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(eds.)

# SYMPOSIUM ON BREEDING FOR DIVERSIFICATION

A Joint Meeting of the EUCARPIA Section  
Organic and Low-Input Agriculture,  
ECO-PB, DIVERSify, INSUSFAR, HealthyMinorCereals,  
LIVESEED, ReMIX and Wheatamix

University of Kassel,  
19<sup>th</sup>–21<sup>st</sup> February 2018  
Witzenhausen

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**Abstract Booklet**

# **SYMPOSIUM ON BREEDING FOR DIVERSIFICATION**

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Jelena Baćanović-Šišić  
Dominic Dennenmoser  
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## Foreword

The knowledge that diversity in agroecosystems increases stability and resilience is age old and the beginning of breeding for diversity go back to the early 20<sup>th</sup> century. Species-rich systems often show higher productivity than monocultures, reduced pest and disease severity, improved resource capture, and greater resilience to environmental fluctuations. However, it is only since the 1980s that some work on within-crop diversification other than the use of mixtures and multi-lines has regained momentum. Since the beginning of this century, there has been an increasing interest in the scientific community in Europe and worldwide in the opportunities in breeding for diversity. Starting with some more or less well financed national projects on population breeding in the UK, France and the USA, often accompanied by participatory breeding, the number of national and international projects addressing issues of breeding for diversity has increased in the last two decades.

One culmination of the recent efforts is the EU Commission implementing decision 150 of March 2014 'on the organisation of a temporary experiment providing for certain derogations for the marketing of populations of the plant species wheat, barley, oats and maize pursuant to Council directive 66/402/EEC'<sup>1</sup>. In the directive, rules are set as to what populations are. For example, at least five genotypes have to be crossed pairwise and the progeny bulked and exposed to natural selection (Vollenweider and Spieß 2018). In addition, rules are set with respect to the amount that may be marketed and there are requirements of traceability.

The possibility to make use of populations legally and the recognition of the need for diversity at all levels has resulted in a significant increase in research efforts. Throughout Europe several current projects have emerged that are working towards breeding for diversity or for greater diversification of agroecosystems. For this first EUCARPIA Section Organic & Low-input Agriculture symposium on Breeding for Diversification, four current EU, one French, and one German project that are engaged in related activities have teamed up. These six projects all have different specific aims and deal with various crops and they are presented in brief below. The overall common view is that for agricultural systems of the future, increased diversity at all levels, from the microorganisms up to the landscape will be crucial in order to be able to reduce inputs and to stabilise—and potentially even increase—overall system productivity.

We are very happy that in this symposium researchers from a multitude of countries around the world are coming together in order to discuss issues about breeding for intra- and inter-specific diversity as well for annuals as for perennials. The submitted abstracts show that the activities are far wider than just the cereals

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<sup>1</sup><http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32014D0150>



that are covered by the EU directive and that there will be a need to expand the activities on the legal side.

We hope that this symposium and the following EUCARPIA/EC-PB session on practical organic breeding as well as the LIVESEED/EUCARPIA/ECO-PB workshop on new concepts and strategies on 21<sup>st</sup> February. will help to bring together some of the many persons interested in breeding for diversity and to show the many different aspects that have to be considered depending on crop, aims, and the social systems concerned. Diversity in approaches and solutions is needed as this is the principle of diversification that there is no one size fits all approach.

We wish you a wonderful symposium with many interesting and inspiring interactions from concepts to practical applications and also on a very personal level. Inspiration in Science as everywhere in life is a personal and interactive process and we hope that we have been able to create a space for this.

G. Backes, J. P. Baresel, J. Enjalbert, M. R. Finckh, L. P. Kiær,  
E. Lammerts van Bueren, I. Litrico, M. Messmer

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## The projects that teamed up for this symposium



**LIVESEED** with the full title '**Improving the performance of organic agriculture by boosting organic seed and plant breeding efforts across Europe**' ([www.liveseed.eu](http://www.liveseed.eu)) is based on the concept that cultivars adapted to organic systems are key for realising the full potential of organic agriculture in Europe. The

LIVESEED project (2017–2021) is coordinated by IFOAM EU with FiBL for scientific coordination and consists of 35 partners and 14 third linked parties from 18 European countries. This project receives funding from the European Union's Horizon 2020 research and innovation program under grant agreement No 727230 and by the Swiss State Secretariat for Education, Research and Innovation under contract number 17.00090.

LIVESEED will help to establish a level playing field in the organic seed market across Europe, improve the competitiveness of the organic seed and breeding sector, and encourage greater use of organic seeds by farmers. LIVESEED will improve guidelines for cultivar testing and strategies for ensuring seed health. It will develop innovative breeding approaches suited to organic farming. Finally, it will investigate socio-economic aspects relating to the use and production of organic seed and their interaction with relevant (EU) regulations.

By connecting several networks LIVESEED will combine **co-construction and exchange of knowledge** integrating biological, technical, legal, organisational, financial and political aspects as well as market development to facilitate fast upscaling and outreach of various tailor made socio-technical, evidence based, innovation tracks. LIVESEED will generate (i) a tool box of measures and interventions to **match production and demand of organic seed**, (ii) validated options for competent authorities to reduce the number of derogation for untreated conventional seed, (iii) technical solutions for national databases of organic seed including an interface to a European wide router database to ease data entry for seed producers, (iv) in depth analysis of formal and informal seed chains, (v) various business models including checklists for setting up new multiplication and breeding initiatives, (vi) technical factsheet on the best practice of seed production for major crops including vegetative plant propagation material, and (vii) an organic seed quality strategy including quality control of farm saved seeds and efficiency of seed exchange networks.

With respect to **organic plant breeding** LIVESEED will provide new concepts for designing and implementing decentralised breeding initiatives for organic and low-input agriculture through (i) establishing networks across Europe (e. g. for apple, brassica vegetables) and fostering public-private partnerships, (ii) combining farmer, value chain or community based system breeding with functional trait-based,

and molecular breeding approaches, (iii) identifying trade-offs between resilience traits and sensoric and nutritional quality, (iv) developing breeding schemes for heterogeneous cultivars and species mixtures, and (v) elaborating different financing models. Considering the plant as a mega organism, including the above and below ground associated microorganism, has the potential to evoke a paradigm shift in plant breeding, and will be elucidated in case studies.



**INSUSFAR** stands for '**Innovative approaches to optimise genetic diversity for sustainable farming systems of the future**'. It is a cooperative research project, funded by the German Ministry for Education and Research (BMBF, 2015–2020) in the frame of the funding initiative "Innovative Pflanzenzüchtung im Anbausystem" (IPAS, Plant Breeding innovations in the Farming System). The Project is being performed jointly by the Technical University of Munich, The University of Kassel and the Julius-Kühn-Institut ([www.insusfar.de](http://www.insusfar.de)).

Sustainable agricultural systems will need an excellent capacity for self-regulation and self-regeneration to allow for overall reduced external inputs while maintaining or increasing overall system output including delivery of ecological services. Biodiversity at all levels is a major system component allowing for efficient self-regulation. However, there is a lack of knowledge about the optimum level of diversity needed for high yield and yield stability, while at the same time achieving maximum self-regulation and self-regeneration to reduce the necessary inputs in a given agricultural system.

The overall aim of INSUSFAR is to achieve a better understanding of how genetic diversity in self-pollinating crops with a focus on wheat and barley grown in production systems characterised by reduced tillage and the use of living mulch crops can be optimised. Adaptation of breeding innovations to agricultural systems differing in input and diversity levels is analysed to determine which types will be necessary for diversified sustainable systems. Also, the potential of plant breeding innovations for the development of sustainable production systems is investigated by analysing their performance when grown in systems characterised by different input levels and different levels of system specific species diversity. Varieties, lines and populations as well as the genetic changes in genetically diverse populations are studied in model cropping systems and on-farm. Besides crop performance, ecological and economical parameters are assessed. The results will be reflected for their potential effects on agricultural practices and breeding methods and goals as well as the political and administrative measures that might be necessary to support sustainable agricultural development. As breeding is a long-term process, a critical aim is also to ensure that the data generated in the project will be available for future research as public and readily accessible source kept in an information system.



**DIVERSify** (2017-2021), '**Designing InnoVative plant teams for Ecosystem Resilience and agricultural Sustainability**' ([www.plant-teams.eu](http://www.plant-teams.eu)) is a four-year project funded by the European Union's Horizon 2020 research and innovation programme under grant agreement No. 727284. The multi-disciplinary consortium consists of 23 beneficiary partners, involving scientists, participatory farmers, advisors, breeders, and technology providers. These are located across diverse pedo-climatic conditions in Europe, and including international partners in Kenya, Palestine and the dry regions (Lebanon, Morocco).

The project aims to develop a novel system for sustainable food production, combining stakeholder expertise, scientific knowledge and farm technology to enhance the productivity, stability and resilience of legume-cereal and species-rich grassland mixtures (combined: 'plant teams'). A novel approach is taken, using ecological theory to explore the associations between plant trait diversity and beneficial mechanisms within these agroecosystems.

Building on knowledge and best practice of innovative practitioners, field trials and modelling are used to optimise trait diversity and identify variety and species combinations that optimise agronomic, environmental and socio-economic performance parameters under conventional and reduced input management across Europe and beyond. Promising plant teams will be demonstrated and validated on field-scale by participatory farmers in diverse pedo-climatic conditions, along with potential new technology. Project outputs include an open data infrastructure on crop plant traits and plant team performance, a decision aid for agronomists and farming practitioners to select suitable plant teams for the current and future climatic conditions in their region, adaptations and/or re-purposing of farm implements for agritechnologists and farmers for plant team management (including PAT), as well as novel tools and concepts to support breeders and crop scientists in plant team optimisation.



**HealthyMinorCereals** is an acronym for '**An integrated approach to diversify the genetic base, improve stress resistance, agronomic management and nutritional/processing quality of minor cereal crops for human nutrition in Europe**'. It is an FP7 cooperative project involving sixteen participants from ten European/Associated countries: Austria, Czech Republic, Estonia, Germany, Greece, Hungary, Poland, Switzerland, Turkey and the United Kingdom.

For the major crops, productivity on the European and global scale has significantly improved due to high external inputs and breeding activities targeting for these

conditions. This both lead to a reduction of diversity within these crops as well as on the level of the distribution of crops in the cultivated area, resulting in low resilience to the changing climatic conditions. The higher variance of yield from year to year is the consequence of this development. On the other hand, minor cereals have retained their ability to grow under more stressful and low-input conditions. Furthermore, they have a higher concentration of micronutrients and other interesting features for a healthy diet.

The main objective of the project is enhancing the exploitation of five of the so-called 'minor cereal' species—spelt, rye, oat, einkorn, and emmer. On the genetic level, the available germplasm of spelt, rye, oat, einkorn, and emmer, as well as of wheat wild relatives, are analysed for genetic structure and diversity in order to better exploitation of these resources. On the phenotypic level, comprehensive phenotyping for yield, resistance to important fungal diseases, nutrient use efficiency and quality is carried out in multi-locus field trials. Further, the content of micronutrients, phenolic and antioxidant content, as well as harmful / anti-nutritive components, are analysed in a large set of genotypes of different minor cereals. On the level of processing, milling is optimised; food preparation methods and novel products are developed for higher consumer acceptance, processability and stability. In order to analyse and improve the use of minor cereals and the acceptance of the respective products, demonstration fields for farmers, market analyses and other dissemination activities are part of the project.



**ReMIX** (EU H2020: 2017-2021) stands for '**Redesigning European cropping systems based on species MIXtures**' ([www.remix-intercrops.eu](http://www.remix-intercrops.eu)). The goal is to exploit the benefits of species mixtures (cereal and legume) to design productive, diversified, resilient and environmentally friendly agro-ecological crop-

ping systems less dependent on external inputs than current systems and acceptable to farmers and actors in the agri-food chain. Species mixtures enhance water and nutrient use efficiency and improve the control of pests, diseases and weeds, while increasing crop productivity and resilience to biotic and abiotic stresses, including those triggered by climate change.

So far, plants have been primarily bred under sole crop conditions. However, the performance of a variety grown as a sole crop does not necessarily represent its performance in a mixed cropping system. Little work has been carried out and formalised on plant breeding approaches for species mixtures. It is necessary to identify these key-traits and their value for mixture performance and introduce them as selection criteria in breeding schemes. Thus, one of objectives of Remix is to pave the way for a renewed genetic improvement of species involved in species mixtures for modern and traditional varieties.



**WHEATAMIX** stands for '**Studying Wheat cultivar Mixtures to assess the impact of within-crop genetic diversity on the multi-functionality and resilience of cropping systems under global change**'. The project is based on a highly multidisciplinary approach, involving geneticists, agronomists, ecophysiol-

ogists, ecologists, economists and management scientists, with a consortium composed of 10 French research labs, as well as key stakeholders (6 Chambres d'Agriculture, farmers: [www.inra.fr/wheatamix](http://www.inra.fr/wheatamix)).

Increasing within-field diversity through the use of cultivar mixtures is a timely option, testified by some significant 'success stories' in the past, and recent bibliographic reviews. Yet, cultivar mixtures are poorly developed worldwide. In this context, the Wheatamix project studies the interest of mixing wheat genotypes to reinforce the sustainability, resilience, and multi-functionality of agriculture. Analysing the interactions among genotypes and with the environment, Wheatamix develops new blending and breeding methods to obtain performing mixtures. Complementary experimental approaches are being deployed: (i) a diversity experiment to quantify the effects of wheat diversity on ecosystem services; (ii) replicates of the same diversity experiment in 4 sites across France under low and high inputs, to test diversity effects under a wide range of soil and climate conditions; (iii) a network of 30 farms, encompassing agro-climatic variability in the Paris basin, to compare the ecological and techno-economic performance of blends and monocultures; (iii) a socio-economic study based on interviews of the wheat supply chain, to characterise the organisational and economic impact of this practice.

Wheatamix has improved the description of the various ecosystem services provided by genetic diversity (yield stability; regulation of foliar diseases; pest and weed biocontrol; maintenance of soil fertility; biodiversity conservation), and is raising important methodological and conceptual issues, crucial when studying the causal link between bundles of functional traits and delivered groups of services. The co-conception of cultivar mixtures developed in the project has also brought both practical knowledge and rules to blend varieties, and theoretical questions to better understand and improve plant-to-plant cooperation in mixtures. Finally, the Wheatamix project emphasises the need of a pluri-disciplinary approach when addressing agroecological subjects, and illustrates the strong mutual benefits of interactions between agronomic and ecological sciences.



