon could lead to misleading interpretation of imaging data if no visual observation of the plants is done in parallel and the cause for the increasing curve under drought treatment is not recognised.

From thermal imaging the canopy temperature over the time period of the experiment was extracted and again a similar pattern over time was observed: very early genotypes showed increased canopy temperature of the stressed plants during the second stress application while for the other maturity groups an increased canopy temperature in the stress treatment was already visible during the first stress application.

Drought stress affected all yield components, but it was obvious that an interaction between genotype and stress response is present in the tested germplasm. While most genotypes significantly reduced the number of fertile tillers, this was not the case for e.g. 'Sofru' (Fig. 4). On the other hand, this variety reacted with a significant reduction in grain weight, whereas e.g. 'Wendelin' and 'Mv Toborzo' showed nearly the same grain weight under drought stress compared to the control treatment. The Romanian varieties 'Glosa' and 'Unitar' showed a low to medium reduction in both grain weight and fertile tillers, resulting in an overall good drought tolerance. Generally, it is obvious that varieties from the EARLY panel showed less reduction in yield components under drought stress. This is mainly due to the high tillering capacity of LATE germplasm which is strongly affected by drought periods resulting in a sharp reduction of fertile tillers. In case of rewatering phases this germplasm tends to regrow and produce green tillers again which usually remain infertile. The energy spent by the plant for regrowth is missing for the grain filling of the fertile tillers, resulting also in a significant reduction of grain weight.



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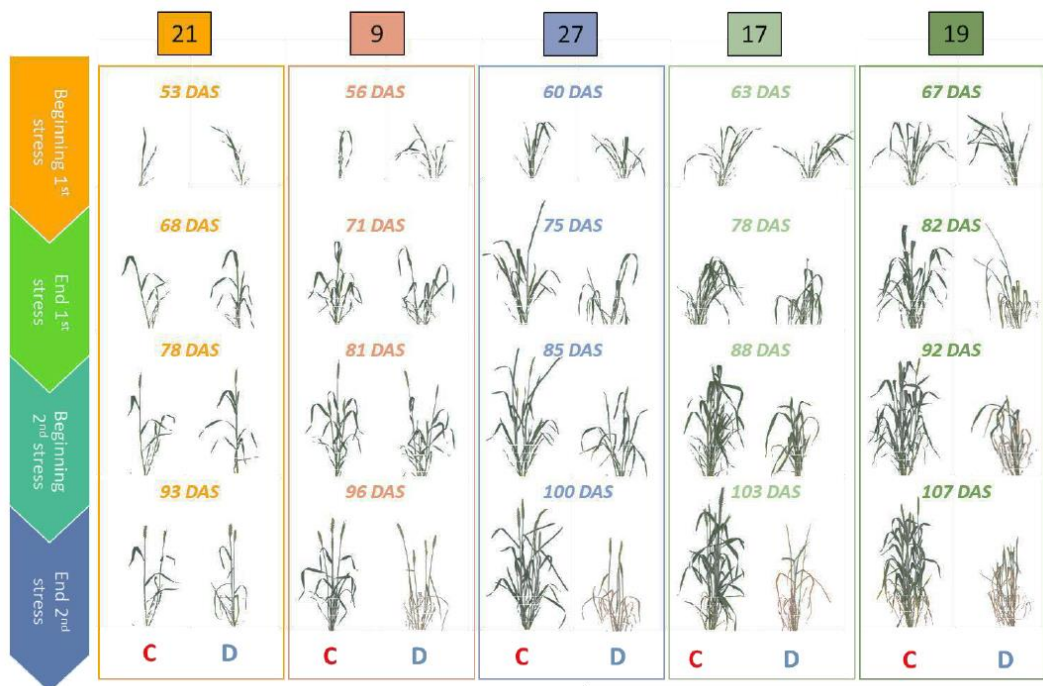


Fig 3 Examples of RGB imaging of digital biomass from side view for genotypic exemplars for each maturity group: 21 = 'Glosa'; 9 = 'Mv Karej'; 27 = 'Capo'; 17 = 'Spontan'; 19 = 'Viki'; C = control; D = drought; DAS = days after sowing.

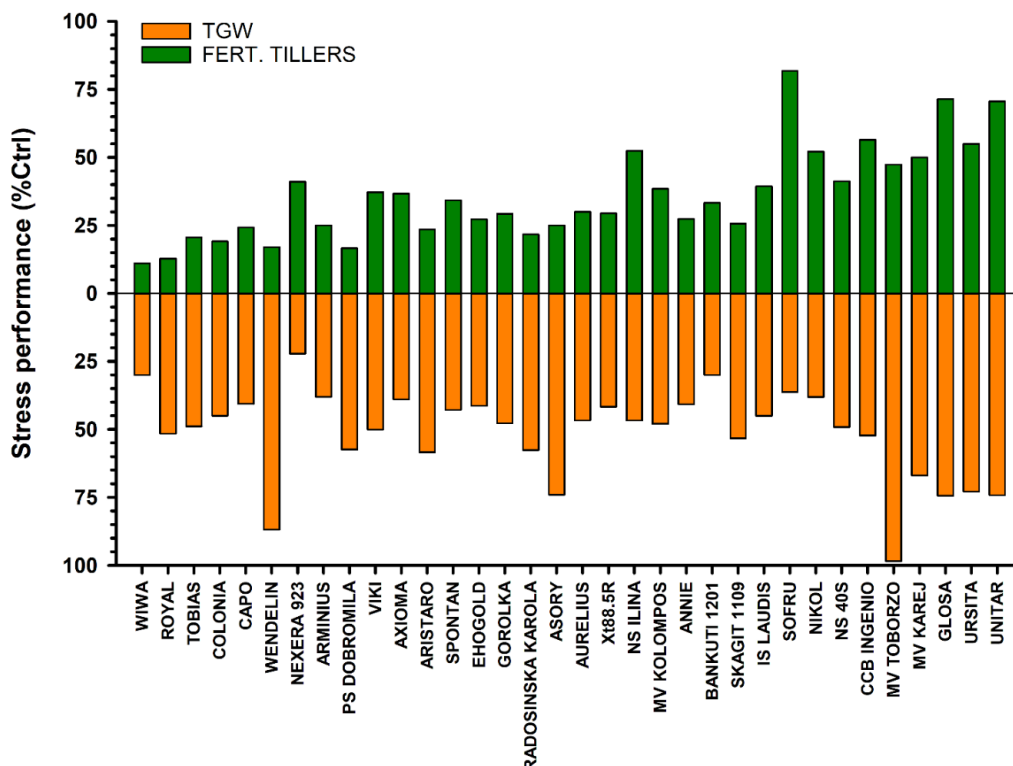


Fig 4 Thousand grain weight (TGW) and number of fertile tillers of common winter wheat varieties after drought treatment shown as percentage of the performance under the control treatment (50% SRWC; soil relative water content).



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The stress experiments in durum wheat revealed that the main effect of salt stress was first a change in the root distribution from depth to horizontal growth, if stress continues for a longer period the whole root system is reduced. However, significant genotype by treatment interaction was detected with 'Jennah Kethifa' and 'Azeghar 2-1' being most tolerant and 'Mv Pelsodur' and 'Cham 1' very susceptible. The good salt tolerance of 'Jennah Kethifa' was also demonstrated by the highest shoot biomass and largest leaf area under both salt treatments. Some root traits even increased significantly for 'Jennah Kethifa' at some time points of measurements.

Grain yield

Variability in grain yield ranged from below 2 t/ha¹ to above 10 t/ha in the EARLY panel. The lowest yields were realised in Slovenia and Romania in 2020 when the trials were significantly affected by drought. The extremely high yields were recorded in Serbia in 2020 and 2021. These trials were carried out a long-time conventionally managed and fertilised field which was converted into low-input management just for this project but clearly showed a legacy effect of mineral fertilisation in the first two years. In 2022 the grain yields were already 40% lower. Grain yields for the LATE panel were between 4 and 8 t/ha with the highest yields realised in Czechia and the lowest in Slovakia. Grain yield of the DURUM panel varied between 3 and 7 t/ha with highest yields realised in Hungary and similar yield levels recorded in Austria and Italy. Across all three diversity panels the lowest and significantly inferior grain yields were observed for the landraces included, i.e. 'Bánkúti 1201', 'A15', 'Radošínska Karola', 'Slovenská 200', 'Stupická Bastard' and 'Cappelli'. This clearly indicates that old landraces are also not competitive with modern varieties under organic management. Growing of landraces by organic farmers may be recommended only if a more than twofold price for the harvested grains can be realised.

Genotypes from the ATK programme in Hungary, 'Mv Káplár', 'Mv Kepe', 'Mv Kolompos', 'Mv Menrot' and 'Mv Karéj' were the highest yielding varieties, followed by French varieties 'Sofru', 'CCB Ingenio', 'Illico' and 'Nikol', and Romanian 'FDL Abund' and 'Unitar'. These varieties realised an overall grain yield of 4.5 to 5.0 t/ha. Yield stability of the top five yielding varieties was acceptable only for 'Mv Káplár' (Table 2). Accounting for yield stability with a factor of 0.3 in a rank sum of grain yield and stability revealed some additional varieties worth to be considered for cultivation under organic farming in the test environments, i.e. 'Mv Uncia', 'Vulkan', 'Ursita' and 'Savinja'.