## Symposium Program

### Monday 19. February 2018

- 13.00–14.00: Registration for Symposium
- 14.00–16.45: ***Breeding for intra-species diversity***
- 14.00: **Welcome to the Symposium**
- 14.05–14.15: **Beat Boller for EUCARPIA**
- 14.15–14.55: **Lars Kiær, University of Copenhagen (DK):** Trait plasticity and G×E challenges when breeding for mixture-ideotypes
- 14.55–15.15: **Enjalbert *et al.*:** WHEATAMIX: increasing within-field wheat diversity to foster the multi-functionality and sustainability of wheat production in the Parisian
- 15.15–15.45: ***Coffee break***
- 15.45–16.05: **Dumalasoová *et al.*:** Disease resistance of spelt wheat—results from the HealthyMinorCereals project
- 16.05–16.25: **Baresel *et al.*:** INSUSFAR: Innovative approaches to optimise genetic diversity for sustainable farming systems of the future. Intra-specific diversity of wheat for increased resilience and high yields and system specific adaptability
- 16.25–16.45: **Horneburg *et al.*:** On-farm management as a tool to maintain diversity and to select superior genotypes
- 16.45–18.00: ***Guided poster session on intra-species diversity***
- 18.00–20.00: ***Diner and social gathering***

### Tuesday 20. February 2018

- 9.00–11.30: ***Breeding for inter-species diversity annual crops***
- 9.00–9.40: **Liesje Mommer, Wageningen University (NL):** Lessons for agriculture: below-ground insights from biodiversity experiments
- 9.40–10.00: **Ayenan *et al.*:** Participatory designing of pigeon pea (*Cajanus cajan* (L.) Millsp.) ideotypes for adaptation to various cropping systems in Benin
- 10.00–10.20 **Arncken *et al.*:** Breeding for mixed cropping and anthracnose resistance of lupins



- 10.20–10.40: Moutier *et al.*:** Breeding for mixtures in organic farming systems: are the traits of pure wheat cultivars predictive of their behaviour in wheat-pea mixtures?
- 10.40–11.30: *Coffee and guided poster session on inter-species diversity***
- 11.30–12.15: *Documentation of diversity breeding***  
**Bülow *et al.*:** Documentation of diversity breeding: The Information system ROBUSTUM
- 12.15–13.15: *Lunch***
- 13.15–14.55: *Breeding for inter-species diversity perennial crops / agroforestry***
- 13.15–13.55: Martin Wolfe, Wakelyns Agroforestry (UK):** Maximising diversification at the agricultural end of short food and energy chains
- 13.55–14.35: Cyril Firmat, INRA Lusignan (FR):** Breeding for mixtures: varieties of approaches, opportunities and limitations
- 14.35–14.55: Cadena González *et al.*:** Traditional agroforestry systems and options of crop-tree combinations, with focus on conservation of native species
- 14.55–15.25: *Coffee break***
- 15.25–18.00: *General concepts for breeding on improved diversity***
- 15.25–16.05: Paolo Annicchiarico, Centro di Zootecnia e Acquacoltura (CREA-ZA) Lodi (IT):** Interspecific competition experiments aimed to define breeding strategies
- 16.05–16.45: Jacob Weiner, University of Copenhagen (DK):** The concept of ideotypes in a crop mixtures context
- 16.45–17.05: Knapp *et al.*:** The relation of intra-genotypic, intra- and inter-specific competition and related canopy architecture traits
- 17.05–17.25: Lingner *et al.*:** The cropping system matters—contrasting responses of winter faba bean genotypes to drought stress
- 17.25–17.45: Janovská *et al.*:** An integrated approach to diversify the genetic base, improve stress resistance, agronomic management and nutritional/processing quality of minor cereal crops for human nutrition in Europe
- 17.45–18.00: Edith Lammerts van Bueren summarising the Symposium**
- 19:00: *Symposium dinner***

## Wednesday 21. February 2018

**9.00–17.00: *Organic breeder's day***

**9.00–10.30:** Open EUCARPIA/ECO-PB Session on Practical organic breeders presentation (invited speakers only)

**10.30–11.00: *Coffee break***

**11.00–12.00:** Open EUCARPIA/ECO-PB Session on Practical organic breeders presentation (invited speakers only)

**12.00–13.00: *Lunch***

**13.00–14.00:** Introduction to the concept of systems-based breeding by Edith Lammerts van Bueren

**14.00–14.20:** Group work to apply concept on your own breeding activities

**14.20–15.20:** World Café (3 × 20 min)

**15.20–15.40: *Coffee break***

**15.40–16.10:** Presentation World Café results

**16.10–17.00:** General discussions on the systems-based breeding concepts, challenges and action needed, examples to illustrate the concept and definition of a roadmap to substantiate the concept and achieve paradigm shift in attitude



## **Breeding for intra-species diversity**



# Trait plasticity and G×E challenges when breeding for mixture-ideotypes

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## Introduction

Plant traits are increasingly being utilised for designing crop mixtures that enhance benefits and services such as improved resource use efficiency, pest and pathogen management and yield stability. Recent efforts to devise mixture ideotypes, assembly rules and mixture breeding design (e. g. Litrico and Violle 2015) are good examples. Being inspired partly by philosophies and findings of the widely successful large-scale biodiversity experiments (e. g. Scherber et al. 2010), these trait-based approaches aim to embrace plant community complexity at levels beyond the assignment of general genotypic traits for mixing ability and competitiveness. To be successful, they should ultimately be able to predict and optimise ecological interactions between contrasting crop components under given environmental conditions based on a diverse array of phenotypic traits (Kiær et al. 2012; Mille et al. 2006).

Studies using the morphological, physiological and phenological properties of genotypes to design specific crop diversity have mainly obtained this information from monoculture trials and experiments. Yet, it is extremely well-documented that most plant traits are characterized by a certain level of plasticity in response to the perceived environment. The phenotypic plasticity that enables plants to adapt to a range of growing conditions also shapes their ecological niche in the community (Berg and Ellers 2010), and ultimately, govern the yield stability of agricultural crops (Lazzaro et al. 2017). Although past breeding may have somewhat reduced the plasticity of modern temperate crop varieties (Gage et al. 2017), significant levels of crop trait plasticity are reported on a regular basis (e. g. Kumar et al. 2017), also in response to mixing (Zuh et al. 2016).

## Material and Methods

We hypothesise that the altered environment in mixed crop stands relative to monocultures can induce plastic adaptations in all crop partners throughout development. In a number of recent projects, we assessed a wide range of morphological, physiological and phenological traits in inter- or intra-specific crop mixtures: variety mixtures of spring barley in organically managed field trials; and

grain legume-cereal intercrops in low-input and conventionally managed field trials. Mixed-effects models were used to test for trait plasticity (factor: diversity level) and its dependence on neighbour identity (interaction factor: neighbour variety) and management (interaction factor: input level).

## Results and Discussion

The level of plasticity differed considerably among traits, species and mixture types. As an example, plant traits of both species in a pea-barley intercrop changed significantly when grown in a mixture. Pea plants responded by having shorter stems, lower biomass, thicker leaves (smaller specific leaf area), and fewer reproductive nodes, but higher total biomass of reproductive organs (close-to-significant). Barley plants grew taller, produced more tillers and consequently more heads and higher leaf biomass, as well as a higher chlorophyll content. The mixing response of the two species differed for a majority of traits, whereas varieties within each species generally responded similarly to mixing. Some significant neighbour effects were found as well. Comparisons between high- and low-input treatments will be made. The results suggest that trait plasticity in response to mixing is larger in legumes than in cereals. Our findings support that plasticity in key diversified traits can contribute to complex Genotype  $\times$  Genotype  $\times$  Environment  $\times$  Management interactions. This challenges the framework used to design and breed for optimal phenotype combinations in the field, pointing to several implications. First, a better understanding of crop trait plasticity in general, and in crop mixtures specifically, is vital in order to define appropriate evaluation methods when breeding for mixtures. Second, the much-desired benefits and services from growing crop mixtures may not be optimal based on monoculture information alone. Third, the finding of 'neighbour effects' suggests that breeding strategies and assembly rules used to obtain mixture-ideotypes must consider various sources of trait plasticity; even genotypes bred within a mixture are likely to interact and perform differently in a different neighbourhood.

Fourth, the complex responses and interactions between mixing partners suggests that it is unlikely to identify genetic markers contributing substantially to general 'mix-ability'.

The continued effort to develop assembly rules and breeding schemes for crop mixtures would benefit from directly addressing and incorporating aspects of trait plasticity. A combination of ecological theory, biophysical modelling and empirical data could be used to derive a new class of field trial designs, the outputs of which would then serve as input for  $G \times G \times E \times M$  interaction analyses. Some of the many unresolved questions that will also be addressed in the presentation include: 'What trait categories are more plastic in modern temperate crop varieties?', 'Does trait plasticity tend to reinforce or reduce niche overlap?', 'To what extent does this influence mixing effect and the ability to predict it?', 'How important is the effect of neighbour phenotype relative to the effect of not growing with

conspecifics?', and 'Should trait plasticity considerations have direct influence on fundamental management decisions such as fertilisation, sowing density and genotype proportions?'

## Acknowledgments

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## Wheatamix: Increasing within-field wheat diversity to foster the multifunctionality and sustainability of wheat production in the Parisian basin

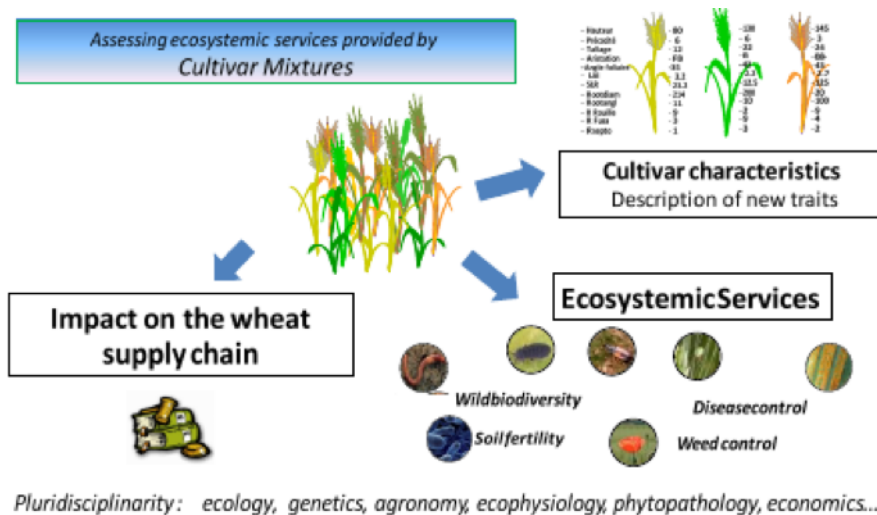
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## Introduction

Among the major changes needed to increase agricultural sustainability, one key lever is to better mobilise crop genetic diversity. Increasing within field diversity through the use of cultivar mixtures is a timely option, testified by some significant 'success stories' in the past, and recent bibliographic reviews. Yet, cultivar mixtures are poorly developed worldwide. In this context, Wheatamix project studies the interest of mixing wheat genotypes to reinforce the sustainability, resilience, and multi-functionality of agriculture. Analysing the interactions among genotypes and with the environment, Wheatamix aims to better understand the impact of intra-specific diversity on various ecosystemic services, and to develop new blending and breeding methods to obtain performing mixtures. Wheatamix is based on a highly multidisciplinary approach, involving geneticists, agronomists, ecophysiologicals, ecologists, economists and management scientists, as well as key stakeholders (Chambres d'Agriculture, farmers). It is based on three complementary research axes: (1) Characterise key morphological / ecophysiological traits and genetic variability of wheat genotypes to test how trait variability affects plant to plant interactions; (2) Quantify multiple ecosystem services provided by variety diversity within wheat fields; and (3) Explore the organisational and economic impact of blends on the wheat supply chain, with a focus on the Paris basin (fig. T2.1).



**Figure T2.1:** The three main research axes developed by the Wheatamix project.

## Material and Methods

Wheatamix has developed complementary approaches: (i) a diversity experiment (88 large wheat plots with 1, 2, 4 or 8 varieties/plot) to quantify the effects of wheat diversity on ecosystem services; (ii) replicates of the same diversity experiment in 4

sites across France under low and high inputs, to test diversity effects under a wide range of soil and climate conditions; (iii) a network of 50 farms, encompassing agro-climatic variability in the Paris basin, allowing to co-design mixtures and to compare the performances of blends and monocultures; (iv) interviews of a representative panel of actors (from breeders to millers) to study the organisational and economic impact of mixtures on the wheat supply chain.

## Results and Discussion

Wheatamix experiments revealed different ecosystem services provided by genetic diversity (yield stability and better nitrogen use; foliar diseases control; aphid predation, or soil nitrification). The project contributed to important methodological issues when analysing: (i) synergies and compromises between ecosystem services; (ii) the role of varietal diversity on multi-service delivery; (iii) link between key varietal traits and basket of services produced. Finally, the Wheatamix project exemplifies the original outcomes provided by highly pluridisciplinary approaches, especially crucial when addressing agroecological issues.

## Acknowledgements

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## Disease resistance of spelt wheat—results from the HealthyMinorCereals project

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### Introduction

Nowadays only three major species make up the majority of cereal food and feed. With this low diversity in cereal production there is a much greater likelihood of production limitations from biotic stresses. Disease resistance is an important and effective component of sustainable cereal production, because it minimises the need for pesticide application and problems associated with pesticide resistance and environmental pollution. The minor species spelt wheat (*Triticum spelta* L.) was investigated for disease resistance traits as a first step of utilising ex-situ germplasm collections of spelt wheat in today's agriculture.

### Material and Methods

A panel of 80 genotypes of winter spelt wheat was established within the framework of the FP7 project HealthyMinorCereals and evaluated for the resistance to yellow rust *Puccinia striiformis* Westend., common bunt *Tilletia caries* (D. C.) Tul. & C. Tul. (syn. *T. tritici* (Bjerk.) G. Winter and *T. laevis* J. G. Kühn (syn. *T. foetida* (Wallr.) Liro., and *Fusarium* head blight (FHB) *Fusarium graminearum* Schwabe, *F. culmorum* (Wm.G. Sm.) Sacc.

Resistance to yellow rust was evaluated in experiments artificially infected with currently appearing races, and in trials with natural infection. The visual symptoms on plants were evaluated according to the methods of Bundessortenamt (Beschreibende Sortenliste 2012).

Common bunt was provoked by artificial inoculation of seeds with a mixture of teliospores of *T. caries* and *T. laevis* originating from three different locations. Seed inoculation and sowing was carried out in early October. The resistant (0 to

**Table T3.1:** List of resistance sources.

Genotype	Disease	Note <sup>a</sup>
ALBIN	Common bunt	bunted ears 0.64 %
SOFIA 1	Common bunt	bunted ears 0.34 %
LANTVETE FRÅN GOTLAND	Yellow rust	1.3 <sup>b</sup>
LONIGO	Yellow rust	1.6 <sup>b</sup>
<i>T. SPELTA</i> ALBUM	Yellow rust	1.3 <sup>b</sup>
EBNERS ROTKORN	FHB	DON 3.0 mg kg <sup>-1</sup>
FARNSBURGER ROTKORN	FHB	DON 2.8 mg kg <sup>-1</sup>
LONIGO	FHB	DON 2.3 mg kg <sup>-1</sup>
LW 12 NÜRTINGEN	FHB	DON 2.6 mg kg <sup>-1</sup>
OBERKULMER ROTKORN	FHB	DON 3.1 mg kg <sup>-1</sup>
ÖKO 10	FHB	DON 3.1 mg kg <sup>-1</sup>
RINIKER WEISSKORN	FHB	DON 2.8 mg kg <sup>-1</sup>
TOESS 6D	FHB	DON 3.4 mg kg <sup>-1</sup>
<i>T. SPELTA</i> KROMERIZ	FHB	DON 2.9 mg kg <sup>-1</sup>

<sup>a</sup> DON results are preliminary; <sup>b</sup> Scale 1–9, 1: resistant, 9: susceptible.

10% spikes infected) or susceptible reaction (11 to 100% spikes infected) was determined in June/July.

*Fusarium* head blight symptoms were evaluated in three terms after artificial infection with *F. culmorum* or *F. graminearum*. The resistance was evaluated also in condition of natural infection after maize as a preceding crop and reduced tillage. Grain samples were analysed for deoxynivalenol (DON) content by ELISA method.

## Results and Discussion

The list of resistance sources is shown in the table T3.1. On the basis of the results from the Czech Republic, Austria and Switzerland the varieties 'Albin' and 'Sofia 1' showed the highest resistance to common bunt.

The evaluation of resistance to yellow rust was affected by the current natural occurrence of physiological races. In 2015, wheat throughout Europe was heavily attacked by yellow rust. 'Holstenkorn', 'Lantvete från Gotland', 'Lonigo' and '*T. spelta* album' showed a high resistance level during the epidemic. In the following years the resistance was confirmed in 'Lantvete från Gotland', 'Lonigo' and '*T. spelta* album'.

On the basis of the evaluation of symptoms after artificial infection and determination of DON, large differences between the tested varieties were observed with respect to *Fusarium* head blight. The highest level of resistance

was found in 'Ebners Rotkorn', 'Farnsbürger Rotkorn', 'Lonigo', 'LW 12 Nürtingen', 'Oberkulmer Rotkorn', 'Öko 10', 'Riniker Weisskorn', 'Toess 6D', '*T. spelta* Kromeriz'.

A combined resistance to common bunt, leaf rust, stem rust, and a moderate resistance against FHB and dwarf bunt was observed in 'Sofia 1'.

### **Acknowledgements**

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# **INSUSFAR: Innovative approaches to optimise genetic diversity for sustainable farming systems of the future. Inter-specific diversity of wheat for increased resilience and high yields and system specific adaptability**

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## **Introduction**

Due to favourable climatic and socio-economic conditions, in Germany extremely high yield levels can be realised. However, in the past decades, there has been a tendency towards simplified rotations, heavier machinery and consequently increased soil compaction, reduced soil cover, increased erosion and decreased biological diversity in the soil. All these factors are known to have a negative impact on productivity. A continuation of these trends will most likely not result in sustainable food production in the longer term. Until now, most likely, increased efforts in plant protection and resistance breeding have played a major role in compensating the negative effects of the changed crop management. However, while yields did not decrease on average, fluctuations have increased considerably. Aim of the INSUSFAR project is to support policy efforts aiming at increasing biological diversity, soil protection and resilience of agricultural systems. The focus is to assess the potential of wheat and barley with increased intra-specific diversity for their adaptability to the proposed future cropping systems. We report preliminary results after two years of experimentation.



## Results and Discussion

A meta-analysis of the results of official varietal trials in ('Landessortenversuche') as was performed in order to assess the role of plant breeding in yield development in the past 20 Years, considering different types of environment and input levels. The analysis revealed increasing yield instability, mainly due to resistance breakdown in the past decades.

Genetic diversity within crop species, e. g. within bulk populations, is a potent means to increase resilience with respect to environmental variation including pathogen pressure (Brumlop et al. 2017, 2013). Depending on the parental genetics, both yields and quality have been shown to be stable in populations over time (Brumlop et al., 2017). However, there are open questions e.g. how breeding progress can be integrated into population breeding. In the frame of INSUSFAR, composite crosses with various parental compositions and that were exposed to widely differing environments by varying the cultivation histories and systems are being examined comprising extensive on-farm-testing. Comparisons of 8 composite cross populations (CCPs) with their parent varieties showed, that both yield, protein content and baking quality were equal or tended to be superior, but never inferior to the varieties they were based on.

Even in the  $F_{15}$  and  $F_{16}$ , under organic conditions, yields and quality were similar or superior to the most recent varieties despite the fact that the populations had been generated from varieties, that almost all had been released more than 30 years ago. On the other hand, yields of single lines extracted randomly from one of these populations were considerably poorer than that of the population itself and were severely affected by the new yellow rust races (*Puccinia striiformis*) and other pathogens. Thus, the diversity within the populations contributed considerably to the stability of their performance, and to reduce economic risks. In addition, it should be useful to integrate modern materials into the CCPs in order to make the populations participate from the current breeding progress. This work is in progress within INSUSFAR.

An important issue when aiming at making use of populations in practical agriculture and breeding programs is the documentation of the often complex population histories. The information system 'ROBUSTUM' has been developed in the frame of the INSUSFAR project by the Julius-Kühn-Institute and is now in the testing stage. It is suitable for documentation of all aspects of the history of populations.

In conclusion, bulk populations such as CCPs, besides being a resource for line breeding, have the potential to be cultivated directly. They contribute considerably to cropping system resilience and decrease the need of pesticides. Their use in practical agriculture will require changes in the organisation of breeding and seed production, especially if also natural selection and adaptation to specific environments is to be exploited. Intellectual property and legal aspects will also

have to be considered. INSUSFAR will continue with further field and on-farm testing, in-depth economic and ecological impact analysis. In addition, solutions concerning organisation, intellectual property rights and legal aspects for the transfer into practical agriculture will be explored.

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## On-farm management as a tool to maintain diversity and to select superior genotypes

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### Introduction

On-farm management of plant genetic resources allows for evolutionary adaptation to site-specific conditions by natural selection. However, knowledge about the development of genetic diversity by on-farm management at multiple sites is still scarce. Lentil (*Lens culinaris* Medik.) is an autogamous species with an outcrossing rate of 0–5% (Horneburg 2006). In a long-term experiment, three old cultivars were exposed to ten generations of natural selection at three farms under rain fed conditions in Central and Northern Germany. Two of the farms were on organic poor or marginal soils (Schönhagen, shallow clay; Tangsehl, sand), the third on a conventional fertile soil (Reinshof, loess). Significant changes in agronomic, morphological, and phenological traits had occurred already after four years of natural selection (Horneburg 2003). Aims of the study were: (i) to monitor temporal and regional changes in population structure during ten generations of natural selection, (ii) to evaluate if site-specific adaptation ('home field advantage') occurred and (iii) to explore the potential of on-farm management to select superior genotypes.

### Material and Methods

Three old lentil cultivars, originally obtained from the genebank IPK Gatersleben, were used for this study. Performance tests of the populations in generation 0, 5, and 10 were carried out in 2015 and 2016 in 5 m<sup>2</sup> field plots at all three farms in a split-plot design with cultivar as main plot and with 4 replications. 100 single plant progenies per population in generations 0 and 10 were tested in mini-plots in a partially replicated block design at Reinshof. 147 SNP molecular markers were used for genotyping. Phenotyping included agronomic, morphological, and phenological traits.

### Results and Discussion

Site-specific changes in seed weight, flowering time (fig. T5.1b) and leaf size were observed in Pisarecka Perla. Natural selection at the most stress-prone location Tangsehl (lowest yield level) led to a lower seed weight and earlier flowering, whereas at the most fertile location Reinshof selection favoured larger seeded genotypes



However, the observed changes did not lead to a significant site-specific adaptation in terms of yield, a so called 'home field advantage'. Across cultivars and locations, an increased yield of generation 10 was observed but it failed to become statistically significant.

The Pisarecka Perla population selected at Tangsehl, the most stress-prone environment, had a yield advantage at all three farms. This may indicate, that on-farm management is not only able to maintain genotypic and phenotypic diversity but can also be used to select for superior genotypes.

## Acknowledgements

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# Modeling competition for light in wheat mixtures

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## Introduction

Over the past 50 years, homogenisation has been the dominant paradigm of agriculture in industrialised countries, associating the cultivation of genetically homogeneous populations with the intensive use of inputs to limit stresses (Tilman 2001). However, it is imperative to reduce the health and environmental impact of agricultural practices, while stabilising the production of crops, despite the great stochasticity of environmental conditions induced by climate change. Agroecology, which relies on the mobilisation of ecological regulations in agroecosystems, allows the development of a new, more sustainable, model of agriculture (Gliessman 2006). Despite its poor use in modern agriculture, the within-field genetic diversity (cultivar mixtures and composite populations) should play an important role in the context of agroecology, contributing to the adaptation and resilience of low input cropping systems, more prone to abiotic stresses.

However many questions are raised by the complex plant-to-plant interactions occurring in such populations, and there is a growing need to develop modelling approaches to improve the design of mixtures and the management of composite populations. Wheat mixtures are a good model to study plant-to-plant interactions, as wheat is a strategic crop, studied by numerous geneticists, agronomists and ecophysicologists worldwide, and because wheat mixtures are more and more grown by French farmers. Up to now, the design of wheat mixtures was based on knowledge acquired by phytopathologists in the eighties, with two main principles: (i) maximise diversity in genetic resistances, to better control diseases, and (ii) minimise the diversity between architectural traits, to limit plant-plant competition (de Vallaville-Pope 2004). However, eco-systems with high functional diversity (inter-specific) are known to be more productive in ecology (Tilman et al. 1997). Thus, there is presently a gap of knowledge on the impact of intra-specific diversity for architecture traits on mixtures performances. Here we explore whether the presence of differences in height between two mixed cultivars could improve the radiation use efficiency (RUE). And as it is experimentally difficult to study the wide variety of heights in wheat cultivars, and wide diversity of height differential in their numerous binary combinations, we developed a modelling approach, using the WALTER model.

## Material and Methods

WALTer is a 3D functional-structural plant model aiming at simulating the development of winter wheat (*Triticum aestivum* L.) from sowing to flowering. WALTer simulates the competition for light within the canopy through the representation of the tillering process. The self adaptation of tillering is modelled by two simple rules: tillering cessation due to an early neighbour perception, and tiller regression due to shading. WALTer has been previously validated (Lecarpentier 2017) for its ability to predict tillering dynamics of wheat cultivars grown in pure stands, for a wide range of sowing densities.

In this study, WALTer was used to simulate binary mixtures at three sowing densities (160, 250, 350 plants  $\text{m}^{-2}$ ), in which the only difference between cultivars was their height at maturity (tillering ability and leaf number, surface and angle were identical). Seven cultivars were simulated with plant height ranging from 40 cm to 160 cm (20 cm steps). They were associated in all possible combinations leading to seven monoculture and 21 binary mixtures and with height differences ranging from 0 to 120 cm. Three outputs of the model were investigated: (i) the cumulated amount of PAR intercepted (PARi) per plant over the cycle, (ii) the tillering dynamics and (iii) the dynamics of Green Area Index (ratio between the total leaf + stem + spike surfaces, and the soil surface covered by the plant). For each density, the over-yielding of the three model outputs previously selected, computed as the performance of the mixture compared to the mean of its components grown in monoculture, was computed to evaluate mixture performance. The plasticity of the three outputs was also studied through the ratio of the performances of a component observed in mixture and in pure stand.

## Results and Discussion

As expected, simulations results have shown that the height of the competitor of a given variety has a significant effect on its performance. These competitive effects were always larger for light interception than for tillering or plot vegetative development and this was true for traits at the level of single genotypes or averaged over the whole plot. Indeed, plasticity curves were characterised by an important asymmetry, especially for light interception (PARi). The higher the cultivar is in the mixture, the more it will produce ears and intercept PAR. This trend was in general agreement with experimental data available in the bibliography. Interestingly, the simulations have highlighted the non-additive properties of mixtures with height differentials, as the over-interception of PAR by the highest genotype was always more important than the under-interception by the shortest.

These results therefore provide interesting insights on how competition for light functions in mixtures and open venues for the optimisation of RUE in cultivar mixtures. We can see that these modelling approaches, and how this relates to the development of each variety of the plot.

## Acknowledgements

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## Participatory approaches: Developing blending rules to design locally adapted cultivar mixtures

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### Introduction

In the context of climate change and pesticides reduction, the use of cultivated diversity is identified as an important lever to develop sustainable agriculture. Among the crop diversification options, intra-specific diversity within the field is more and more used by farmers (Borg et al. in press). Compensation, complementarity and competition between varieties into mixtures (Barot et al. 2017) confers better resilience to biotic and abiotic stresses and contributes to yield stability (Kiær et al. 2009).

However, despite available scientific knowledge on mixtures, this practice is impeded by the lack of blending rules. Translating existing knowledge in practical rules is a prerequisite for helping farmers and advisory services to design cultivar mixtures.

The WHEATAMIX project aims at better evaluating the possible roles of wheat mixtures to reinforce the multi-functionality and resilience of cropping systems under global change. Here we describe how we developed a variety mixture co-design and evaluation with farmers, advisory services and scientists.

### Material and Methods

An ideotyping approach was proposed with researchers, 6 Chambers of Agriculture of the Parisian region and farmers engaged in low-input agriculture. The objective of ideotyping is to identify variety profiles (defined by physiological and morphological traits) that would be interesting to mix for adaptation to local farming system. We adapted the Durel and Gauffreteau (2014) plant ideotype design strategy to the case of variety mixtures, with 3 steps: (i) The definition of specifications assigned to mixtures in a given production context. It was assessed by a bibliographical study, analysis of 3 farmers' forums (ACE, Agricool and Agriavis), and interviews with farmers practicing mixtures. (ii) The design of blending rules and mixtures conducted during three workshops organised successively with researchers, agricultural advisors and farmers. The mixtures designed in the second and third year were improved in light of results obtained in the first year of

evaluation. (iii) The field evaluation of mixtures. The performance of mixtures and their components in pure stands were assessed in both farmers' plots and more classical repeated trials carried out by chambers of agriculture during three years (2014–2017), in around 50 sites in the Paris region with highly contrasted conditions. Mixtures were compared to their components for yield, quality, and diseases resistances.

## Results and Discussion

**Step i)** Interviews of farmers growing mixtures revealed three main motivations: (1) secure yield, (2) reduce inputs (mainly fungicides) and (3) simplify wheat crop management within the farm. These items diverge from the ones studied in scientific papers, which focus firstly on the over-yielding (yield gain of a mixture compared to the mean of its components in pure) rather than production stability.

**Step ii)** During the first workshop, researchers proposed innovative varietal blending rules, such as coupling height and resistance criteria to enhance pathogen regulation. The second workshop with agricultural advisors assessed the technical feasibility of the association rules proposed by the researchers. For example, introducing diversity in plant height has been discarded in some areas, as it implies to lower the cutting of the harvester which slows down the harvest and is risky in stony soils. The last workshop with farmers allowed the design of 160 mixtures, and remarkably, with little replications in the network.

**Step iii)** The assessment of the 160 mixtures over a three years period shows that the yield of the mixture is higher than the average yield of the pure varieties in 70 % of the cases. This difference is more important when cropping conditions are hard as in 2016 where strong diseases' pressure has strongly impacted yield. In this year the median over-yielding reached 4.6 % compared with 1.5 % and 0.3 % in 2015 and 2017 respectively. There are no differences between mixtures and pure varieties in terms of protein content. These results are in full accordance with the literature (Borg et al. 2017). We also compared the performance of each mixture to the one of a variety chosen by the farmer at the sowing time. The yield of the mixture was higher than the one of the chosen variety in 54 % of the cases, but is systematically higher in 2016 showing clearly that mixtures minimise the risk of yield loss due to poor varietal choice in unpredictable cropping conditions.

This participatory ideotyping highlights the need to better understand the interactions between varieties in a mixture to refine blending rules, and provide relevant performance criteria for the evaluation of variety mixtures.

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# Network for the dynamic management of winter barely genetic resources

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## Introduction

Genebanks apply static *ex situ* management systems to conserve plant genetic resources for food and agriculture. By doing so, genebanks facilitate users' access to germplasm and related data. While genebanks maintain accessions, i. e. a spatial and temporal part of the evolutionary process, dynamic management strategies aim at promoting the continued adaptation of crops to changing environmental conditions (Bretting and Duvick 1997). An effective and efficient approach for a dynamic management system with wheat was described by (Goldringer et al. 2001) and is being developed by the INRA at Le Moulon (France) towards an on-farm management system involving farmers (Enjalbert et al. 2011). The transfer of this approach to barley was recommended by the German Federal Ministry of Food and Agricultural in its expert program for plant genetic resources. The Julius Kühn-Institut was subsequently requested to develop an institutional network for the dynamic management of winter barley genetic resources. Populations and sub-populations are cultivated at different locations and exhibit a wide genetic variation within and between populations, adapted to regional agricultural conditions, and with potential for future adaptation to climate changes.

## Material and Methods

From a total of 227 German winter barley varieties released between 1914 and 2003, a set of 58 varieties was genetically analysed using SSR markers. Among these, 32 genotypes representing the genetic diversity of the whole set were selected to produce a highly recombinant winter barley population. In the years 2008 to 2015, the 32 selected winter barley varieties were crossed according to the Multi-parent Advanced Generation Inter-Cross (MAGIC, Cavanagh et al. 2008) scheme resulting in a set of lines with each line being a descendent of all 32 initial varieties and thus harbouring parts of all 32 initial genomes. Aliquot amounts of seeds from 324 of these lines were combined in 2015 to form a highly heterozygous population and grown for multiplication.