In the LATE panel 20 varieties realised a mean grain yield above 6 t/ha. 'Liseta', 'Ibarra' and 'KWS Milaneco' were top yielding varieties with also good yield stability. 'Wendelin' and 'Naturastar', two varieties from a breeding programme for organic farming and released after organic VCU, showed medium-high grain yields and medium-good stability, whereas varieties from organic breeding programmes, e.g. 'Graziaro' and 'Roderik', as well as organic heterogeneous material, e.g. 'Brandex Population', were performing below average (Table 3).

In the DURUM panel, high and stable yields above 5 t/ha were realised by 'Mv Pelsodur', 'Sambadur', 'Mv Vekadur', 'Lunadur', 'Mv Masnidur', 'Iride' and MvTD16-19. This is not surprising as the majority of test environments were in Central Europe and almost all of these varieties were selected there. Besides 'Iride' varieties from the Mediterranean



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genepool worth to be mentioned are 'Miradoux' as high yielding variety, and HFN-94n, 'Levante' and 'Ouasloukos 1' with excellent yield stability.

Table 2 Grain yield and yield stability of the top varieties of the EARLY diversity panel. Sum of ranks was calculated by weighting grain yield and stability with factors 1.0 and 0.3, respectively.

Variety	Grain yield (kg/ha)	Rank Grain yield	Rank Imperfect correlation	Rank sum
Mv Káplár	4964	1	35	11.5
FDL Abund	4532	10	23	16.9
Illico	4566	8	34	18.2
Mv Uncia	4473	11	31	20.3
CCB Ingenio	4608	7	49	21.7
Mv Kepe	4739	2	70	23.0
Vulkan	4240	23	8	25.4
Mv Menrot	4631	4	72	25.6
Mv Kolompos	4757	3	76	25.8
Ursita	4419	15	37	26.1
Sofru	4745	6	69	26.7
Unitar	4419	13	50	28.0
Mv Karéj	4641	5	77	28.1
Nikol	4613	9	66	28.8
Savinja	4174	30	3	30.9

Table 3 Grain yield and yield stability of selected varieties of the LATE diversity panel. Sum of ranks was calculated by weighting grain yield and stability with factors 1.0 and 0.3, respectively.

Variety	Grain yield (kg/ha)	Rank Grain yield	Rank Imperfect correlation	Rank sum
Liseta	6626	3	12	6.6
Elixer	6758	2	18	7.4
Ibarra	6243	9	10	12.0
KWS Milaneco	6256	8	17	13.1
Turandot	6811	1	42	13.6
Viki	6397	4	34	14.2
Asory	6268	7	28	15.4
SG-S269-09	6362	6	38	17.4
Sultan	6205	11	27	19.1
Wendelin	6075	15	15	19.5
Naturastar	6165	14	20	20.0
Capo	6004	20	30	29.0
Graziaro	5990	21	49	35.7
Brandex	5915	25	46	38.8
Roderik	5838	30	45	43.5

Protein content

Mean protein content across all environments in the EARLY panel was low and would have not been suitable in many environments for marketing as wheat for baking. Only 'FDL Amurg' realised a mean protein content >10%. 'PS Dobromila', 'Mv Suba' and 'Arnold' can be regarded as varieties with high and stable protein content. Mean protein contents



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above 10% in the LATE panel were realised by 'Prim', 'Tengri', 'Philaro', 'Pizza', 'Wiwa', 'Butaro', 'Arnold' and 'Wendelin'. Considering also stability, 'Tobias', 'Wital' and 'Thomaro' are worth mentioning besides the others.

In the DURUM panel only 'Cappelli' realised mean protein contents >13%. Generally, genotypes from the Mediterranean gene pool had higher protein contents than those from Central Europe. However, considering also stability the Hungarian breeding lines MvTD16-19 and MvTD20-19 need to be mentioned with a mean protein content of \approx 12.5% and excellent stability. The best combination between mean protein content and stability was observed for 'Sebatel 2 (45)' with 12.8%.

1.3 Summary and conclusions

Organic multi-environment trials

In the LATE panel varieties from breeding programmes for organic farming, e.g. 'Wendelin' and 'Naturastar', showed both good grain yields and yield stability, outperforming all varieties derived from organic breeding programmes. The latter ones, however, were best with respect to weed competitiveness and protein content which emphasises that the currently existing organic breeding programmes select especially for high weed competitiveness and high end-use quality. Contrary to Eastern European countries, organic farmers in Central Europe (i.e. Austria, Germany and Switzerland) may already select from a broad range of commercially available varieties which are adapted to organic farming conditions. It can be assumed that specific organic VCU tests as introduced by Austria and Germany more than 20 and 10 years ago, respectively, are an attractive measure to promote also the breeding of varieties specifically adapted to organic farming. Considering the weed suppression index besides grain yield, only 'Mv Kolompos' and 'Nikol' could be recommended for organic farming in the test environments in Eastern Europe. In the DURUM panel a clear distinction was observed between the Mediterranean and Central European germplasm and test environments. Varieties from Austria and Hungary were outperforming the Mediterranean germplasm across all test environments, however, considering only the Italian test site some Mediterranean varieties were also top yielding. Similar to the results obtained for bread wheat, the landrace 'Cappelli' showed outstanding protein contents, however, significantly inferior grain yields. The tested landraces from Crete showed no valuable agronomic or quality traits which would justify a reintroduction of this germplasm. However, this germplasm was early in development and showed a fast juvenile growth; traits which are favourable in organic farming with respect to weed suppression. Unfortunately, the germplasm was also very tall and prone to lodging even under organic management.

Imaging of drought stress

All imaging data provided an excellent picture of the individual genotypic reactions on drought stress over time and can be of added value for the wheat breeder selecting for drought tolerance. However, imaging by various kinds of sensors and over time creates big data which can be hardly processed by practical breeders. Biometric pipelines need to be developed which allow a real-time assessment and easy interpretation by the



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practical wheat breeder. Preferably response curves over time from different sensor measurements should each end up in only one figure which can be used subsequently in univariate and multivariate analyses to provide an easy picture about genotype performances/interactions for the final selection.

Durum drought and salt stress

Although all genotypes responded to abiotic stress during the whole experiment very differently, at the end of the experiment nine of the thirteen traits of the landrace 'Jennah Khetifa' were significantly higher than in other genotypes. Therefore, it can be concluded that 'Jennah Khetifa' has better adaptation strategies regarding root plasticity under cyclic abiotic stresses. In order to find out what wheat root system traits are responsible to tolerate cyclic abiotic stress, 'Jennah Khetifa' could help as a control plant. Since root system plasticity plays a key role in adaptation to abiotic stress, root systems diversity analysis among landraces, and modern varieties, can assist in breeding programmes. The local landraces can provide new alleles, which can be introgressed into modern cultivars to improve valuable traits.

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2 AMF-compatibility evaluation

Arbuscular mycorrhizal fungi (AMF) form a symbiotic relationship with many crop species providing important benefits such as enhanced nutrient acquisition, tolerance to biotic and abiotic stresses and the promotion of soil structure. Significant interactions between wheat genotypes and soil microbial populations in the rhizosphere have been observed and some studies have shown that AMF colonisation and plant benefit from the symbiosis are dependent on genotype. Selecting for increased AMF colonisation has the potential to reduce phosphorus fertiliser inputs and the environmental consequences of increased phosphorus supply to water systems.

2.1 Methodology

Greenhouse experiments

Pot experiments were carried out with 40 winter wheat genotypes selected mainly from the EARLY and LATE diversity panels. The growth medium (clay, sand, peat) was either treated with a commercial AMF inoculant containing *Rhizoglyphus irregularis*, *Funneliformis mosseae* and *F. caledonium* or with the isolated *F. mosseae* strain BEG12. Plants were harvested after 6 or 8 weeks of growth, the potting substrate was removed from the roots which were then washed under tap water, and root and shoot fresh weight was measured. Root samples were divided for dry weight determination and analysis of AMF colonisation. For the latter, fresh roots were stored in ethanol until microscopic analysis. As AMF colonisation failed in two attempts with a commercial inoculant, another pot experiment was carried out using field soil from a certified organic field with its naturally occurring AMF population. DNA was extracted from the soil sample in order to identify the major AMF species/strains present by next generation sequencing (NGS).

Roots were stained according to an existing protocol and stored in ethanol until further use. For each sample one microscope slide with 30 root pieces was prepared. Afterwards, AMF root colonisation was determined via light microscopy (Fig. 5). Thirty root pieces per sample were evaluated for intra-radical hyphal abundance (H%), vesicle (V%) as well as arbuscule abundance (A%). From these data, parameters such as mycorrhizal frequency (F%) in the root system and intensity of mycorrhizal colonisation (M%) in the root system were calculated.