## Results and Discussion

In order to promote the development of differently adapted germplasm, 12 ecogeographically contrasting locations within Germany were selected to form a network for the dynamic management of winter barley genetic resources. These include 3 locations under organic management practices. Since 2016, sub-populations of the material have been continuously cultivated under high and low input conditions at these locations. Adaptation of winter barley sub-populations to different climatic, soil and agricultural input conditions will be monitored over a period of 6 to 8 years. Based on samples taken in each year, changes in the allele frequencies within and between locations will be monitored at the DNA level. Simultaneously, ROBUSTUM has been developed as an information system for the consistent documentation of varieties and lines, crossing schemes, composition of (sub)populations, cultivation conditions, characterisation and evaluation data, and for subsequent data analysis. The complex pedigree of the initial winter barley population has already been recorded within ROBUSTUM. All data as well as plant genetic resources developed by the network will be publicly available according to the rules of the Multilateral System of the International Treaty.

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# Estimation of mixing ability for variety mixtures: Statistical models and experimental results

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## Introduction

Challenges in agriculture such as climate change and the urge to reduce the use of synthetic inputs can partly be addressed by diversifying agroecosystems, such as introducing within-field inter- and intra-specific diversity. In particular, variety mixtures represent an agro-ecological practice easy to implement and of great potential interest for organic or low input farming systems due to their capacity to buffer biotic and abiotic stresses (Finckh and Mundt 1992; Wolfe 1985). However, when designing mixtures, farmers grapple with the lack of references to identify the varieties which are best adapted to this practice. Indeed, for winter wheat (*Triticum aestivum*) it has been shown that mixture performances can be poorly correlated to the pure stand performances, calling for estimating mixing ability of the varieties. Moreover, the varieties have been bred for pure stand performances without considering their potential for mixed stands, so mixing ability might be improved by integrating this parameter in breeding programs. Assessing mixing ability nonetheless address methodological and statistical challenges due to the high number of combinations to assess when the number of genotypes increases.

## Material and Methods

Two approaches can be used to assess mixing ability. A first approach for defining mixing ability is based on an analogy with combining ability in hybrid breeding (Griffing 1956), where mixing ability is partitioned between General Mixing Ability (GMA, which is the mean performance of a genotype in mixture) and Specific Mixing Ability (SMA, which is the interaction term for a particular combination of genotypes in mixture). GMA and SMA are usually assessed by evaluating a panel of genotypes in mixture with each other, in a complete diallel design. Here we propose to generalise this approach to incomplete designs and to any mixture order (binary mixtures and higher order mixtures) by using mixed models. We developed an

appropriate statistical framework based on mixed models and variance-component framework, and we proposed an original modelling of SMA taking into account neighbouring probabilities, as well as the intra-variety competition (present in pure stand).

The second approach is based on competition models, and especially a Producer-Associate model previously developed to study competition bias in breeders' nursery trials using a 'smart' experimental design (Goldringer et al. 1994). It consists of using a single-row trial where each genotype is observed with different neighbours to estimate a Producer effect of this genotype, i. e. its mean ability to withstand inter-genotypic interactions and an Associate effect which is the mean ability of a genotype to impact the performance of their neighbours.

These two approaches have been applied to a diverse panel of 58 genotypes of winter wheat in (i) a single-row trial at Le Moulon to evaluate the complete panel for Producer-Associate effects, (ii) an incomplete diallel experiment of binary mixtures at Le Moulon for a restricted panel of 25 genotypes, (iii) another experiment carried out at Clermont-Ferrand of binary and higher order mixtures for a subset of 16 genotypes; (ii) and (iii) using both GMA-SMA models.

## Results and Discussion

For the two mixture experiments, the use of mixing ability models improved the correlation between observed mixtures and predictions in comparison to predictions from the mean of the pure stand components, especially in the first experiment (from 0.51 based on pure stands to 0.88 based on mixtures). Genetic variability for GMA was detected for yield and its components, whereas variability for SMA was lower. GMA estimated in the two experiments were highly correlated for yield (0.90).

When using single-row trials, the Producer and Associate effects estimated for yield appeared mildly correlated ( $-0.16$  only) opening the possibility to improve both parameters jointly. The correlation between Le Moulon observed mixtures and predictions based on Producer-Associate effects estimated in single-row trial was satisfactory (0.59 for yield).

In Le Moulon experiment, where yield components were recorded, spike density gave significant responses with both high SMA and Associate effects, attesting the high plasticity of tillering in mixtures.

These statistical and experimental methods of mixing ability assessment might be further adapted and integrated in breeding programs for mixture development according to the genetic structure of mixing ability, and adapted to inter-specific mixture development.

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# Application of molecular markers for plant breeding and variety investigation and diversification

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## Introduction

The application of molecular markers has enabled breeders to select superior genotypes for traits that are difficult to select based solely on phenotype or to pyramid desirable combinations of genes into a single genetic background. Since the 1990s SSR markers have been extensively used in constructing genetic linkage maps, QTL mapping, marker-assisted selection (MAS) and germplasm analysis in plants. Most of the important agronomic traits are polygenic or controlled by multiple QTLs (Campbell et al. 1999). MAS for the improvement of such traits is a complex and difficult task because it is related to many genes or QTLs involved, QTL × Environment interaction and epistasis. Usually, each of these genes has a small effect on the phenotypic expression of the trait and expression is affected by environmental conditions. Over the past three decades, the total number of the proposed molecular markers reached such a level, which makes it possible to construct high density genetic maps. In the present study, we have applied microsatellite markers (Röder et al. 1998, 2002) for the association with major loci connected with controlling of main wheat agronomical and morphological characteristics in a core collection from modern Ukrainian bread wheat varieties (Kolesnyk et al. 2013).

## Material and Methods

The analyzed material consists of 48 bread winter wheat varieties (*Triticum aestivum* L.) originated in Plant Breeding and Genetic Institute (PBGI) and registered in State Register of plant varieties suitable for dissemination in Ukraine during different years: Hospodynja (2007), Scarbnytsia (2007), Kosovytsia (2008), Antonivka (2008), Zamozhnist' (2008), Blahodarka odes'ka (2009), Misiia odes'ka (2009), Dal'nyts'ka (2005), Yednist' (2008), Kiriia (2004), Liona (2005), Kuial'nyk (2003), Poshana (2004), Zaporuka (2008), Bunchuk (2009), Podiaka (2008), Oksana (2007), Zahrava odes'ka (2010), Epokha odes'ka (2010), Lytanivka (2008), Sluzhnytsia odes'ka (2009), Hoduval'nutsia odes'ka (2009), Istyna odes'ka (2010), Zmina (2007), Dovira (2009), Krasen' (2009), Otaman (2008), Borvii

(2010), Turunchuk (2008), Diuk (2008), Nebokrai (2011), Khyst (2013), Pylypivka (2011), Zorepad (2011), Zhaivir (2010), Uzhynok (2010), Hurt (2013), Dobrochyn (2013), Vatazhok (2011), Pol'ovyk (2009), Holubka odes'ka (2011), Kniahynia O'lha (2011), Lebidka odes'ka (2 samples, 2011), Zhuravka odes'ka (2011), Bezmezhna (2008), Lastivka odes'ka (2011). Varieties Albatros odes'kyi (1990) and two collection samples of variety Bezosta 1 (1955) were taken into research as standard (etalon) samples according to recommendation of Ukrainian Institute of Examination of Plant Varieties of Ministry of Agriculture and Food of Ukraine as a national standards provided for distinctness, uniformity and stability (DUS) of new varieties of bread wheat (*Triticum aestivum* L.) for the purpose of granting the Breeders' Right.

It is assumed that in consequence of breeding these varieties are adapted to the conditions of a certain region, i.e. the genetic basis of such varieties is respectively narrowed compared to the complete gene pool of the culture. Each year the obtained grain material was analysed for a number of morphological and colorimetric characteristics (grain area projected on a plane, perimeter, length, and width, circularity, grain color intensity) by the optical methods using the digital images of kernels. In addition important agronomical traits (heading time, plant height, 1000 kernel weight, awn size, ear color, ear size) were evaluated during three growing seasons.

## Results and Discussion

Genetic diversity in studied varieties of interest was evaluated based on (i) allelic richness (range of allele size and number of alleles per locus) as a measure of genetic variation; (ii) presence of specific alleles found in one variety but absent in others as a measure of genetic distinctiveness and uniformity; and (iii) PIC of each SSR marker as a measure of genetic diversity. A total of 114 alleles were detected at 17 SSR markers among the 48 winter bread wheat varieties, and the number of alleles per locus ranged from 4 to 10 with an average of 6.7. Allelic polymorphism was manifested by the occurrence of numerous alleles per SSR locus appearing in the whole pool of varieties, and by their frequency varying. The largest number of alleles (10) was found at Xgwm155-3A, Xgwm325-6D and Xbarc126-7D loci, whereas only four alleles were detected at Xgwm190-5D locus in the investigated varieties. Besides, the average PIC value was 0.67 with a range of 0.48 (Xgwm190-5D)–0.84 (Xgwm155-3A).

On the studied material there was confirmed the previously shown presence of statistically significant associations of allelic state revealed by microsatellite loci with the expressiveness of a number of parameters of agronomical and morphological traits. There was determined the hereditary nature of some associations found in previous years. Development of research in this area is promising in terms of a more complete disclosure of genetic predetermination of the key breeding traits (plant height, photoperiod response, resistance to various diseases). Based on the

experimental results of this study, the practical cooperation with the department of wheat breeding has been started in order to improve the variety control while breeding and primary seed production and to increase the competitive ability of Ukrainian varieties on the global seed market.

## Acknowledgements

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# Genotypic characterisation of spelt (*Triticum spelta* L.)

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## Introduction

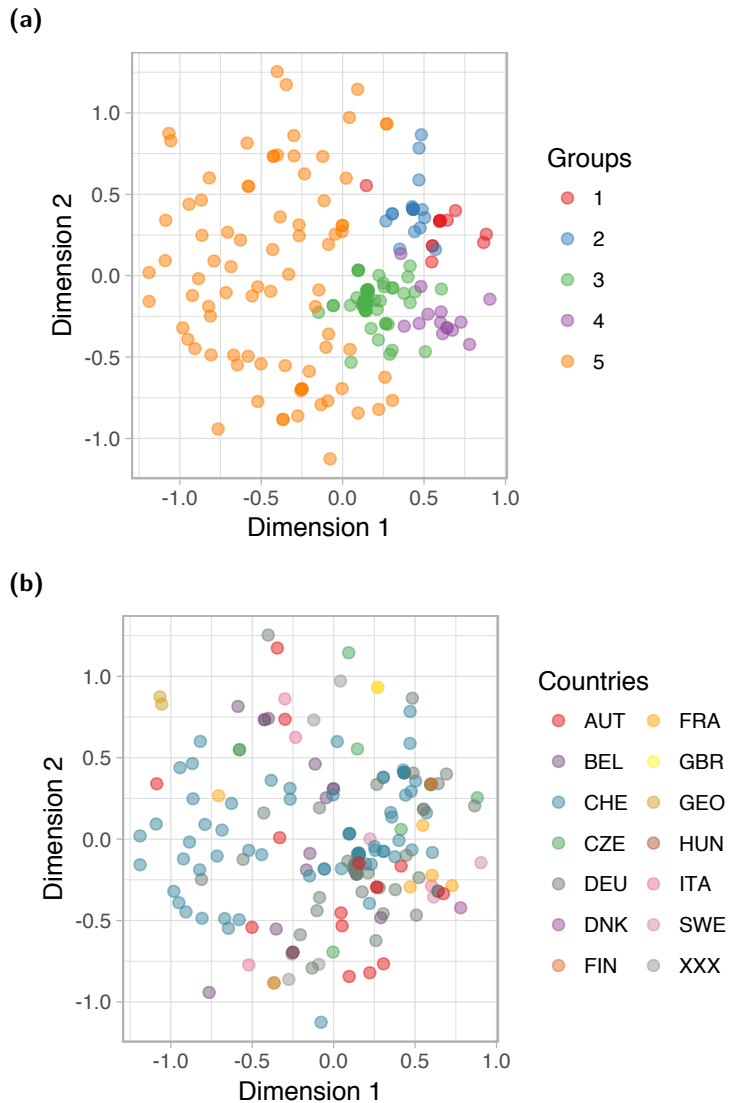
In Europe cereal production is focused on major cereals like common wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.) and maize (*Zea mays* L.). This leads to a low diversity in cereal production that may lead to a low resilience of crop plants meeting biotic and abiotic stresses as a consequence of climatic changes. Moreover, most varieties of major crops often depend on high input of mineral fertilisers. Minor cereals are rarely cultivated cereal species, for instance spelt (*Triticum spelta* L.), rye (*Secale cereale* L.), oat (*Avena sativa* L.) and wheat wild relatives. Minor cereals are important for organic agriculture because they tend to be better adapted to more stressful conditions and because more diverse crop rotations require a higher crop portfolio. Furthermore, minor cereals show nutritive features interesting for food and feed. In September 2013, supported by the European Union FP7 programme, the project 'HealthyMinorCereals' started in order to improve and diversify the production of small grain cereals in Europe. In this multidisciplinary project minor cereals are genetically and morphologically characterised.

In regard to spelt marker-based diversity hasn't yet been as intensively studied as in common wheat (Würschum et al. 2017). The aim of this study is to analyse the genetic diversity and structure in a European spelt collection by a wheat SNP array.

## Material and Methods

Seeds of 265 spelt varieties (incl. *T. macha*, *T. spelta* × *T. macha*, *T. aestivum* × *T. spelta*) from 14 countries were multiplied by the project partner at the University of Natural Resources and Life sciences (BOKU) at Vienna. BOKU provided spelt varieties which originate from old varieties, landraces and genetic resources from genebank collections (Austria, Switzerland, Czech Republic, Hungary and Sweden). Furthermore, accessions from the collection of LSA Hohenheim, comprising modern varieties and breeding lines from various breeding programmes, e. g. GZPK, were included. Spelt DNA was isolated from plant leaves by SDS method. Based on SSR marker results, 184 genetically diverse spelt accessions were chosen and analysed

by TraitsGenetics GmbH, Gatersleben with a 15k SNP array (Wang et al. 2014). This analysis resulted in data from 7299 SNP markers. Of those, 381 markers were monomorphic and were thus excluded from the analysis.



**Figure P6.1:** iMDS showing population structure of spelt; genotyping by SNP markers; colour-coding corresponding to groups (a) and countries of origin (b).

Further 2,178 markers were excluded due to a frequency of one of the alleles of less than 7% of the accessions to avoid false positives. For a further diversity analysis to base the correction of genetic structure on, 96 evenly distributed markers were selected from the remaining 4740 markers. Based on these SNP markers, a simple matching distance matrix was calculated. Further to optically control for genetic structure an interval multidimensional scaling (iMDS) with MM algorithm

(alternative SMACOF algorithm) and Torgenson standard configuration for 2 dimensions was performed using R. The optimal number of groups was determined using Bayesian clustering with the software 'STRUCTURE' v.2.3.4. The genetic characteristics of the groups (e. g.  $F_{ST}$ ) were calculated with a Microsoft Excel macro provided by G. Backes.

## Results and Discussion

Population structure and genetic relatedness were assessed by iMDS based on simple matching distance. The best number of clusters, as revealed by structure was 5. For this case, the  $F_{ST}$  value was 0.187. The  $F_{ST}$  value for the origin of the lines was evaluated as 0.095. Figure P6.1 shows the results of the iMDS, colour-coded by groups (a) and the same arrangement with colour-coded countries (b). No major population structure and no clear grouping according to the origin of the lines was apparent. The results of the marker-based clustering resulted in groups that separated the spelt collection with a better resolution, still genetic structure was weak.

## Acknowledgements

The authors wish to thank the European Union, FP7 for the financing of this work in the project 'Healthy Minor Cereals: An integrated approach to diversify the genetic base, improve stress resistance, agronomic management and nutritional / processing quality of minor cereal crops for human nutrition in Europe'.

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## Yield stability of barley mixtures and populations

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### Introduction

Development of integrated and organic farming systems promotes increase of genetic diversity within crop varieties. Genetically diverse crops are expected to be more stable–yielding over time and across different locations than genetically uniform varieties currently dominating in commercial production (Döring et al. 2011). For self–pollinating cereals there are several methods to increase genetic diversity. One is to create mixtures (Lopez and Mundt 2000) and another is creation of composite cross populations (CCPs) (Sunenson 1956).