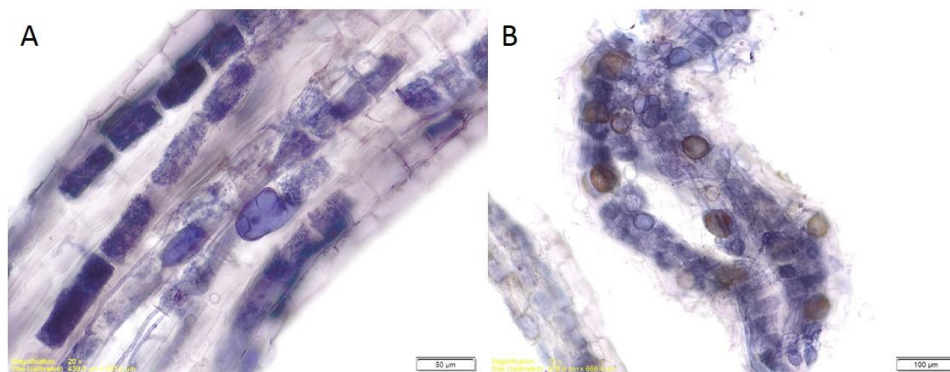


Fig 5 Arbuscular mycorrhizal structures in wheat roots from field samples stained with ink and vinegar: varieties 'Aurelius' (A) and 'Arminius' (B).



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Field experiment

In order to screen for native AMF colonisation in the field, an organic field trial with 6 common wheat and 6 rye varieties was established in Austria in 2020. The pre-crop was alfalfa; rye was selected as a second cereal to provide a comparison. The rye varieties were selected to include old landraces, modern OP (open pollinated) varieties and a modern hybrid variety. Root samples were taken at the middle of heading (BBCH 55). In each plot, 4 root samples were taken at a depth of 10 cm and stored in a cool box until further preparation in the laboratory. Roots were washed under tap water and root fractions were split into two parts. One part was analysed for AMF colonisation and the second part was stored at -80°C until further processed for AMF community analysis via metabarcoding.

DNA extraction and sequencing

For DNA extraction frozen roots were lyophilised and ground to a fine powder. DNA was extracted from root powder and samples were outsourced for library preparation and amplicon sequencing. Clustering and counting sequences per cluster was done by using a 97% sequence similarity. The OTU sequences were aligned and a phylogenetic tree was calculated. Taxonomic affiliation of OTUs was done with BLASTN and the GenBank database. Only assignments of the sub-phylum Glomeromycotina were used further.

Statistical analyses

For the genomic data of the field experiment OTU richness and Shannon index were calculated. For principal coordinate analysis (PCoA), the distances between fungal communities from separate samples were based on weighted UniFrac distances. The influence of environmental variables such as AMF parameters and yield data on OTU abundances was tested by performing redundancy analyses.

2.2 Results

Pot experiment with natural organic soil

There was a significant difference between varieties of a first screening in all colonisation parameters recorded. The highest AMF frequency (F%) was observed for 'Mv Elit CCP' followed by 'Aszita' and 'Tengri'. In terms of mycorrhizal intensity (M%) 'Aszita' had the highest level followed by 'Mv Elit CCP' and 'NS Ilina'. Arbuscule abundance (A%) was greatest in 'NS Ilina' followed by 'Aszita' and 'Mv Elit CCP' whereas hyphae abundance (H%) was greatest in 'Mv Elit CCP' followed by 'Aszita' and 'Arminius'. Vesicle abundance was at a very low level and in half of the varieties no vesicles were recorded. 'Wendelin', 'Wiwa' and 'Glosa' had generally the lowest AMF parameters recorded. For all parameters studied there was no significant effect of soil sterilisation for 'Wendelin' and 'Glosa' which suggests potential contamination or an ineffective sterilisation process.

The intensity of AMF in the root consists of the sum of relative vesicle, arbuscule and hyphal abundances. 'Wendelin' and 'IS Laudis' had a low relative abundance of arbuscules when compared to vesicles and intra-radical hyphae (Ar%) while 'Skyfall' and 'PS Dobromila' showed the opposite in terms of high Ar%. Pairwise Pearson correlation analysis between plant growth and the main root colonisation parameters were all non-



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significant however there was a trend ($0.05 < p < 0.1$) for a negative correlation between root fresh weight and F%.

In the second screening A%, M% and Ar% were all much higher than for the first screening (F%: 35.9 vs 94.1; M%: 4.9 vs 11.84; A%: 3.3 vs 7.1, respectively). Higher colonisation parameters were also recorded for V% and Vr%. With respect to colonisation parameters the only significant difference between varieties was for F% whereby 'Rübezahl' (SEC290-08-1a-1) showed significantly lower values than 'Nexera 923' (Xt 9.23), Xt189FH182 and Xt 865. Pairwise correlation analysis between plant growth and the main root colonisation parameters were all non-significant.

Pot experiment with *Funneliformis mosseae* BEG12

All 40 wheat genotypes were colonised by *F. mosseae* during the 6-week growth period. The genotypes did not differ significantly with regard to frequency of AMF in the root system. With regard to intensity of mycorrhizal colonisation in the root system, 'Spontan' and 'Bauermeister' of the first screening and 'NS_Mila' from the second screening showed the highest intensities (M%: 20-26%) whereas 'Ehogold' and Xt88.5R had the lowest intensities of 2% and 1%, respectively. At the same time, 'Spontan' and 'Bauermeister' also showed higher relative vesicle abundance (Vr%) compared to the other genotypes (Fig. 6A). Similar dynamics were observed in the second screening (Fig. 6B). This also indicates that AMF are already entering the vesicle stage important for storage and survival without a living host plant.

Field experiment

Contrary to the pot experiment with *F. mosseae* BEG12, all genotypes showed similarly high levels of AMF frequency in the field. Only 'Bauermeister' with very high intensities in the greenhouse trial showed indeed here slightly lower values. In all genotypes, AMF had formed all three structures i.e. hyphae, arbuscules and vesicles to nearly equal amounts. When comparing wheat and rye AMF parameters from the field experiment, most of the samples tended to show a technical maximum for the field plots. This could be especially observed for the rye varieties where no differences with regard to frequency or intensity could be observed. With regard to AMF development, the relative number of vesicles was much higher compared to the relative number of arbuscules. The same could be observed for wheat and rye. Overall, it can be concluded that the selected field plots had a high prevalence of native AMF and that all genotypes formed readily arbuscular mycorrhiza with the local AMF strains.



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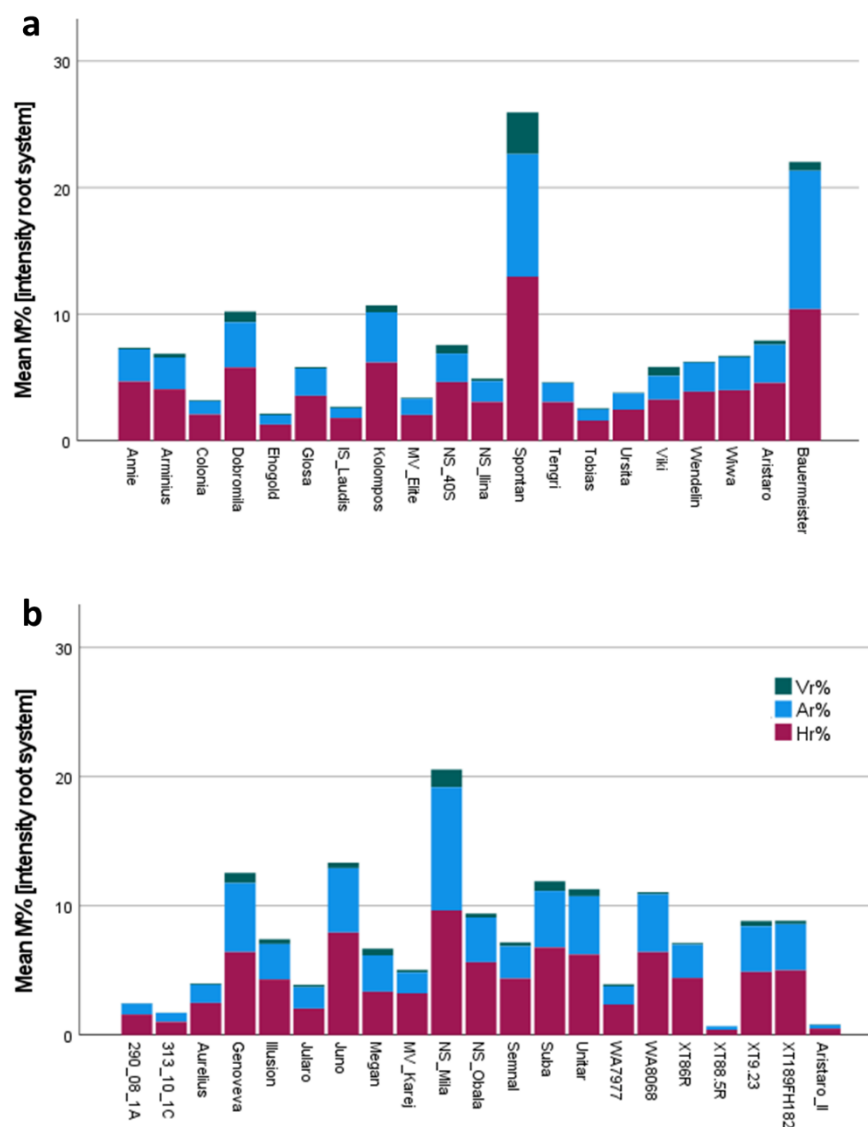


Fig 6 Mean intensities of AMF in the root system of wheat genotypes ($n=5$) of sub-set 1 (A) and sub-set 2 (B) presented as relative amounts of vesicles (Vr), arbuscules (Ar) and hyphae (Hr). Inoculation of the growth medium was done with *Funneliformis mosseae* BEG12.