The aim of this study was to assess yield stability of spring barley (*Hordeum vulgare* L.) genotype mixtures and three types of populations.

### Material and Methods

Field trials were carried out during 2015–2017 under organic (O) and conventional (C) growing conditions in two locations in Latvia differing for meteorological and soil conditions. Yield results from 11 environments were obtained (five O and six C). Eight mixtures M1–M8 consisting of two, three and five components, 16 mixture components in pure stand, four simple populations SP1–SP4 (two parents), five complex populations CP1–CP5 (3–5 parents), two CCPs (10 parents, CCP III—crosses to male sterile accessions) and three local check varieties were used in the experiment (one component = check variety; overall n = 37). Mostly advanced breeding lines and some varieties with potential adaptability to organic farming were used as initial material in mixtures and populations. Yield stability was assessed using regression (Eberhart and Russel 1966) and rank analysis (Fox et al. 1990).

### Results and Discussion

Five out of eight tested mixtures can be characterised as suitable to different growing conditions or with wide adaptability to environments; four of them (M1, M2, M5, M8) yielded on average above the all genotype mean (4.71 t ha<sup>-1</sup>) over the environments and M7 significantly surpassed it (table P7.1). Mixtures M1 and M3

can be described as suitable to high yielding (conventional) environments; both of them yielded above the all genotype mean with significant difference for M3. Only mixture M6 showed adaptability to lower yielding organic environments, however, its mean yield was slightly below the average of the trial. Mixtures in most of the cases ranked either in the top of the list or in the middle, which confirmed that mixtures can ensure yield stability over years and different environments. All four SPs yielded significantly below the all genotype mean and in most of cases under both farming systems ranked in the lower level. CP1 showed adaptability to favourable environments with yield above the all genotype mean.

**Table P7.1:** Average yield of genotypes over 11 environments and yield stability indicators

Genotype	Average yield (t ha <sup>-1</sup> )	b	s <sup>2</sup> dj	Number of rankings					
				Organic (n = 5)			Conventional (n = 6)		
				I	II	III	I	II	III
M1	4.94	1.14 <sup>2</sup>	0.07	–	5	–	4	2	–
M2	4.90	1.00	0.10	2	2	1	2	3	1
M3	5.03 <sup>1</sup>	1.10 <sup>2</sup>	0.06	2	2	1	5	1	–
M4	4.87	1.07	0.04	1	4	–	4	1	1
M5	4.87	1.06	0.05	2	2	1	3	3	–
M6	4.65	0.83 <sup>2</sup>	0.10	3	–	2	–	2	4
M7	5.09 <sup>1</sup>	1.03	0.06	5	–	–	4	1	1
M8	4.95	1.01	0.10	3	2	–	3	2	1
SP1	4.13 <sup>1</sup>	0.89 <sup>2</sup>	0.03	–	–	5	–	–	6
SP2	4.28 <sup>1</sup>	0.89 <sup>1</sup>	0.02	–	1	4	–	–	6
SP3	4.40 <sup>1</sup>	0.98	0.06	–	1	4	–	2	4
SP4	4.43 <sup>1</sup>	1.00	0.10	–	3	2	1	1	4
CP1	4.79	1.17 <sup>2</sup>	0.11	–	3	2	3	3	–
CP2	4.53	0.96	0.06	1	1	3	–	3	3
CP3	4.13 <sup>1</sup>	1.01	0.14	–	–	5	–	1	5
CP4	4.67	0.90	0.08	1	4	–	1	4	1
CP5	4.54	1.07	0.10	–	2	3	–	4	2
CCP I	4.82	0.90	0.06	4	–	1	1	5	–
CCP III	4.50	1.03	0.06	–	–	5	–	4	2
Rubiola	5.01 <sup>1</sup>	1.15	0.13	3	2	–	4	1	1
Abava	4.47	0.80 <sup>2</sup>	0.08	2	2	1	–	1	5
Rasa	4.48	0.98	0.14	–	–	5	1	2	3

<sup>1</sup> significantly distinctive from average yield over 11 environments ( $p < 0.05$ ,  $LSD_{0.05} = 0.27$ );

<sup>2</sup> significantly different from 1 ( $p < 0.05$ ); b – coefficient of regression; s<sup>2</sup>dj – deviation from regression; I, II, III – ranked in the upper (I), middle (II) and lower (III) third.



Other CPs can be characterised with wide adaptability and lower yield levels. Both CCPs can be characterised as widely adaptable to various growing conditions. CCP I showed a trend to yield advantage under organic environments and yielded above the average level, but CCP III had yield below the average.

All of the tested mixtures, both CCPs and four CPs were more stable than check varieties 'Abava' and 'Rasa'. Better yield stability than the best check variety 'Rubiola' showed M7.

Yield differences between types of populations can be explained not only by diversity level but also by different genetic background.

We can conclude that in case of our specific genetic material mixtures and also CCPs can be the most stable and highly ranked under organic farming system.

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# Evolutionary-participatory breeding generates wheat populations adapted for organic agriculture in Italy

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## Introduction

Evolutionary Populations (EP) of crops can adapt to climate change and different agro-ecological environments, increasing farmers' resilience and enhancing on-farm biodiversity. The extent of initial genetic diversity, selection intensity, and population size, determine the adaptation rate. However, the suitability of crop EPs, and their acceptance by farmers in developed-world countries such as in Western Europe, remains unclear. To investigate the possibility of using EPs as a tool for climate-adapted varieties of *Triticum aestivum* L. (bread wheat), we developed populations in farmers' fields in different regions of Italy, and tested their performance under organic growth conditions.

## Material and Methods

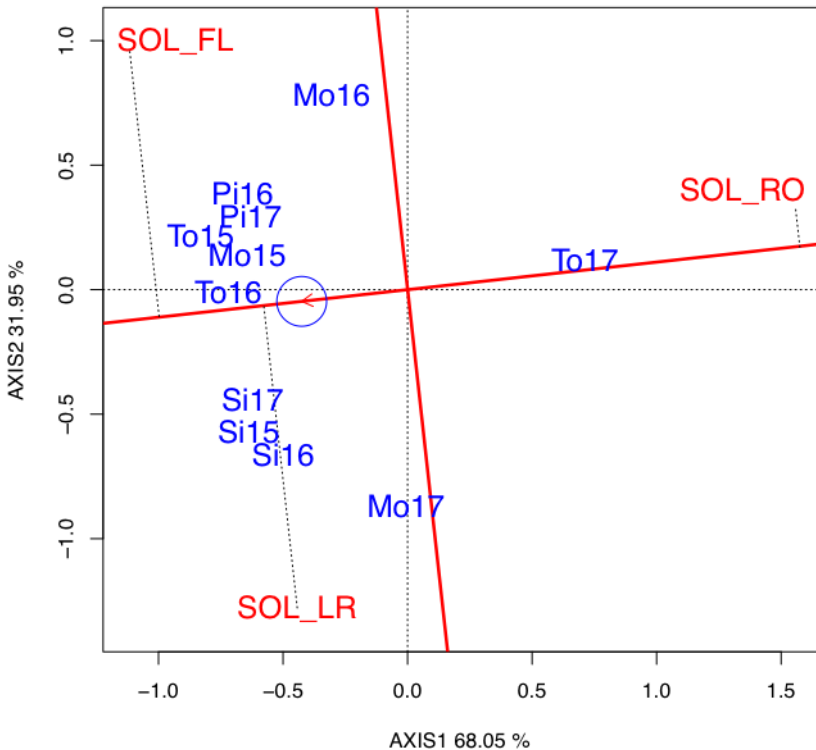
The same bread wheat EP (Ceccarelli et al. 2010) evolved for five growing seasons at sites in Tuscany (To) and Sicily (Si), resulting in two EPs, SOL\_FL and SOL\_LR respectively. A farmer's phenotypic selection within SOL\_FL resulted in a third EP named SOL\_FLS. The EP evolving in Sicily was moved to Abruzzo after three years, where it was called SOL\_RO.

We conducted comparative trials for three years in each of the two regions of adaptation, and at two further locations in Molise (Mo) and Piedmont (Pi). In addition to the four EPs, the trials included three other EPs obtained in the UK, three variety mixtures, three local varieties and one control, a modern variety recommended for use under organic growth conditions. Field trials followed a row-column design in two replications with optimised randomisation (Cullis et al. 2006). A spatial analysis, adapted from Singh et al. (2003), was run to generate Best Linear Unbiased Estimates (BLUEs). Genotype  $\times$  Environment and Genotype  $\times$  Trait Interactions were then derived using GGEbiplot in statistical software R.

## Results and Discussion

All three of the SOLIBAM EPs were found to be specifically adapted to the regions in which each had been cultivated: this was evidenced by a high level of performance stability in the environment in which each population evolved and by higher instability across environments. During the three years of the experiment, more than 350 farmers and visitors evaluated the germplasm being tested. The preference was often for some of the highest yielding EPs and varieties, such as SOL\_FL, SOL\_FL and Maiorca respectively, although some low yielding mixtures, such as the mixture of old varieties, were also favoured, possibly because they were very tall.

There is a clear relationship between dietary diversity and health (Dwivedi et al. 2017). Dietary diversity in turn depends on cultivated biodiversity, which is also essential to improving resilience in scenarios where biotic and abiotic stresses may increase due to climate change.



**Figure P8.1:** The three SOLIBAM EPs generated by years of natural selection (SOL\_FL, SOL\_LR, SOL\_RO) were specifically adapted to the regions where they evolved. As expected, this was accompanied, by a high level of stability in the environment in which each population evolved, and by a high instability across environments. SOL\_RO was poorly adapted to all tested environments, having evolved in a different region from those where trials were carried out.

The EU COMMISSION IMPLEMENTING DECISION (2014/150/EU), provides for certain derogations for the marketing of populations of wheat, barley, oats and maize, and hence establishes a legal framework for marketing of SOL\_LR and SOL\_FL through decentralised seed systems. There is preliminary evidence that the use of adapted bread wheat EPs results in improved culinary and baking properties. Our results suggest that EPs could provide organic farmers with a low-cost technology to derive stable-yielding populations thus confirming the work of Raggi et al. (2017). Where there is a lack of crop breeding programs specifically addressing the needs of organic agriculture, EPs have the potential to provide populations specifically adapted to both the physical and agronomic conditions of organic farmers.

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# Future proofing decentralised evolutionary wheat populations' seed systems in Italy using a climate analogues approach: The example of Tuscany

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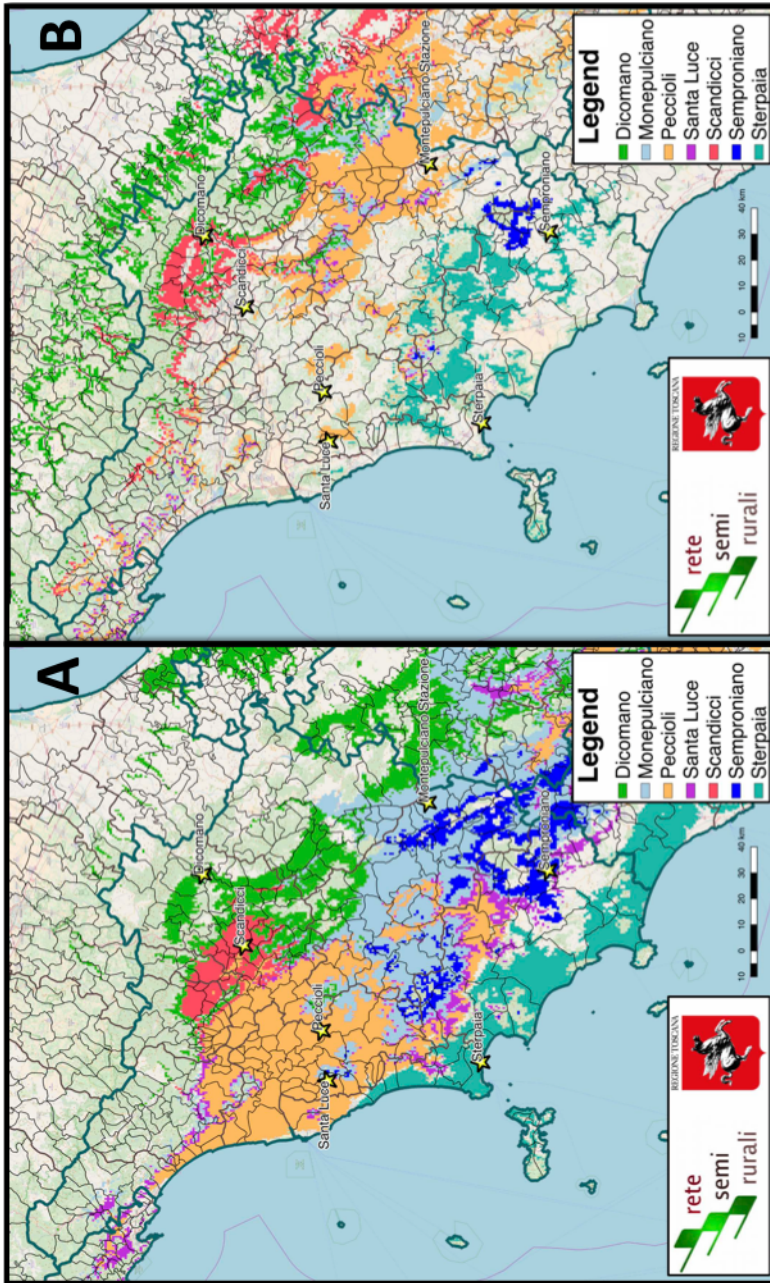
## Introduction

Crop varieties specifically bred for organic agriculture are lacking, imposing limits on the uptake and efficiency of organic production systems (Wolfe et al. 2006). The ability of Evolutionary Populations (EP) of food crops to evolve and adapt to different agro-ecological environments makes them ideally suited to obtaining specifically adapted plant material, allowing farmers to better harness the full potential of organically managed and marginal lands. A *Triticum aestivum* L. (bread wheat) EP developed at ICARDA (Ceccarelli 2012) has been evolving for five years at a farm in Tuscany, taking the name of 'SOLIBAM Tenero Floriddia'.

Thanks to the EU COMMISSION IMPLEMENTING DECISION (2014/150/EU), providing for certain derogations for the marketing of populations of wheat, barley, oats and maize, this genetically heterogeneous population is now for the first time been marketed as certified seed. A precondition for successful deployment of EPs is that the seeds be produced in the same region in which they will be utilised. This can typically be best achieved through decentralised seed systems, wherein farmers play central roles as both seed users and producers. Defining the boundaries of these micro-environments, as opposed to the wider 'mega-environments' considered during breeding programmes associated with the Green Revolution, is therefore key for establishing networks of farmers and growers who can establish networks for the exchange or trade of EP seed within specific areas of adaptation.

## Material and Methods

Current and future climatic similarities between Tuscan seed-hubs and the rest of the region within Italy were investigated using the Climate Analogues approach,



**Figure P9.1:** (A) Current climatic similarities between seed-hub centres and their surrounding area; and (B) how these same areas are projected to shift under climate change RCP 8.5 emission scenario by the 2030's. Deploying EPs within decentralised seed systems (A), should allow them to adapt to climate changes as they occur preventing a (B) scenario from negatively impacting on agricultural yields.

whereby the climate of a chosen location is compared to that of a search range. Non-directional simulations (current → current) were carried out to assess the current climatic similarities between each seed-hub and the rest of the region. Forward simulations (current → projected) were used to assess how the current growing conditions were shifting due to climate change. The desktop analogues R package developed at CIAT, Colombia (Ramírez-Villegas et al. 2011) was used to calculate a climate similarity index (SI) for each raster point on the grid. The dataset used for the simulations consisted of the mean monthly values (temperatures and rainfall) for the season October–June derived from: (i) baseline climate data, extracted from WorldClim (1950–2000) (Fick and Hijmans 2017), and (ii) projected mean climate data (2020–2049) of 33 Global Climate Models (GCM). The spatial resolution for the simulation was 30 arc seconds, which can be approximated to one km<sup>2</sup>. A threshold of 0.75 SI was set for each simulation, and resulting rasters rendered into maps using GIS software QGIS.