AMF community analysis

In the field experiment, 305 fungal OTUs were retrieved from 192 root samples. To investigate AMF compatibility, only OTUs assigned to Glomeromycotina were used for further analysis. In total, 87 OTUs were identified as Glomeromycotina. The OTUs could be assigned to the following 8 genera of the Glomeromycotina: *Ambispora*, *Claroideoglossum*, *Diversispora*, *Funneliformis*, *Glomus*, *Rhizophagus*, *Scutellospora* and *Septoglossum*. Among these genera, the highest relative abundances were observed for *Funneliformis*, *Claroideoglossum* and *Rhizophagus*. *Funneliformis* accounted for 47% and 37%; *Claroideoglossum* for 25% and 30% and *Rhizophagus* for 24% and 26% of the rye and wheat samples, respectively. Species of these genera are common in agricultural field soils. Reads assigned to *Ambispora*, *Diversispora* or *Septoglossum* species were only observed <1%. *Scutellospora* made up 2% and 5% of the relative abundance in rye and



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wheat, respectively. When observing the different genotypes, rare taxa reads belonging to the genus *Ambispora* were observed to a greater extent in rye varieties 'Elias' and 'Norddeutscher Champagnerroggen' and in wheat variety 'Mv Kolompos'. Reads from *Scutellospora* species could be found to a higher degree in wheat varieties 'Aristaro' and 'Aurelius'.

As α -diversity measures, observed OUT richness and the Shannon index were calculated. Observed OTU richness was significantly affected by crop species with rye plants hosting on average 35 OTUs compared to 30 OTUs in wheat. Among genotypes, rye variety 'Sangaste' had the highest observed richness with 37 OTUs. The highest value in wheat was observed for 'Mv Kolompos' with 34 OTUs. The Shannon index which considers both species richness and evenness did not reveal any differences between varieties or crop species. β -diversity is presented as PCoA bi-plots based on weighted UniFrac distance matrices. Each distance matrix is most powerful for certain scenarios in microbiome data. In order to combine several distance matrices in one test, PERMANOVAG was performed for crop species and cultivar on the d0, d0.5 and d1 distance matrices. In the PCoA bi-plot, the first axis explains 62.8% of the variation (Fig. 7). The influence of crop species and genotype can be considered as low as the samples overlap to a great extent. In order to test for the influence of environmental variables such as AMF parameters and yield data on OTU abundances, redundancy analysis was performed. The full model was simplified by forward selection. Constrained ordination was tested by the Monte Carlo permutation test that did not give a significant result. Thus, species abundance could not be explained by the used environmental variables, i.e. yield components and AMF parameters.

2.3 Summary and conclusions

Most field-based studies on wheat report 20-40% AMF root colonisation which is in line with the results obtained in our project. There were clear differences in colonisation parameters between the different screenings of the experiment with natural organic soil. In both pot experiments clear varietal differences in colonisation parameters between varieties were observed. However, there was no real consistency in the performance of the varieties between the two experiments. For example, 'Spontan' had the highest F%, M% and second highest A% when inoculated with *F. mosseae* BEG12, however, was near the bottom of the list for all three parameters when natural organic soil was used as growth medium. A vice-versa reaction was observed for 'Mv Elit CCP'.

In the field study the predominant AMF genera present in wheat and rye were Funneliformis, Claroideoglomus and Rhizophagus and species of these genera are common in agricultural field soils. Colonisation differences between wheat and rye plants observed in this study in Austria might be explained by their different root system architectures. Rye forms a much denser root system and is also better suited to grow under marginal conditions. All genotypes tested in the field showed root colonisation with native AMF. However, the effects on plant physiology remain unclear in this setting. Furthermore, high colonisation rates do not necessarily result in a yield effect but might have other positive effects like improved plant fitness. AMF growth responses can depend on the applied AMF community ranging from positive over neutral to negative.



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Thus, genetic markers for the nutritional function of AMF would be interesting for future breeding programmes. Differences between AMF communities of plant varieties were in our study only minor.

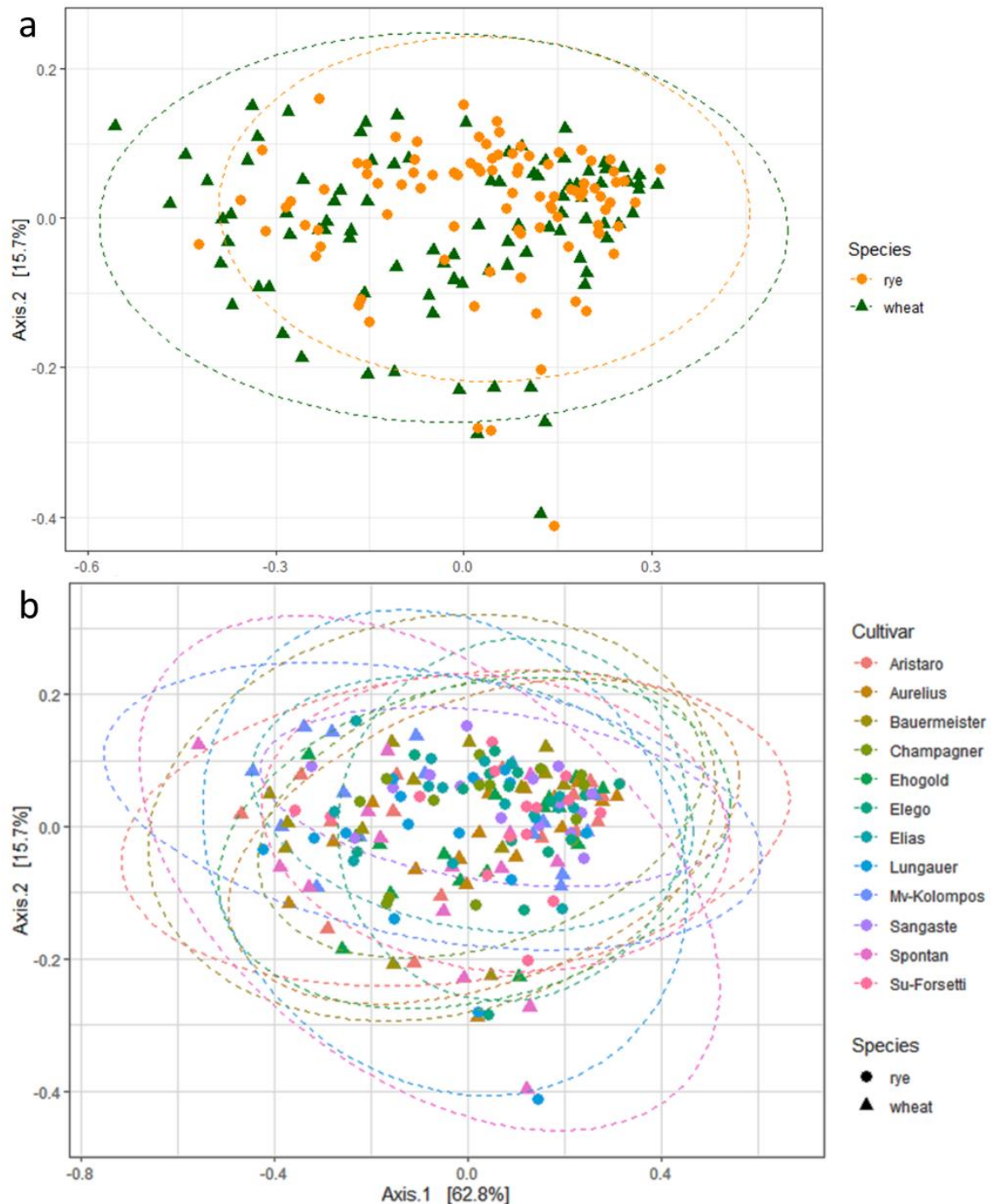


Fig 7 PCoA bi-plot based on weighted UniFrac distance matrix with 95 % confidence ellipses for crop species (A) and cultivar (B).



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3 Allelopathic activity screening

Synthetic herbicides may be harmful to the environment and human health, but are widely used due to their rapid efficacy on weeds. However, plants may also have the ability to produce natural phytotoxic compounds that can replace chemicals for weed control. This phenomenon is called allelopathy; the natural compounds produced by the plants are called “allelochemicals” and their effect on neighbouring plants may be directly or indirectly through microorganisms’ activity. Allelochemicals can be released by air (volatilisation), the soil (plant residues and root exudates), leaching or transformed by microorganisms in the soils to create other molecules (Fig. 8). Some plants release these chemicals to compete with other species, while others promote growth and protect them from pests and diseases.

3.1 Methodology

The allelopathic potential of 30 wheat varieties against two very aggressive and herbicide-resistant weeds in Europe, the dicot common purslane (*Portulaca oleracea*) and the monocot annual ryegrass (*Lolium rigidum*), was evaluated after *in vitro* co-cultivation of wheat varieties with the weeds. Germination rate (G%), plant weight (PW), shoot (SL) and root length (RL), and plant height of the weeds were measured to obtain information on the inhibition or stimulation of weed growth, the ability of weeds to colonise the space through germination and development (SIC: shoot invasive capacity; RIC: root invasive capacity) and how weed plants can develop in the next generation (SVI: seedling vigour index). Shoot and root length, and plant weight were also measured for the wheat plants. Second, allelochemicals from the shoots and roots, as well as root exudates from wheat and weeds were quantified to evaluate the accumulation or release of these compounds by different wheat varieties in presence of the two weed species.

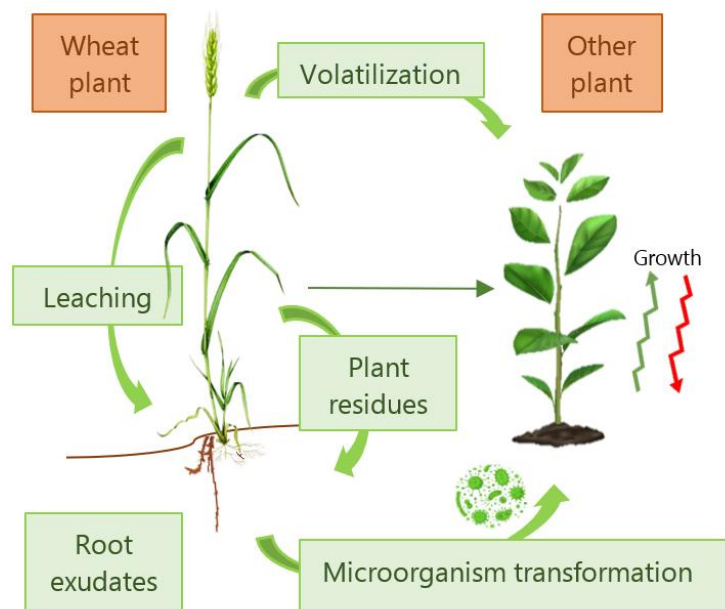


Fig 8 Allelopathy and how it works: release pathways from the wheat plant and direct and indirect effects of wheat allelochemicals on competing plants, e.g. weeds.



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Nine wheat varieties were also tested in semi-natural conditions in the greenhouse against annual ryegrass. The plants were grown in pots and two watering treatments were applied: (i) manual irrigation in order to check how root exudates behave in soil and can affect weeds, and (ii) artificial rain to measure the allelopathic potential of leaf leachates. Both weed and wheat parameters were measured and the soil solution was extracted to analyse the presence and abundance of allelochemicals.

3.2 Results

In general, the wheat varieties showed a greater ability to control annual ryegrass than common purslane. The *in-vitro* bioassay showed that all genotypes except 'Glosa' inhibited at least one of the tested parameters in annual ryegrass. The varieties 'Ludwig', 'Maurizio' and 'NS Azra' inhibited all seven parameters (G%, PW, SL, RL, SIC, RIC and SVI); six varieties ('Capo', 'Midas', 'NS 40S', 'Proteinka', 'PS Dobromila' and 'Tobias') inhibited 6 out of 7 parameters, and another three varieties ('Bezostaya 1', 'Ehogold' and 'IS Laudis') inhibited 5 out of 7 parameters. Interesting are also the results of 'Alessio', 'Arminius' and 'Element' which stimulated RL without altering PW or SL. The most affected parameter in annual ryegrass was RIC which was inhibited by 22 out of the 30 wheat varieties. Most of the varieties (21 out of 30) induced inhibition of germination and just 'Element' stimulated germination. In summary, the greatest allelochemical effect against annual ryegrass was exhibited by 'NS Azra', followed by 'Ludwig', 'Maurizio', 'PS Dobromila', 'Midas' and 'Tobias'. In contrast, the bioassay with common purslane showed that none of the tested varieties inhibited more than four measured parameters; just 'Capo' and 'Glosa' were able to inhibit four parameters (G%, PW, RL, RIC and PW, RL, RIC, SVI, respectively), while 10 varieties ('Adesso', 'Annie', 'Capo', 'Ludwig', 'Midas', 'NS Azra', 'Spontan', 'Tobias', 'Ubicus' and 'Ursita') inhibited three parameters. Surprisingly, 'NS 40S' strongly stimulated RL, SIC, RIC and SVI of the dicot weed.

On the other hand, root and shoot growth of wheat was generally unaffected when grown in the presence of both weeds. Just 'Element' and 'PS Dobromila' showed reduced shoot or root length in the presence of both weeds, 'Annie' and 'Edison' showed reduced growth only when grown together with annual ryegrass. In contrast, 'Ehogold', 'Ludwig', 'NS Futura' and 'Proteinka' showed increased root length in the presence of both weeds; 'Ludwig', 'NS Futura', 'Proteinka' and 'Ursita' showed also increased shoot length in the presence of annual ryegrass.

The chemical analysis also showed different profiles of accumulation and/or exudation of allelochemicals in the different wheat varieties which could be related to their ability to compete with the weeds. Different allelochemicals (benzoxazinoids and polyphenols) were detected in the shoots, roots and agar (root exudates) of the wheat varieties when grown alone as a control. However, the allelochemicals profile changed in the presence of weeds depending on the variety studied. This was especially true for the benzoxazinoids BOA, DIBOA, HBOA, MBOA, DIMBOA and HMBOA. For example, 'Maurizio' exuded higher amounts of some allelochemicals from the roots to the growing medium in the presence of annual ryegrass or common purslane than when grown alone, whereas the content of allelochemicals in the roots was lower after cultivation with both weeds. 'Ursita' accumulated high concentrations of allelochemicals in shoots and roots but also



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exuded a great quantity to the growing medium when grown together with weeds. Indeed, 'Ursita' exuded higher amounts of benzoxazinoids when the number of weed plants was higher than that of wheat compared to when both species were at the same density. Contrary, 'Element' accumulated allelochemicals in the roots rather than exude them into the growing medium. 'Midas' did not change its chemical profile when grown alone or with weeds; this variety naturally produces and exudes more allelochemicals than any other variety and does not need to stimulate their exudation or accumulation. Regarding phenolic compounds, a similar pattern of accumulation or exudation was observed for the different varieties and co-cultivated target weed.

In the greenhouse experiments, a reduction in the germination of annual ryegrass was observed after co-cultivation with all tested varieties ('Adesso', 'Ehogold', 'Element', 'Emilio', 'Ludwig', 'Maurizio', 'Midas', 'NS Azra', 'Ursita') in both watering methods. The rainfall method was more effective controlling the weed, as SIC of annual ryegrass was significantly lower when grown together with all varieties. Some varieties also reduced shoot length ('Adesso', 'Ehogold', 'Ludwig', 'Maurizio' and 'Midas') and shoot weight ('Adesso', 'Element', 'Emilio', 'Maurizio' and 'Midas'). Only when grown with 'Ludwig', SW of annual ryegrass was significant higher. In the manual watering treatment, SIC was also the most affected parameter, being reduced by all varieties except 'Ludwig' and 'Ursita'. However, shoot length and weight was less affected as only 'Adesso' and 'Midas' inhibited shoot length and 'Adesso', 'Ludwig' and 'Midas' shoot weight. Wheat growth was almost unaltered as in the *in-vitro* bioassays.

3.3 Summary and conclusions

The studies carried out within this project reveal that different wheat varieties have different abilities to manage neighbouring weeds by allelopathy and that the same variety can behave differently depending on the weed species. The screening of wheat varieties for allelopathic effects is crucial to move forward in organic weed management as allelopathy can support other control measures of weed management, e.g. harrowing or utilising plant traits such as plant height, early vigour etc. In particular the latter control measures might have limitations, e.g. harrowing may not be possible at the right time due to weather conditions or may damage the crop stands due to inappropriate usage or time. In addition, too tall a plant height may cause severe lodging and, therefore, grain yield and quality losses. In our research, a correlation between root exudation and weed inhibition was found. For example, the large amount of root exudates from 'Ursita' resulted in a strong inhibition of both weeds. The same was true for 'Maurizio' and 'Midas', which exuded higher amounts of benzoxazinoids than other varieties, resulting in a low number and very small weeds. However, not all varieties have allelopathic potential. Co-cultivation of annual ryegrass with 'Element' resulted in the stimulation of both germination and root length of the weed. 'Element' did not exude benzoxazinoids from its roots to the growth medium, but accumulated them in the roots, probably as another strategy of defence. Summarising, the most promising varieties to control annual ryegrass according to the *in-vitro* bioassay are 'NS Azra', 'Ludwig', 'Maurizio', 'PS Dobromila', 'Midas', and 'Tobias', while 'Capo', 'Glosa', 'Adesso', 'Annie', 'Ludwig', 'Midas', and 'NS Azra' were the most phytotoxic against common purslane. Under semi-natural



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conditions in the greenhouse a reduction in annual ryegrass was seen without relevant growth changes in the wheat plants. The allelopathic potential of leaf leachates which induced a reduction in annual ryegrass were highest for 'Adesso', 'Ehogold', 'Element', 'Emilio', 'Ludwig', 'NS Azra' and 'Ursita'.