## Results and Discussion

Our findings suggest that this approach can inform farmers' networks & organisations, as well as policy makers and regulatory authorities on optimal delineation of agro-climatic zones, characterised by highly similar climatic condition. Engaging with Evolutionary Participatory Breeding (EPB) and EPs seed production within the boundaries of these zones' will help accelerate the specific adaptation of EPs' as required for optimal agronomical results under organic conditions.

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# Mixing susceptible and resistant wheat cultivars with contrasted stem height can reduce progression of disease dispersed by rain-splash

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## Introduction

In a context of climatic uncertainty and demand for more sustainable cultural practices, alternative diseases management techniques have received renewed interest. In particular, increasing the diversity of host plant population has shown interesting results in disease management (Finckh and Wolfe 2006). Cultivar mixture is an efficient way of increasing diversity in agricultural systems with moderate modifications of farmer's organisation (Borg et al. in press; de Vallavieille-Pope 2004). Important criteria considered for mixture design often include the level of resistance of cultivars as well as the proportion of resistant and susceptible plants. However, cultivar architecture has been little taken into consideration in mixture design despite the fact this factor has been shown to be a relevant lever in the control of splash dispersed diseases such as Septoria Tritici Blotch (STB) (Robert et al. 2008; Vidal et al. 2017b).

## Material and Methods

We designed a field experiment to investigate the effects of mixture canopy structure on microclimate, disease propagation of STB blotch caused by *Zymoseptoria tritici*. Treatments included cultivar mixtures with similar or contrasted straw height, in different proportion and pure stands of component cultivars. Canopy structure was characterised in detail. Disease assessments were carried out weekly in order to quantify disease progression in mixtures and pure stands. Classical weather measurements were carried out and microclimate was assessed through temperature, relative humidity, vapour pressure, wetness duration (Vidal et al. 2017a).

Also, we used a mechanistic modelling approach within an explicit description of canopy architecture and splash dispersal processes (Gigot et al. 2014) (Saint-Jean et al. 2004; Vidal 2017). Dispersal patterns in cultivar mixtures with either similar



(70 cm height) or contrasted straw height (120 cm and 70 cm) were compared with experimental results.

## Results and Discussion

Results emphasise the interest of mixtures with contrasted height in the reduction of STB propagation. In the field experiment, susceptible plants were less affected by disease when grown with a tall resistant cultivar, compared to a more classical, homogeneously short, cultivar mixture. Also, microclimate and in particular leaf wetness duration were less favourable to disease development in heterogeneous mixture. Modelling results showed that a difference in plant height could modify dispersal mechanisms and enhance the barrier effect provided by the resistant cultivar that prevent spores to reach susceptible cultivars in the mixture. These results suggest that intra-specific architectural diversity can be a lever to improve the management of plant diseases using cultivar mixtures.

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# Management histories of wheat composite cross populations (CCPs) drive yield in two tillage systems

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## Introduction

Breeding for within-species diversity can be achieved through the use of heterogeneous composite cross populations (CCPs) that are exposed to evolve in response to biotic and abiotic stresses over time. This will lead to the development of so called 'modern landraces' that are locally adapted (Murphy et al. 2005) and should finally result in a greater yield stability and resilience (Brumlop et al. 2017). We tested the performance of 6 wheat CCPs with different management histories under different tillage systems, clover intercropping, and compost application in an organic crop rotation. Results of the first year are presented.

## Material and Methods

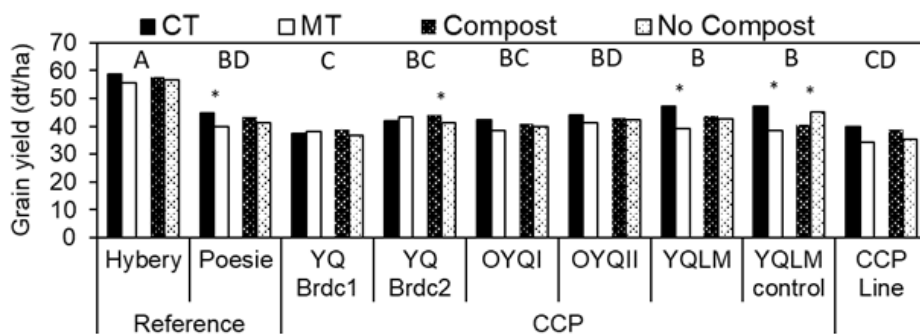
Six CCPs originating from the same original cross but with different management histories were used. In 2001, 11 high baking quality (Q) wheat varieties were intercrossed with eight high yielding (Y) varieties and 'Bezostaya' was crossed with all 19 varieties (total of 107 crosses) to form the CYQ Population. Since the  $F_5$  the CYQ has been maintained at the Technical University of Munich (TUM) and University of Kassel in Germany (UniKassel). At TUM, the CYQ was split in the  $F_9$  and grown either in the presence of a living mulch of clover without weed control (YQLM) or in the absence of clover with weed control (YQLMcontrol). At UniKassel the  $F_6$  was split into two parallel non-mixing populations that were managed organically (OYQI and OYQII). In addition, in the  $F_8$  two parallel populations were created that were managed organically without mechanical weed control but sown broadcast for maximum weed suppression (YQBrdc1, YQBrdc2). The populations used in the experiment were in the  $F_{16}$ . A single line selected randomly out the CYP at TUM in the  $F_9$  and two reference varieties, 'Hybery' and 'Poesie', were also used.

The populations and controls were sown in  $1.5 \times 15$  m plots into a three-factorial long-term field experiment at the experimental farm of the University of Kassel in Neu-Eichenberg. The experiment was set up with four replicates in 2012. Factor I is differential tillage (ploughing at 25 cm depth vs. minimum tillage at <15 cm

depth, respectively in 12 × 60 m strips). Within tillage, factor II in wheat is the combination of a living mulch undersown in wheat compared to sole wheat and factor III is regular fertilisation with 5 t ha<sup>-1</sup> yr<sup>-1</sup> dry matter of a yard waste compost versus equivalent amounts of mineral potassium and phosphorous fertiliser. Wheat entries were sown together with white clover living mulches on October 17<sup>th</sup> 2016 and harvested on August 4<sup>th</sup> 2017.

## Results and Discussion

The season 2016/17 was characterised by a very dry fall, winter, and spring that prevented adequate establishment of the clover living mulch. Therefore, the factor living mulch did not play an important role throughout the season and no effects of undersown clover could be assessed. Tillage and wheat entries interacted significantly ( $F_{8, 158} = 2.54$ ,  $P < 0.02$ ) (fig. P11.1). While 'Poesie' and the conventional YQLM and YQLMcontrol yielded significantly lower under reduced tillage and this trend was also observed for the selected line, all other CCPs and 'Hybery' were not affected by tillage (fig. P11.1). The variety 'Hybery' performed best (57 dt ha<sup>-1</sup>) under all conditions and yielded 14–20 dt ha<sup>-1</sup> more than the other entries. Very short tillers of 'Hybery' (73 cm) compared to the other entries (87 cm) indicate a better nutrient use efficiency for kernel formation.



**Figure P11.1:** Grain yields (dt ha<sup>-1</sup>) of two reference varieties, 6 composite cross populations (CCPs) and one CCP Line affected by plough (CT) versus minimum tillage (MT) and compost versus mineral potassium and phosphorous fertilisation (no compost). Different capital letters above bars indicate significant differences between wheat entries (Tukey-test ( $P < 0.05$ ) after a linear mixed effects model (lme)). \* indicate significant differences between two factor levels (lme at  $P < 0.05$ ).

Both YQBrdc had been managed organically with minimum weed control since 2008. The resulting adaptation to high weed pressure and low nitrogen contents (little soil movement), conditions that generally occur under minimum tillage in Europe (Holland 2004), was probably giving the YQBrdc an advantage over all other entries. In contrast, the YQLM and YQLM control CCPs had been managed conventionally the past years, probably allowing for better adaptation to higher soil nitrogen levels and thus, explaining the great yield gap between plough and

minimum tillage. Most wheat entries responded positively to compost application, potentially induced by increased micro nutrient availability, water holding capacity, and soil warming in spring (Tilston et al. 2005).

In conclusion, selective forces during the management histories of CCPs coming from identical gene pools lead to adaptations to specific environmental conditions.

## Acknowledgements

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# A participatory approach to breeding for diverse and adapted wheat mixtures on farm

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## Introduction

During the last century wheat (*Triticum aestivum*) cultivated diversity in France has collapsed, due to several mechanisms such as an intra-varietal homogenisation, an increase in varieties genetic similarity and the prevalence of few largely cultivated varieties in the landscape (Bonnin et al. 2014; Perronne et al. 2017). The fact that to be sold a variety has to be registered to the official catalogue and meet the ‘Distinction, Uniformity and Stability’ criteria has largely contributed to this loss in diversity, excluding heterogeneous populations from the seed market. Moreover, farmers in organic farming or low-input agriculture do not always find in the official catalogue varieties that are adapted to their practices and objectives. Therefore, some farmers of the French farmers’ seed network (Réseau Semences Paysannes, RSP), started to cultivate and breed on-farm wheat populations. Since 2006 the DEAP team (INRA GQE le Moulon), farmers and facilitators from the RSP are working in close collaboration on a participatory plant breeding project, aiming at developing populations adapted to each farmers’ practices and environment (Dawson et al. 2011); Rivière et al. 2014). These farmers are interested in increasing diversity in their fields, and many of them mix the populations they developed, taking advantage of mixtures benefits such as yield stability (Creissen et al. 2016), disease resistance (Finckh et al. 2000; Mundt 2002) and resilience to climatic stochasticity (Kiær et al. 2012). Still many questions remain as to how to select on-farm for adapted mixtures. The objective of the paper is to compare different selection practices that have been co-designed with farmers and evaluated on-farm since 2015.

## Material and Methods

A dozen farmers are participating to this evaluation, and each created one or several mixtures from populations of his / her choice. The first year they sowed the mixtures and their components. Then several practices were tested: (i) two years of mass selection within the components before mixing, (ii) one year of mass selection within the components, mixing those selections and then one year of mass selection within this new mixture, and (iii) two years of mass selection within

the mixture. These practices were compared with the same mixture let to evolve without mass selection. Agronomic and morphological variables were measured on the plants, spikes and grains. The following analyses have been done on these data: compare mixtures to their components, analyse which variables are selected by farmers during their mass selection, and evaluate the impact of mass selection on mixtures behaviour.

## Results and Discussion

The comparison of mixtures to their components showed that most mixtures provided larger values than what was predicted based on the components' mean behaviour for many variables (spike weight, spike length, mean number of grains per spike and last leaf to spike base distance LLSD), with a positive and significant mean overyielding for these variables. While mixtures produced smaller grains compared to their components' mean, there was no significant difference for protein content. For most variables, very few mixtures behave worse than their worst component, indicating that a farmer would take less risk by growing a mixture than a randomly chosen single variety as pure stand. The study of selection differentials showed that farmers selected mainly heavier spikes with more grains and in a lesser extent spikes with larger seeds. When comparing the mixtures created from different selection practices to the non-selected mixtures, we saw that on the one hand selecting directly inside the mixture gave the strongest response to selection for some variables, and a response more correlated to the selection differential. On the other hand when comparing the observed variability for variables measured in the mixtures coming from the selection within the mixtures and in the mixtures coming from selection within the components, we saw that more diversity was maintained by mixing selections done within components. The third year of experiment will give more insights on the impacts of these practices, and will permit the comparison with the mixtures coming from two years of selection inside components. These results will contribute to improving methods and tools for on farm participatory plant breeding not only for this particular group of farmers but also for any farmers' organisations who want to reintroduce within-field genetic diversity and become more autonomous in their agroecological systems.

## Acknowledgements

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# Molecular-genetic polymorphism of glycinin encoding genes in Ukrainian and Serbian soybean varieties

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## Introduction

Soybean (*Glycine max* L.) is the main source of vegetable protein and amino acids in the diet of humans and animals of the world. It is one of the most common crops in world agriculture, which yields only rice, corn and wheat in terms of sown area. It is grown on an area of about 93.5 million hectares of the world (Wang et al. 2016). In recent years, soybean has become very significant in Ukraine. The most promising proteins for the production of soybean products are globulins, namely, 11S globulin fractions due to the presence of glycinin. The quality of the products depends on the ratio of these fractions and the directions of their usage (Hsiao et al. 2015).

Polymorphism of *Gy1*, *Gy2*, *Gy3*, *Gy4*, *Gy5* genes, which are encoding glycinin is studied on the American soybean varieties (Jegadeesan et al. 2012; Kim et al. 2010). The aim of our conducted research is to detect polymorphism of glycinin encoding genes in Ukrainian and Serbian soybean varieties.

## Material and Methods

Polymorphism of *Gy1*, *Gy2*, *Gy3*, *Gy4*, *Gy5* genes was studied by polymerase chain reactions (PCR) on the samples of Ukrainian (Anatoliivka, Antares, Apolon, Berehynia, Chernivets'ka 9, Farvater, Kyivs'ka 98, Mel'pomena, Odes'ka 150A, Ustia, Valyuta, Vasyl'kivs'ka, Yuh 30) and Serbian (Proteyinka) soybean varieties. As reference variety there was used the American variety Harovinton.

## Results and Discussion

As results, all studied varieties have *Gy1*, *Gy2*, *Gy3*, *Gy4*, *Gy5* gene alleles that were found to be similar to the soybean variety from the USA. The results of the conducted research are indicating that during the breeding of the studied varieties there were used the same origin samples.

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# Intra-specific diversity of wheat composite cross populations (CCPs) maintains yield in a low input system

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## Introduction

Unpredictable climatic conditions and increasing pressure on non-renewable resources mean that alternative agricultural systems able to cope with increasing biotic and abiotic stresses, as well as resource costs, are needed. A focus of sustainable agricultural systems is self-regulation, which helps to reduce external inputs, while maintaining or increasing system output and providing ecosystem services (Altieri 1999). Such sustainable agricultural systems are characterised by a high degree of inter- and intra-specific diversity (Moreau 2010). Evolutionary breeding through the introduction of composite cross populations (CCPs) encourages intra-specific diversity, enabling CCPs to adapt to changing environmental conditions. Here we ask whether genetically diverse CCPs that have been managed in Neu-Eichenberg since 2005 under both organic and conventional conditions without conscious selection, are capable of adaptations to various environmental forces. Therefore, CCPs and other genotypes were tested in two successive years at the University of Kassel under differing N input levels in order to test past adaptation and suitability to differing fertilisation levels as well as the yield stability of the CCPs in comparison to modern varieties encoding genes in Ukrainian and Serbian soybean varieties.