We conclude that different plant genotypes have different capacities to synthesise and exude specialised metabolites, resulting in a wide range of allelopathic or competitive effects on weed germination/seedling growth depending on the prevailing weed species. We demonstrated that different wheat varieties can behave differently towards the same weed. Based on our results a few varieties were identified as promising varieties to control both tested weed species, annual ryegrass and common purslane, via the inhibition of the germination and growth of the weeds and the exudation of higher concentrations of benzoxazinoids from the roots to the rhizosphere. It is essential to understand the mode of action of allelochemicals (benzoxazinoids and polyphenols) as well as the role of other traits, e.g. early vigour, plant height, growth habit, etc., to identify and select wheat varieties suited to organic and low-input farming systems. Considering all traits determined in our studies and the field phenotyping, the most promising varieties for weed control of the tested diversity panel are 'Ludwig' and 'Tobias'.

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4 Marker Assisted Selection

Improving crop traits is highly required for the development of new varieties that can face the challenges of climate change and reduced input of fertilisers and pesticides by maintaining at the same time a satisfying yield potential and end-use quality. Marker-assisted breeding and other genomics approaches can help to reach breeding targets more efficiently and rapidly.

4.1 Resistance genes

Resistance breeding against fungal pathogens as the most environment-friendly and cost-efficient way of plant protection is generally a major goal in wheat breeding. In organic wheat breeding it is of utmost importance as no fungicides can be used in case of epidemics. Especially seed-borne diseases, e.g. bunts and smuts, can devastate organic wheat if mitigation strategies such as seed hygiene, crop rotation or the use of resistant varieties are ignored.

Common bunt

Common bunt is a fungal disease of wheat caused by *Tilletia caries* and *T. foetida* with the primary inoculum source being contaminated seeds. Infection occurs right after seedling emergence, followed by systemic growth of the pathogen in the host until wheat plants reach the stage of fruit development. Infestation with common bunt leads to a replacement of wheat grains by so-called “bunt balls”, which are sori consisting of fungal teliospores. These spores contain trimethylamine, causing a distinct odour resembling rotting fish. Organic wheat production without chemical seed treatment is especially prone to infection by common bunt.

Resistance QTL were identified in a previous project in varieties from the United States and a landrace from Turkey. Based on this work KASP (competitive allele-specific PCR) markers were developed. In total, 31 different markers were used to select for bunt resistance loci. Wherever possible (depending on polymorphisms between donor and crossing parents) at least two markers at flanking positions were used per QTL region. More than 500 breeding lines derived from different back-crosses and multi-parental crosses, grouped in 3 populations, were finally screened for the homozygous presence of resistance QTL and subsequently tested in artificially inoculated field trials across two years (2021-2022) and two locations (Austria and Czechia). In addition, bi-parental mapping populations were used to characterise a new resistance source originating from wheat accession PI 166910 and unlock it for use in breeding programs via MAS.

Results in all 3 populations were similar: resistance loci on chromosomes 1A and 1B provided good resistance levels and a combination of loci generally resulted in a higher resistance than a single QTL (Fig. 9). In the bi-parental mapping populations a major resistance locus was found on chromosome 6D and a second locus with a smaller but still consistent effect on chromosome 4B. The locus on chromosome 6D partly overlaps with a region previously identified to confer dwarf bunt resistance. By a parallel testing of various varieties from the EARLY and LATE diversity panels, as well as from the United States, a high level of resistance across environments and using different races of common bunt, was also identified in ‘Aristar’ from Germany and ‘Deloris’ and ‘UI SRG’



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from the United States. A good level of resistance against most races was also demonstrated for 'Unitar' from Romania.

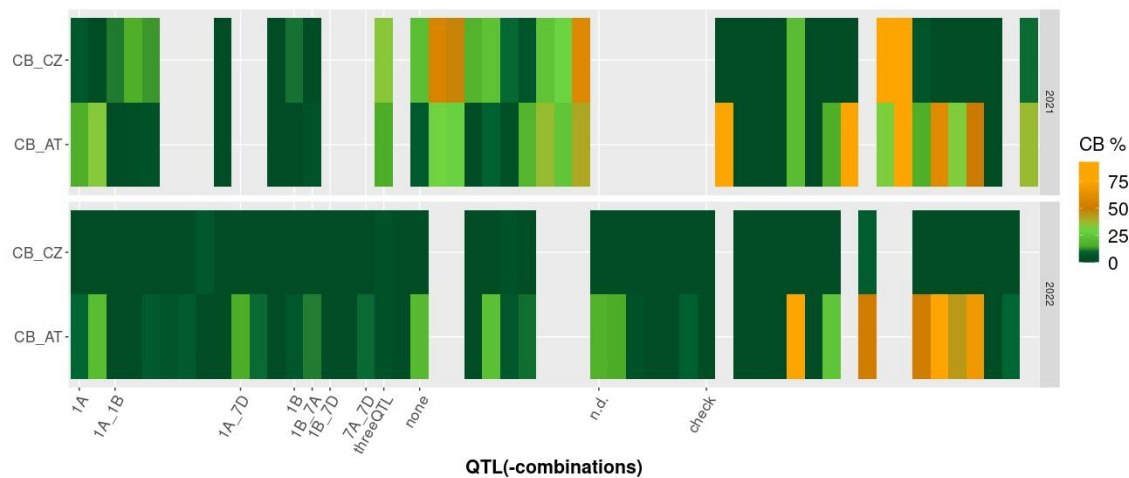


Fig 9 Common bunt incidence of wheat lines scored both in Austria (CB_AT) and Czechia (CB_CZ) in 2021 and 2022. Infection severity is indicated by the colouring of the heatmap and each line is represented as a bar. QTL (-combinations) harboured by individual lines are indicated on the x-axis. The locus on chromosome 7D originates from 'Blizzard'. Lines harbouring three out of the four different selected QTL are marked by "threeQTL", whereas negatively selected lines are indicated by "none". Lines in class "n.d." were not subjected to MAS. Lines in class "check" include varieties and/or breeding lines developed by ECOBREED partners.

Wheat rusts

Rust diseases in wheat are an economically important fungal disease and have a worldwide prevalence as they are able to spread and travel long distances by dispersal of wind-borne spores. Leaf rust (*Puccinia triticina*) and yellow rust (*P. striiformis* f.sp. *tritici*) outbreaks are regularly reported in Europe, while stem rust (*P. graminis* f.sp. *tritici*) was absent in Western Europe for more than 60 years. However, regional outbreaks in Europe in durum and common wheat have been reported in recent years.

More than 100 winter wheat (*T. aestivum*) genotypes representing different gene pools from Europe were subjected to screening for specific rust resistance genes by various molecular marker systems. In this study, molecular markers were applied for leaf rust (*Lr1*, *Lr10*), yellow rust (*Yr5*, *Yr15*), stem rust (*Sr2*, *Sr15*, *Sr26*, *Sr31*, *Sr1A.1R*, *Sr36*, *Sr38*) and complex (*Lr34/Sr57/Yr18/Pm38/Ltn1/Bdv1*, *Lr46/Sr58/Yr29/Pm39/Ltn2*, *Sr31/Lr26/Yr9/Pm8*, *Sr38/Lr37/Yr17*) resistance genes.

A wheat-rye chromosome translocation and thereby the presence of the *Sr31/Lr26/Yr9/Pm8* gene complex was observed with a frequency of 8.1%. While the 1BL.1RS translocation was present in various breeding programmes, the 1AL.1RS translocation was found only in the German and Romanian breeding programmes. Most widely exploited in the ECOBREED diversity panels is the *Sr38/Lr37/Yr17* gene complex from *T. ventricosum* which is present in 35.5% and 48.3% of the EARLY and LATE panel, respectively, followed by *Lr1* (28.9% and 31.7%). *Lr10*, *Lr34* and *Lr46* are present in about 20-30% of the EARLY diversity panel, while these genes were rarely present in the LATE panel. Genotypes with equal or more than 3 resistance genes against leaf rust were



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observed only in the EARLY panel (i.e. 'Mv Uncia', 'CCB Ingenio', 'Solehio', 'BC Lira', 'FDL Amurg', 'NS Mila', 'NS Frajla', 'NS Obala', 'Asory', 'Alex', 'Semnal'). Resistance genes against stem rust were more or less not present in the two diversity panels with the exception of gene complexes transferred either via the *T. ventricosum* or the wheat-rye translocations. Only in varieties 'Graziaro', 'Vulkan' and the populations 'Brandex' and 'Liocharls' (all *Sr15*) other *Sr* genes than *Sr31* or *Sr38* are present.