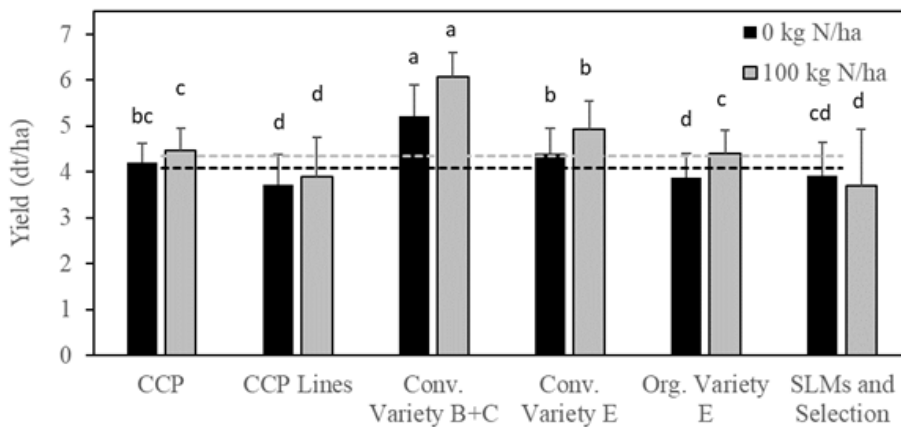
## Material and Methods

The field experiment was set up in Oct. 2015 and replicated in Oct. 2016 at the organically managed experimental farm of the University of Kassel in Neu-Eichenberg. 35 entries of 10 reference varieties, 11 CCPs, 10 CCP Lines selected from CCPs, and 4 Danish sister line mixtures (SLM) and Selections were grown in a split-plot experiment with the main factor nitrogen fertilisation (0 and 100 kg ha<sup>-1</sup> levels applied as hair-meal pellets prior to sowing) with four field replicates. This resulted in 280 (1.5 × 10 m) plots. Foliar diseases, biomass, plant length, percent of lodging and yields were recorded. For the statistical analysis, the wheat entries were compared by groups: CCPs (11 entries), CCP Lines (10), SLM and Selections (4), baking quality (E class) wheat varieties bred conventionally (4), E varieties bred for

organic conditions (4), and conventionally bred high yielding varieties (B+C class) (2). The analysis of variance was performed across seasons using a split (season) - split (replicate) - split (fertiliser) - plot model followed by protected least significant difference (LSD) tests with Bonferroni corrected P-values.

## Results and Discussion

Both seasons 2015/16 and 2016/17 were influenced by dry conditions from March until May. About 100 mm rainfall occurred in June 2016 accompanied by cool conditions that supported a late season yellow rust epidemic, while in June 2017, the drought continued resulting in an overall low foliar disease severity. Averaged over both seasons and all entries, yields were  $0.3 \text{ t ha}^{-1}$  greater after 100 kg N than after 0 kg N fertilisation ( $F_{1,6} = 19.95$ ,  $P < 0.005$ ). There was a significant interaction between fertiliser input and groups ( $F_{1,6} = 8.817$ ,  $P < 0.025$ ) and therefore, yields were analysed separately for each fertiliser level (fig. P14.1). The yield decline for SLM and Selections after 100 kg N application can be explained by their high susceptibility to yellow rust in the first experimental year, while the failure to significantly increase yields through 100 kg N input in the CCPs and CCP Lines was due to more than 50% lodging compared to 4% in the 0 kg N level.



**Figure P14.1:** Means and standard deviations of grain yields ( $\text{t ha}^{-1}$ ) separated by entry groups and N-input levels (black bar: 0; grey bar: 100 kg N/ha/yr. Means and statistics across the two experimental years are shown. Group sizes are CCPs: 11; CCP Lines: 10; conventional varieties B+C: 2; conv. Variety E: 4; organic varieties E: 4; sister line mixtures (SLMs) and Selections: 4. Different letters above bars indicate significant differences between wheat groups; lower case: 0 kg N, upper case: 100 kg N. Protected LSD-test at  $P < 0.05$  with Bonferroni correction.

Both entry groups responded to N with the production of longer tillers and more biomass than with development of more seeds, thus increasing their susceptibility to lodging. Interestingly, yields of CCPs generally exceeded the respective averages

of the fertiliser levels and not statistically different from the conventional E varieties at 0 kg N. Along with the low standard deviations, CCPs showed a high degree of adaption to low input systems and yield stability over both cropping seasons.

In conclusion, CCPs proved to be a (drought and foliar disease) stable source of production that can compete with modern varieties, particularly under low input conditions. The low yield increase for CCPs under high input conditions supports the general hypothesis that natural selection processes for CCPs over time rather increase their competitive ability (plant height, biomass) than their grain yields (Costanzo and Bàrberi 2014).

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# **Breeding for inter-species diversity annual crops**



## Learning from nature: A below-ground perspective on plant-plant interactions in diverse grassland communities

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Biodiversity is increasingly threatened by human actions such as climate change, exploitation and pollution. This causes great concern, not only about the species that are threatened, but also about the functions that ecosystems can provide.

The question how biodiversity loss would affect ecosystem functioning was first raised in the 1990s and led to the emergence of a new field of ecological research that investigated this question experimentally by manipulating plant species richness in experimental grassland communities (Hector et al. 1999; Tilman et al. 2001; van Ruijven and Berendse 2005). After many experiments across the world, there is consensus among ecologists that primary productivity, an important ecosystem function, increases with plant species richness (Cardinale et al. 2012). In other words: monocultures perform worse than plant species mixtures.

Although it is now well established that plant diversity is important for ecosystem functioning, the underlying mechanisms remain debated. Initially, the explanation for the pattern was sought in resource partitioning among plant species. If plant species that differ in the ways they acquire and use resources are grown together, they would acquire and use these resources more effectively than the species can do separately, and consequently, produce more biomass. However, direct experimental evidence for resource partitioning among species (e. g. based on tracer studies, (Bachmann et al. 2015; von Felten et al. 2009) and its contribution to increased biomass production in mixtures is mixed at best, at least below-ground (Mommer et al. 2010; Oram et al. 2018).

The limited evidence for resource partitioning, sparked the development of an alternative hypothesis, centred around the idea that interactions between plant roots and pathogenic soil biota are key to understand the biodiversity-productivity relationship (de Kroon et al. 2012; Maron et al. 2011; Schnitzer et al. 2011). The pathogen hypothesis is built upon two assumptions about plant-pathogen interactions within plant communities. The first is that plant species accumulate species-specific pathogens, referred to as host-specificity. These species-specific pathogens can reduce the performance of their host species, but have little impact on other plant species. The second assumption is negative density-dependence,



which suggests that the accumulation of plant species-specific pathogens and the negative impact on host performance declines with decreasing relative abundances of their host plants. Hence, along a gradient of plant species richness, pathogen pressure, potentially limiting biomass production, is high at low species richness (i. e. in monocultures where the relative abundance of the host is 100 %) and expected to decrease in plant species-rich mixtures, where the relative abundance of host plants will be lower. This ‘pathogen hypothesis’ is analogous to crop yield reductions from pests in agriculture, which typically increase with repeated cultivation of the same crop on the same field, and can be reduced by rotations with different crops (Bullock 1992).

My presentation at the EUCARPIA symposium will introduce the state-of-the-art of biodiversity-ecosystem functioning research (Mommer et al. in press, 2016), with the aim to provide an integrated perspective for breeders on this topic. The insights that ecologists have developed over the last three decennia are ready to be translated into agricultural practices (Mariotte et al. 2018). For example, the use of different genotypes and (mixtures of) cover-crops may help to increase both plant biodiversity and soil biodiversity and therefore develop sustainable agricultural practices.

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# Participatory designing of Pigeon pea (*Cajanus cajan* (L.) Millsp.) ideotypes for adaptation to various cropping systems in Benin

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## Introduction

Pigeon pea is a perennial multipurpose legume shrub. The crop is grown for its produces (grains, fodder, and firewood) and services (ability to improve soil fertility and to control weeds). Pigeon pea is drought tolerant, well adapted to semi-arid and arid regions and to low input cropping systems (Odeny 2007) and integrated in various cropping systems. In Benin, farmers grow pigeon pea varieties without taking into account the cropping systems the crop is integrated to (Ayenan et al. 2017). Varieties adapted to cropping systems limit the extent of competition with other intercropped species. It is, then, important to design and promote the use of varieties with adapted plant architecture, growth type and cycle for each cropping system. This will enable farmers to optimise the benefit derived from the crop either in sole cropping or in intercropping. This study aimed at analysing pigeon pea based cropping systems and designing ideotypes for each cropping system.

## Material and Methods

We conducted a survey with 302 farmers in four major pigeon pea growing regions to document and analyse the various pigeon pea-based cropping systems. Data were collected through semi-structured interviews, focus group discussions, field visit and observation. The designing of pigeon pea ideotypes based on land availability, gender and production objective was jointly done with farmers. In addition to the participatory rural appraisal (PRA), 39 local pigeon pea accessions were evaluated along with 9 breeding materials obtained from the International Institute of Tropical Agriculture (IITA) to assess their diversity based on important traits for adaptability to various cropping systems. The evaluation was conducted during the cropping season of 2015-2016 at IITA sub-station in Benin.

## Results and Discussion

Findings from the PRA revealed that most farmers grow pigeon pea in association with up to four crops species on the same plot. The associated crops were mainly maize, vegetables, groundnut, cassava and yam. The crop association was either in temporal intercropping or mixed intercropping (association with other crops on the same row). Combination of such a high number (up to 4) of crop species requires special plant architecture to limit competition for space, water, light and soil nutrients.

Against the backdrop of the pigeon pea-based cropping systems, we asked farmers to design pigeon pea ideotypes considering the following criteria: production objectives, land availability and gender (table T7.1). It came out that female farmers are likely to prefer early maturing, compact and short stature varieties amenable to intercropping. This could be explained by the fact that women have less access to land and by adopting early maturing varieties, they can harvest within 4 months and save space for other crops. Further, intercrop compact and short stature varieties with other species would limit interspecific competition and could increase the land equivalent ratio. In regions where land availability is not a constraint and the production objective is soil fertility improvement / and or weed control is the main objective, high branching and late maturing varieties are likely to be preferred. Actually, late maturing varieties with high branching produce high biomass and homogenous soil coverage, which are key to increase organic matter and prevent soil erosion. Any of the options, however, goes with a trade-off.

**Table T7.1:** Ideotypes of pigeon pea varieties based on land availability, gender and production.

	<b>Soil fertility improvement</b>	<b>Weed control</b>	<b>Agroforestry</b>	<b>Forage</b>
Land scarcity/female farmers <sup>a</sup>	Compact, short stature and early maturing	Compact, short stature and early maturing in pure stand	compact, short stature	Semi-compact, short stature
Land availability	Semi-compact, High branching varieties with high biomass production	Semi-compact, Medium branching in pure stand or in association	Compact, Medium branching	Spread or semi-spread, High branching varieties with high biomass production

<sup>a</sup> Regardless of the region, female farmers have limited access to land, reason why in the designing of the ideotype they were linked to land scarcity.

For example, adopting early maturing, low branching and short stature varieties goes with low ability for improving soil nutrients content as compared to medium to late maturing (Mapfumo and Mtambanengwe 2004).

From farmers' ideotypes designing, we evaluated a set of local accessions and breeding materials of pigeon pea using phenological and plant architectural traits. We found that all the local accessions were tall ( $260.35 \pm 11.33$  cm), semi-determinate or indeterminate, semi-compact with high branching ( $15 \pm 3$  primary branches) with medium to late maturity ( $176 \pm 9$  days). The breeding lines were short stature ( $140.30 \pm 22.7$  cm), semi-spreading or compact and extra or early maturing ( $70 \pm 5$  days). This finding suggests that materials could be selected from both the breeding lines and the local accessions for specific pigeon pea-based cropping system.

In conclusion, even though from the literature, adoption of pigeon pea varieties does not solely depend on their growth pattern and architecture, it is important to consider these traits in breeding and introducing pigeon pea varieties to better harness the potential of the crop.

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# Breeding for mixed cropping and Anthracnose resistance of lupins

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## Introduction

In Switzerland, the organic sector has been trying to reduce the massive import of protein crops for feed by encouraging grain legume cultivation during the past years (Clerc et al. 2015). The main limiting factors of low yield stability and high weed pressure were successfully overcome through intercropping of peas and faba beans with barley and oats, respectively. Thus, the production of peas and faba beans grew continuously from 2010 to 2015 from 200 t and 90 t to 1170 t and 560 t, respectively (Bioaktuell 2017). For more diversification of domestic grain legume cultivation and supply, FiBL has started field trials with blue (*Lupinus angustifolius*) and white lupins (*L. albus*) in 2014. The high risk of anthracnose, caused by the fungal agent *Colletotrichum lupini* (Bondar) (Damm et al. 2012), is presently preventing white lupin cultivation in Switzerland, although organic feed mills are highly interested in domestically grown lots of this valuable plant protein source for food and feed.

The objectives of the study were (i) identification of suitable crossing partners for blue and white lupins, (ii) suitability of cultivars and breeding lines of blue and white lupin under organic growing conditions in Switzerland in mixed cropping systems, (iii) identification of genetic resources of white lupins with partial resistance against anthracnose, and (iv) development of a composite cross population and prebreeding material of white lupins with improved resistance.

## Material and Methods

Four different lupin trials with plots of 7.5 m<sup>2</sup> size in a randomized block design with three replicates were conducted from 2015–2017 on the biodynamic farm Bioböhler in Mellikon/Rümikon (“high” Rhine valley, Kanton Aargau), Switzerland: Trial 1 testing 6–7 different crops as cropping partners with the blue lupin cv. Boruta; Trial 2 testing 8–12 different blue lupin genotypes in mixed cropping with summer oat cv. Buggy; Trial 3 testing 6–7 different crops as cropping partner with the white lupin cv. Feodora, Trial 4 testing 8 different white lupin genotypes in mixed cropping with summer oat cv. Buggy. Additionally, genetic resources and breeder’s lines of white lupin were tested for anthracnose resistance in single rows planted between spreader rows of the susceptible cultivar Amiga. 111 rows were sown in 2017 harvested

from infected plants that had already been assessed and selected in 2015 and 2016, and 101 rows were sown in 2017 with new genetic resources.

## Results and Discussion

**Trial 1:** For blue lupins, mixed cropping is beneficial as it reduces weed pressure and has a higher land equivalent ratio resulting in higher financial return than pure stands. Depending on the year, the cropping partners were very competitive and reduced the percentage of blue lupin below 30 %. This was the case with oats and in 2015 and 2016, with barley in 2015 and 2017, and with wheat in 2017. Triticale proved to be the best partner over the three years.

**Trial 2:** The only determinate cultivar Boruta yielded less than the indeterminate types but was much more reliable concerning lodging resistance and homogenous ripening, allowing an earlier harvest than the indeterminate types.

**Trial 3:** For white lupins, the mixtures tested to date had no yield benefits compared to the pure stands. In some cases, disease scores were slightly reduced in mixed cropping compared to the pure stands especially when the partner crops were below the lupin canopy (e. g., for triticale and the dwarf summer oat Buggy). Seed maturation was accelerated in mixed cropping with tall, vigorous oat cultivars.

**Trial 4:** All cultivars and breeders' lines were highly susceptible to anthracnose, which was most obvious in the more humid early summer of 2016. Yield differences mainly reflected the maturation regime, late ripening allowing more yield if conditions were favourable, but early ripening being the best escape from anthracnose. In the dry summer of 2015, the late maturing cultivar Energy was the best with a total (lupin + partner) yield of  $4 \text{ t ha}^{-1}$  (of which 72 % were lupin seeds). In 2016 with high anthracnose incidence and overall low lupin yields, the early maturing cultivar Feodora with determinate growth had the highest total yield ( $1.3 \text{ t ha}^{-1}$ ), combined with the best lodging resistance. However, yield differences were small. In 2017, the early ripening but indeterminate cultivar Zulika showed high yield, but was prone to lodging.

Out of the 111 infected lines, two breeding lines from Chile and three genebank accessions continue to show lower disease score index by  $\geq 1.25$  compared with cv. Amiga. Of the 101 new lines, 8 were clearly superior to Amiga, but will have to be tested again in a subsequent year. Five of these were obtained from the genebank and three were breeding lines from Italy.

Various genetic resources, including lines with improved resistance capacities, are crossed in a hierarchical order to generate a heterogenous composite cross population. The  $F_2$  of 10 single crosses between white lupins were bulked and multiplied in the field in 2017 to allow for natural selection against anthracnose.

For blue lupins, cultivation can be recommended in Switzerland especially in mixed cropping with triticale. In contrast, white lupins cannot yet be recommended due to the high risk of anthracnose, although yields can be very high in individual years. Our breeding goal for white lupin is an anthracnose resistant, early ripening, lodging resistant, low alkaloid cultivar. Only a small percentage of the genetic resources showed higher resistance against anthracnose than the commercial varieties. They will be used as parental lines for crossing schemes to establish a composite cross population. Artificial inoculation at the seedling stage will allow screening of a larger number of genetic resources for resistance. Mixed cropping could help reduce the late weed pressure in white lupin but further research is needed with less competitive crops or under-sowing.