4.2 End-use quality

End-use quality of common wheat is usually associated with bread making quality which is in turn largely influenced by the quantity and the quality of the wheat protein. In organic wheat production, protein content is generally lower than in conventional production due to lower nitrogen availability. In durum (*T. durum*) a major quality issue is cadmium accumulation in the grain. This heavy metal has negative health impacts and is, therefore, regulated by the EU allowing only a contamination of 0.05 mg/kg.

Grain protein content

The functional *Gpc-B1* allele from wild emmer is responsible for a higher grain protein content, tightly linked to *Yr36* and showed in some genetic backgrounds a pleiotropic effect on grain Zn and Fe concentrations but also a negative effect on grain yield by reducing grain weight. Both PCR-CAPS markers (Xucw71, Xucw79, Xucw83) and a KASP marker (GCP-DUP) were applied to screening screen more than 500 F_{3:4} plants of two crosses (BTX501 Spontan/Glupro and BTX502 Mv Kolompos/Glupro) for the allelic status of *Gpc-B1*. The donor of the functional allele *Gpc-B1b*, 'Glupro', was originally provided by North Dakota State University. MAS was used to develop two bulks homozygous for both *Gpc-B1* alleles in each cross. Analysis of the F₅ bulks grown in Austria in 2022 for protein content by NIRS revealed an increase in grain protein content *Gpc-B1b* bulks compared to the bulks where the allele is absent (+1.1% for BTX501 and +0.6% for BTX502). *Gpc-B1b* also improved significantly dough strength as revealed by rheological properties of the dough. In 2023 F₆ bulks were grown together with some additional isolines in US spring wheat background in organic multi-environment trials (Austria, Germany, Hungary, Romania, Slovakia, Slovenia). Results of these trials were contradictory to the previous year and revealed increased grain protein contents between 0 and 0.9% ($p=0.061$) and a non-significant ($p=0.467$) increase in grain yield (Table 4).



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Table 4 Effect of the functional allele *Gpc-B1b* from wild emmer on grain protein content and grain yield in organic multi-environment trials in 2023. $\Delta\%$ indicates the increase/decrease of the respective trait in percentage relative to the *Gpc-B1a* wild type.

Genetic background	Protein content (%) $p=0.061$			Grain yield (dt/ha) $p=0.467$		
	<i>Gpc-B1b</i>	<i>Gpc-B1a</i>	$\Delta\%$	<i>Gpc-B1b</i>	<i>Gpc-B1a</i>	$\Delta\%$
Choteau	12.7	12.0	+5.8	36.37	34.96	+4.0
Explorer	13.3	12.4	+7.3	30.54	29.14	+4.8
Hank	12.2	12.2	± 0.0	23.60	22.19	+6.4
Mv Kolompos	12.6	12.3	+2.4	35.21	33.81	+4.2
McNeal	12.4	11.3	+9.7	34.07	32.67	+4.3
Overload	10.6	10.3	+2.9	39.72	38.32	+3.7
Spontan	12.9	12.0	+7.5	37.59	36.19	+3.9
UC 1110	11.5	10.9	+5.5	35.34	33.94	+4.1

Cadmium accumulation

The gene *Cdu1* is primarily responsible for low or high grain accumulation of cadmium in durum wheat. Molecular markers were developed to select for the favourable allele 'low' to avoid a high Cd accumulation in durum wheat products, e.g. semolina and pasta.

Leaf samples of 50 genotypes of durum wheat from the DURUM and CRETE diversity panels plus some additional control varieties were collected directly from the Austrian field trial in spring 2022. For 9 genotypes the status of marker *usw47* was known from previous analyses; these nine genotypes were subsequently used as references. For the development of PCR/HRM compatible markers, sequence and primer information was used from several publications. Five primer pairs were adopted or developed and used for the analyses. The five different primer pairs were evaluated concerning their performance in PCR and HRM (high resolution melting). Two eligible candidates were chosen (Cad-5B and ScOPC20) to be used for analysis. At both markers, three clusters could be categorised in the melting profiles, one corresponded with the status 'high' in the reference samples and both others corresponded with the favourable status 'low' in the predisposition for cadmium uptake and accumulation. Significant different, but non the less categorisable melting profiles were also observed and documented. 'Saragolla' was found to carry the 'low' allele, however, other molecular evaluation studies revealed the 'high' allele and/or a heterozygous status for *Cdu-1*. Hence, the exact genotype of 'Saragolla' seems to be largely depending on the seed source as this variety was most probably heterozygous for *Cdu-1* in the origin. More than half of the tested germplasm contained the favourable allele for low Cd uptake. Interestingly, this allele was not only present in modern durum wheat but also in almost all landraces from Crete. Contrary, widely grown durum varieties (e.g. 'Levante', 'Sambadur', 'Simeto') or progenitors (e.g. 'Parus', 'Cappelli') contain the unfavourable 'high' allele. This fact together with the application of MAS should be considered when using this germplasm for crosses.

4.3 Adaptation traits

Wheat is globally cultivated and the most important crop with respect to acreage. Thus, wheat production requires varieties adapted to a broad range of environments. Besides resistances to biotic and abiotic stresses, phenology and plant architecture traits are of



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key importance for providing the necessary variation for adaptation to different environments. Reaction to daylength is determined by the homoeologous *Ppd1* genes located on chromosome group 2. Wild-type alleles of *Ppd1* are associated with day-length sensitivity, i.e. longer days accelerate flowering. Alleles that confer day-length insensitivity are associated with earlier heading and flowering date and also with an increased rate of spikelet development and higher spike fertility. Dwarfing genes in wheat enabled spectacular increases in grain yield arising from the 'Green Revolution'. The gibberellin-insensitive genes *Rht-B1b* and *Rht-D1b* are the primary sources of semi-dwarfism in wheat. Besides their positive effect on lodging tolerance, they are associated with increased susceptibility to Fusarium head blight.

Molecular markers for *Ppd-A1* and *Ppd-D1*, as well as for *Rht-B1* and *Rht-D1* were applied to screen the EARLY and LATE panel. Almost all tested genotypes from the LATE panel carry the *Ppd-A1b* allele (sensitive), a few the null allele from 'Cappelle-Desprez'. Five LATE genotypes (12.8%) carry the *Ppd-D1a* allele ('Dagmar', 'Liseta', KM72-18, KM78-18, 'Wital'), whereas in the EARLY nursery 62.3% of the tested genotypes carry the allele for photoperiod insensitivity. The dwarfing allele *Rht-B1b* is present in 43.8% of the tested genotypes of the EARLY nursery; this dwarfing allele is especially prevalent in modern Hungarian and Romanian varieties and breeding lines, while the dwarfing allele *Rht-D1b* is present only in Hungarian 'Mv Karizma' and French 'Anapurna'. Contrary, in the LATE nursery *Rht-B1b* is present only in 'Asory', *Rht-D1b* has a slightly higher frequency, being present in four varieties, i.e. 'Asory', 'Curier', 'Moschus' and 'Rübezahl'. Hence, 'Asory' is the only variety of the ECOBREED diversity panels carrying both dwarfing alleles. Within *Rht-B1a*, insertions predominate in the LATE nursery. The 160 bp is present in more than half of the investigated genotypes, the 197 bp insertion in 13%.

4.4 Summary and conclusions

The low frequencies of *Sr* genes in the ECOBREED germplasm reflects the low importance of stem rust in the last decades in Europe but also the danger of high losses due to increased susceptibility in case of the appearance of *Ug99* and other new races as already observed in recent years in Mediterranean environments. Contradictory results from different marker systems with respect to *Yr15* demonstrated that some published markers may not provide reliable results in other than the originally studied germplasm, hence, results from MAS should not be taken for granted.

The *Gpc-B1b* allele showed contradictory results in the two test years 2022 and 2023. While in the first year a significant increase in grain protein content and a decrease in grain yield was recorded, the increase in protein content was not significant in 2023 at $p=0.06$. Otherwise, a negative effect on grain yield was not observed which is a good message that this allele could indeed be exploited in European wheat genetic backgrounds without reducing grain yield. However, it has to be considered that the original donor of *Gpc-B1b* is low yielding and not adapted to European growing conditions. Application of MAS in targeted back-cross programmes may be the best strategy for a fast and efficient exploitation of this favourable allele in organic breeding programmes. Similarly, MAS can be applied to efficiently select for low cadmium



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accumulation in durum wheat as also for this gene MAS proved to be very efficient in identifying germplasm with the favourable allele for low Cd grain accumulation.

Analysis for photoperiod sensitivity and dwarfing genes revealed that especially in the Eastern European breeding programmes favourable alleles for both traits are enriched. However, with respect to organic farming it is questionable if the currently used dwarfing genes are suitable to guarantee good weed suppression. Other dwarfing genes, e.g. *Rht24* with less significant effects on plant height and no negative effect on Fusarium head blight might be more favourable in that respect.

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5 Development of new wheat germplasm for organic farming

5.1 Multi-parent advanced generation inter-cross populations

Two 8-parent MAGIC populations were developed for further selection of either pure line breeding lines or development of organic heterogeneous material (syn. evolutionary bulks or populations) under organic conditions. The first crosses started in 2019 and the final cross was done in 2021. Afterwards, the two populations were multiplied in 2022 and 2023 as bulks in Hungary, and in Austria as ear-to-row progenies. Material was further distributed in autumn 2023 as bulks to partners in Germany, Czechia, Slovakia and Slovenia. The first MAGIC population contains mainly varieties from the EARLY panel, i.e. 'Glosa' and 'Ursita' from Romania, 'NS Ilina' and 'NS 40S' from Serbia, 'Mv Karej' from Hungary, 'PS Dobromila' from Slovakia, 'Ehogold' from Austria and 'Nexera 923' from Slovenia. The second MAGIC population includes mainly varieties from the LATE panel, 'Wendelin' and 'Spontan' from Germany, 'Arminius' and 'Tobias' from Austria, 'Viki' from Czechia, 'IS Laudis' from Slovakia, 'Mv Kolompos' from Hungary and Xt88.5R from Slovenia. The detailed pedigrees are:

- (i) Glosa/NS Ilina//Mv Karej/PS Dobromila/3/Ursita/NS 40S//Ehogold/Nexera 923
- (ii) IS Laudis/Mv Kolompos//Arminius/Xt88.5R/3/Viki/Wendelin//Tobias/Spontan

Ear-to-row progenies were evaluated in 2023 for heading date, plant height and disease resistance. Rows with similar development stage and plant height were harvested together in order to create sub-populations (Table 5). These sub-populations are currently multiplied in Austria on both an organic and a conventional managed field. Additionally, 159 and 151 ear-to-row progenies of the Early and Late MAGIC populations, respectively, are multiplied for harvest in 2024. This germplasm should be the basis for a future long-term experiment on the response to selection in evolutionary bulks versus pure/inbreeding lines.

Table 5 Sub-populations of the ECOBREED MAGIC populations created by BOKU.

Bulk	Early MAGIC		Late MAGIC	
	BBCH 24/05/2023	Plant height (cm)	BBCH 24/05/2023	Plant height (cm)
I	58-59	80-90	47-49	75-85
II	51-59	95-125	47-49	90-110
III	61-67	65-90	51-53	80-90
IV	61-65	95-130	51-57	95-115

5.2 Organic heterogeneous material

Composite cross populations (CCPs) were developed in Slovenia using wheat accessions with increased male sterility and accessions which carry the *Rf3* fertility restoration gene. Thereby, natural out-crossing should be favoured. Grain yield of these CCPs, however, was inferior compared to the average of the EARLY panel when tested in Hungary. Hence, this germplasm was not followed up in further trials. The Mv BIO2020 POP was developed in Hungary by mixing some F₂ populations of crosses between mainly Hungarian and



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Romanian genotypes from the ECOBREED nurseries. This new CCP was delivered to Hungarian organic farmers in autumn 2020 for sowing within farmer's participatory trials. A similar approach was used in Austria to develop nine CCPs including up to 10 different parental genotypes. Field tests in Austria and Slovakia revealed that none of these CCPs outyielded the control varieties 'Aurelius', 'Capo' and 'Arminius'. Moreover, the protein content of these CCPs was in almost all cases lower than for the control varieties. Hence, most of these CCPs were discarded and only two were sown in autumn 2023 for further multiplication. Another population called NS OBI-CCP was created in 2022 in Serbia from mixing segregating progenies of some crosses carried out in ECOBREED material. This population was distributed to an organic farmer for further selection and was resistant against *Septoria tritici*, leaf and stem rust and showed a low susceptibility against yellow rust in 2023. Grain yield was medium-low but quality traits were good.

5.3 Breeding lines

More than 100 crosses within the ECOBREED germplasm were carried out by 8 partners in winter common wheat from 2020 onwards. From these crosses more than 1000 ear-to-row progenies are sown in the field for further selection in 2024 by all partners. The most advanced lines are currently in F₇. One advanced line from Czechia, ST 1518/19 successfully passed the first year of organic VCU and seeds were delivered to the national authority UKZUZ for further official testing. Parallel to the official testing, maintenance breeding of this line was started at the newly established organic trial site of Selgen a.s. in Stupice, Czechia. Besides common wheat, a few crosses and back-crosses were also carried out with material from the DURUM diversity panel by partners in Italy and Hungary.

5.4 Perennial wheat

Advanced perennial bulks (#1, #6, #13 and #16) derived from inter-specific crosses of common wheat with intermediate wheatgrass (*Thinopyrum intermedium*) were originally received from Washington State University. The material was tested in Austria and Germany. After 3 years of growing the trial was abandoned in Austria due to winter damage and reduced regrowth which in turn caused significant weed infestation. In Germany, however, selection within the bulks was carried out for regrowth, biomass, and grain yield. Different sowing dates with these perennial bulks showed that sowing in spring resulted in lack of uniformity with respect to heading and maturity and therefore, grain was not harvested. Under-sown clover had no positive effect on weed suppression in the perennial wheat plots as expected. *Trifolium repens* developed too much biomass and, therefore, competed with the perennial wheat, while *T. subterraneum* did not suppress the weeds. Fertilising with 40 kg N/ha horn manure resulted in an increase in grain yield from 0.89 (control) to 1.18 t/ha. Generally, grain yield of the perennial wheat bulks was 50% or more lower compared to the organic common wheat check 'Wendelin' (2.5 t/ha). Two seed multiplications with in total 1.5 ha were harvested in 2022. In 2023 a third multiplication was done by a farm in Saxony which plans to grow perennial wheat on a larger acreage for a nature conservation project. The seeds of these selections are marketed under the ECOBREED trademark.



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In Slovenia more than 20 intermediate wheatgrass accessions were tested since 2018 along with more than 100 different other *Triticeae* accessions. More than 40 crosses and back-crosses between *T. aestivum* and *Th. intermedium* were carried out. Finally, F₃ progenies of two crosses (i.e., Xt220.1 = *T. aestivum*/2**Th. intermedium*; Xt220.2 = *T. aestivum*/*Th. Intermedium*) were selected for further selection and development. While regrowth and spike morphology of these progenies is good, grain yield is very low due to reduced spikelet fertility and low grain weight.

In Austria a small observation plot was established in 2020 with Kernza® *Th. intermedium* material received from The Land Institute, Salina, Kansas. Regrowth of this material is excellent and no problem with weed infestation appeared since then. However, grain yield is very low and due to the hulled nature of the seed a tedious dehulling process would be necessary for processing. Moreover, new crosses were carried out in 2021 between different genotypes derived from inter-specific crosses. For two crosses (MT-4/Salish Blue and Salish Blue/Agrotana) hybridisation and regeneration of F₁ plants was successful, however, only 3 and 1 plants were recovered, respectively, which was most probably due to chromosome abnormalities in the hybrids as chromosome number between the used × *Agrotriticum* types varied between 28 and 56. The alien genes for perenniality in this material were originally introgressed from *Th. intermedium* and *Th. elongatum*. The 4 plants are since 2022 multiplied vegetatively (Fig. 10) by regular cutting. Regrowth proved to be excellent, however, as for the other germplasm seed set is low.



Fig 10 Perennial wheat plots sown at different dates in Germany (left) and regrowth of progenies of × *Agrotriticum* crosses end of October 2022 in Austria after harvest in July (right). The already heading plant is derived from the cross Salish Blue/Agrotana, the other three plants are from the cross MT-4/Salish Blue. The plot in the background is *Thinopyrum intermedium* Kernza® in its 3rd year of cultivation

5.5 Summary and conclusions

MAGIC populations are created by several generations of inter-crossing among multiple founder lines. Multiple founders contribute more allelic diversity and multiple cycles of inter-crossing give greater opportunities for recombination. Such multi-parental populations are nowadays widely used for the mapping of genes/QTL, however, may also be used for the creation of heterogeneous material for on-farm selection. Within the project two MAGIC populations were developed and distributed among partners for further selection work. In future projects these populations may be used for studying



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long-term effects of both natural and human selection on allele diversity within and between (sub)-populations.

Work within this project has shown that perennial wheat is far from becoming a successful grain crop in near future. However, it may play a role in providing ecosystem benefits by avoiding soil erosion, increase humus content and soil biodiversity, increase carbon sequestration, provide biomass for sustainable grazing/forage systems, etc. Definitely more research is needed to improve the currently existing germplasm. Further research should also focus on the development of new diploid and tetraploid perennial germplasm in order to probably overcome problems of spikelet fertility.

Bi-parental crosses and back-crosses done by the project partners were especially carried out with germplasm from other breeding programmes and gene pools. Thereby genetic diversity was increased. Although some diversity will be lost in the forthcoming site-specific selection cycles, some favourable alleles will be exploited by the respective breeders and therefore help to increase the genetic diversity in their own material. The project made a significant impact in the exchange of germplasm as many partners had no material exchange with each other before this project.

Further reading

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