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## Breeding for mixtures in organic farming systems: Are the traits of pure wheat cultivars predictive of their behaviour in wheat-pea mixtures?

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### Introduction

In France, demand for organic wheat flour is higher than supply. On average, 30 % of organic bread wheat is imported. Despite having a financial interest in producing organic wheat, farmers face quantitative and qualitative difficulties among which low grain protein content because of lack of nitrogen in organic farming systems. One solution to overcome this difficulty is to cultivate wheat with a companion legume crop.

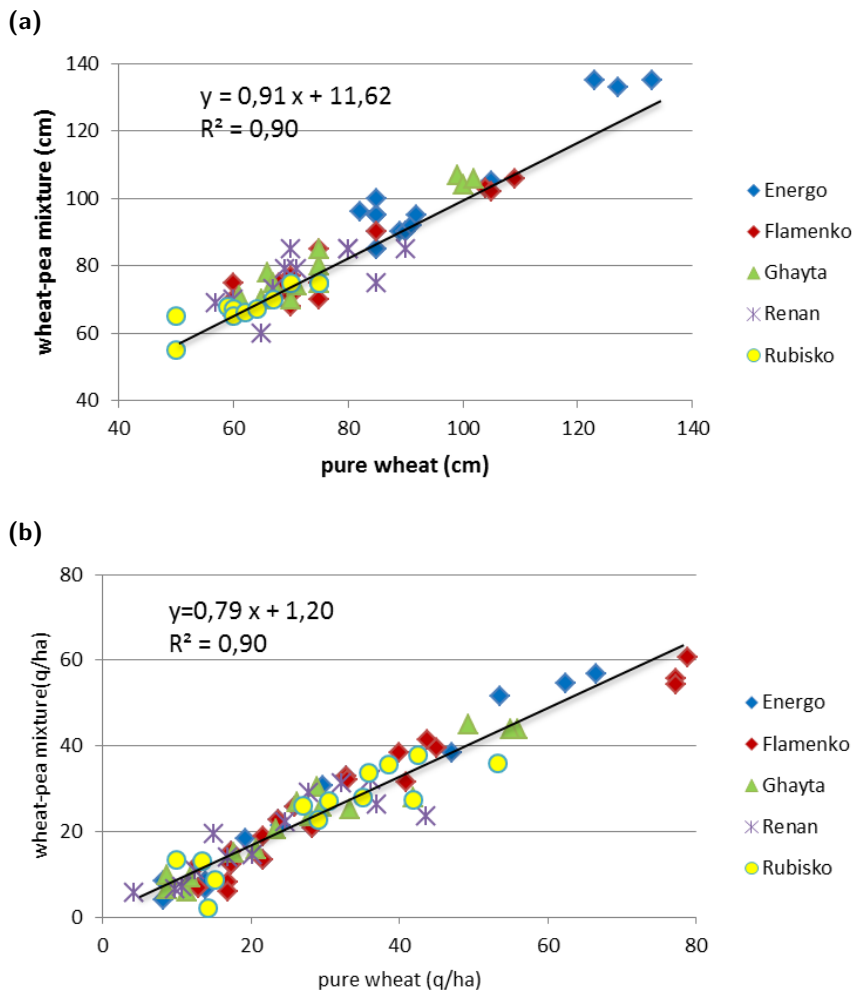
The question of the choice of the wheat cultivar that would be best suited to be mixed with the pea arises. Can this choice rely on traits only assessed in pure crop? To what extent are these traits and performances of a cultivar grown in sole crop predictive of traits and performances in mixtures? And finally, is it necessary for some of these traits to consider specific breeding approaches for adaptation to mixtures?

### Material and Methods

Our work aimed evaluating bread winter wheat-pea mixture traits compared to those of pure wheat crop. Five field trials were carried out during 2015–2016 and / or 2016 / 2017 seasons, under various pedoclimatic conditions in the North-Western (near Ancenis, near Poitiers and at Rennes) and South-Eastern (near Valence) parts of France.

Five wheat cultivars with contrasted phenological, architectural and performance traits were grown as sole crops and full mixed on the row with a single protein pea cultivar.

Agronomic traits (canopy height at heading date BBCH55, final canopy height, soil coverage at three different stages), grain yield, specific weight and grain protein content have been measured both on sole crops and mixtures.



**Figure T9.1:** Wheat canopy height at harvest (a) and wheat yield (b) in wheat-pea mixture in relation to pure wheat values based on the 82 couples sole crops / mixtures measured.

## Results and Discussion

First, we observe that, as usual, mixtures increase the soil coverage of crops (which likely allows a better control of weeds), reduce wheat yield and increase wheat protein content.

Pearson correlation coefficients, calculated between sole crops and mixtures, all cultivars together, were:

- very low for specific weight ( $R^2 = 0.16$ ),
- medium for soil covering power at early and medium stages and grain protein content;
- and high for plant height at heading date, soil covering power at heading date, final plant height (fig. T9.1a) and grain yield (fig. T9.1b) ( $R^2 = 0.69$ , 0.67, 0.90 and 0.90 respectively).

This means that only for the four latest traits the assessments in sole crop are really predictive of the ones in mixtures. These results show that some characters evaluated in sole crops can be predictive of the behaviour in mixture in our environmental conditions

We have investigated a range of wheat cultivars mixed to a single pea cultivar using a unique additive sowing density. Further investigation is needed to extend our conclusions to a larger range of pea cultivars in various environmental conditions.

## Acknowledgements

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## Minor cereals exhibit superior antioxidative effect in human cancer cell lines compared to common wheat

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### Introduction

Reactive oxygen species (ROS) are known to be highly harmful to cellular systems and produced in living organisms, particularly under stress conditions. Exposure to excessive levels of ROS might lead to the disruption of lipid membranes, unfolding of proteins, degeneration of organelles such as mitochondria and result in DNA damage and related accumulation of mutations. Consequently, ROS contribute to several pathologies, including inflammation, neurodegenerative diseases and cancer in human body. In order to neutralise excessive ROS, cells use diverse of antioxidant mechanisms which are highly affected by the composition of the consumed diet. Regular dietary intake of antioxidant molecules is, therefore, crucial for organismal and cellular health. Cereals are a major component of the daily energy intake. Consequently, the content and composition of antioxidants of the cereals consumed have critical effects on the antioxidant status of the body. Nowadays, consumers pay special attention to consumption of antioxidant-rich foods, because of increasing awareness of the positive effects of antioxidants and related bioactive compounds (i) on minimising deleterious effects of natural ageing and (ii) mitigating adverse impacts of many chronic human diseases in body such as cancer and cardiovascular diseases.

In the present study, the antioxidant capacity of commonly consumed modern wheat cultivars were investigated in different human cancer cell lines in comparison to different minor cereals including spelt wheat, rye, oat and wild wheats. This knowledge is important for the selection of suitable material in future breeding programs.

## Material and Methods

Antioxidant capacity of common wheat (*Triticum aestivum*) and minor cereals including oat (*Avena sativa*), rye (*Secale cereale*), spelt (*T. spelta*) and wild wheat was studied in human epithelial cell lines. The genetic materials used in this study were selected from the collections of genetic resources of oat, rye, spelt and wild wheat species on the basis of the previous results.

Ethanol-soluble extracts made from twenty genotypes each of oat, rye, spelt and wild wheat and 12 genotypes of common wheat were used in the biological tests conducted. The antioxidant effects of the grain extracts were studied on HeLa (cervical) and HCT116 (colorectal) cancer cell lines as human epithelial cell models. In these cell cultural tests, cellular levels of reactive oxygen species (ROS), mitochondrial health, DNA damage and cell survival were analysed by using standard biological tests. In the tests, N-Acetyl Cysteine (NAC) has been used as control antioxidant molecule

## Results and Discussion

The majority of genotypes of the minor cereal species tested showed higher antioxidant effects in human cell cultures compared to the common wheat genotypes. Many of the minor cereal genotypes with high antioxidant capacities protected DNA from damage, blocked mitochondrial depolarisation and prevented cell death in HeLa cells and colon-derived HCT116 human epithelial cells. The results indicate that minor cereals contain astonishingly high levels of certain antioxidant substances.

Most of the rye, oat, spelt and wild wheat genotypes had also much better protective potential against both generation and oxidative attack of ROS in human cell cultures than the modern wheats. Modern wheat cultivars usually failed to protect cell damage and death catalysed by ROS. The results obtained indicate clearly that increased dietary intake of foods made by using minor cereal species will be more effective in fighting against oxidative cell damage and improving organismal health.

## Acknowledgements

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# Effects of organic and conventional management for five years on early seedling traits of three winter wheat composite cross populations

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## Introduction

High levels of within-crop diversity, such as composite cross populations, (CCPs) of wheat are expected to buffer against biotic and abiotic stresses. Increased in-field diversity provides higher stability and flexibility and allows for continuous adaptation over time (Finckh 2008). In diverse CCPs, early vigour is of special importance for performance in conditions of limited nutrients, and where weeds need to be outcompeted. To determine if seedling vigour can be enhanced over time by exposing CCPs to differing growing environments, Bertholdsson et al. (2016) successfully compared the 3 CCPs of  $F_6$  and  $F_{11}$  in a hydroponic system. They found differences in seedling traits due to the differing genetic composition of the CCPs and due to the organic and conventional management system. However, they used seeds that had been frozen for various lengths of time. In addition, there was a large difference in seed size among the  $F_6$  and  $F_{11}$ , because the CCPs had been grown in separate fields with variable soil and weather conditions. Therefore, we grew the seeds of the two generations for one year under the same conditions in one field and retested the seeds of the ensuing  $F_{6.1}$  and  $F_{11.1}$ .

## Material and Methods

In 2001, three very heterogeneous winter wheat CCPs (Y, Q and YQ) were created based on 9 high yielding (Y), 12 baking quality (Q), and the intercross of all 20 parental lines (YQ) in the UK. Since the  $F_5$ , these CCPs have been maintained at the University of Kassel under organic (org.) and conventional (conv.) conditions and since the  $F_6$ , each population was separated into two parallel populations within system, resulting in a total of 12 CCPs that were managed without conscious selection. In 2016 / 17, seeds of the 12 CCPs of the  $F_6$  and  $F_{11}$  (harvested 2007 and 2012) were increased at the Technical University of Munich, Freising, resulting in the  $F_{6.1}$  and  $F_{11.1}$ . The early vigour of these CCPs was assessed using the

hydroponic system based on Bertholdsson et al. (2016) at 2 mM N concentration. Eight replicates and ten plants per replicate of the 12 CCPs of  $F_{6.1}$  or  $F_{11.1}$  were used. Plants were grown in a heated greenhouse with 18 °C day and 12 °C night temperature for 14 days. The longest seminal root length (SRL) and the shoot length (SL) are presented here.

## Results and Discussion

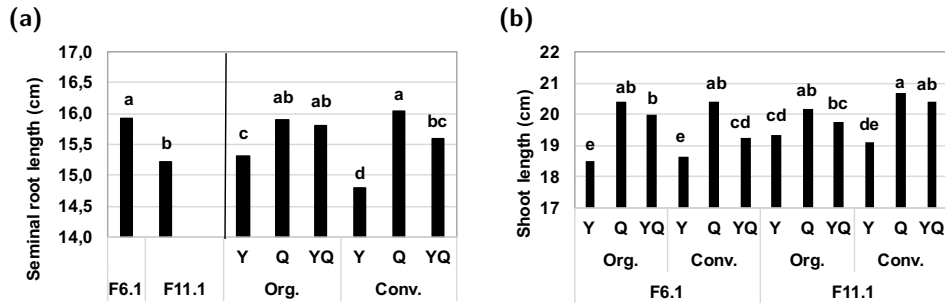
Under the same field conditions, the seed size of  $F_{11.1}$  (44.2 g) was larger than  $F_{6.1}$  (42.4 g) and it was larger for org. (43.7 g) than conv. (42.8 g), and also larger for Q populations (44.1 g) than Y (42.6 g). The effects of generation, system, population interacted in various ways (table P16.1). Within systems (org. or conv.), the two parallel CCPs of Y, Q and YQ were similar for length traits (data not shown). Seminal root length (SRL) was significantly shorter in the  $F_{11.1}$  than in the  $F_{6.1}$  (fig. P16.1a). In contrast, shoot length (SL) increased by the  $F_{11.1}$  (19.9 cm) from  $F_{6.1}$  (19.5 cm). Among populations, SRL and SL was longest for the Q (16.0 and 20.4 cm) followed by YQ (15.7 and 19.9 cm) and shortest in the Y (15.0 and 18.9 cm). Across generations, SRL was shortest in conv. and org. Y populations and longest in Q and YQ of both systems (fig. P16.1a). By the  $F_{11.1}$ , SL of all three conv. populations increased (only significant for conv. YQ) whereas, in the org, SL increased only for org. Y (fig. P16.1b).

**Table P16.1:** Analysis of variance for seedling traits from CCPs of  $F_{6.1}$  and  $F_{11.1}$  grown under organic and conventional system.

	Seminal root length	Shoot length
Generation	***	***
System	ns	ns
Population	***	***
Generation * System	ns	*
System * Population	*	ns
Generation * System * Population	ns	**

Overall, genetic and management system effects on the length traits follow similar trends as those found in the tests with the frozen seeds by Bertholdsson et al. (2016). Thus, despite prolonged freezing for different times and effects of growing environment on seed size the results are consistent. However, results of the fresh seeds of the parallel populations were more similar making it easier to determine management system effects. The strongest system effects were observed between the org. and conv. Y populations suggesting that these populations were genetically distinct and most reactive to the growing system. For low-input or organic systems, longer SRL is particularly crucial for soil exploration and water and nutrient uptake. Besides, the quick and fast emerging shoot will increase photosynthetic rate, which

will further enhance the translocation flow of assimilates to the roots (Bertholdsson et al. 2016).



**Figure P16.1:** A: Seminal root length in two generations and separated by system and population. B: Effects of generation, management system and population on shoot length. Columns with the same letters are not significantly different ( $P < 0.05$ ) (Duncan test).

## Acknowledgements

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## Overview SRUC related research on breeding for diversity

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### Introduction

Plant interactions in agricultural systems can be defined according to crop genetic structure including mono-genotypic (monocultures), multi-genotypic (e. g. mixtures of cultivars) and multi-species (e. g. intercropping systems). Understanding the physiological and genetic basis of intra- and inter-specific interactions in the crop or competition environment is of prime importance for developing plant breeding programmes to support a wider diversity of crop growing systems, in which crop genetic diversity can be exploited to increase yield and crop resilience in the face of climatic challenges.

For application to plant breeding, it is important to establish the effect and heritability of intra- and inter-specific plant interactions on traits that influence crop vigour, yield and grain quality. Analysis of different genotypes grown under a range of crop environments will allow us to consider how knowledge of plant interactions can support breeding for more diverse crop systems. Our experimental approaches include the use of field phenotyping and genotyping to study the genetic basis of crop yield and quality in crops of different genetic structure. We are also exploring novel approaches to breeding for intercrop scenarios involving orphan crops that can diversify low input production systems in developing countries.

For enhancing crop resilience, multi-genotypic mixtures and multi-parental populations, diverse mixed or inter-crossed lines respectively, have the ability to adapt to and utilise environmental variable conditions. Any heritable response of these populations under low input scenarios such as nutrient use efficiency and disease resistance would enable us to develop more resilient systems. Breeding for diverse crops is also a main target in Integrated Pest Management (IPM). The development of material that is more locally adapted and more variable than the current genepool will benefit sustainable crop management. This ongoing and proposed work will be presented and discussed.

# Assembling rules for the control of *Ascochyta* blight in winter wheat/pea mixtures

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## Introduction

Among the services expected from species mixtures are the control of pathogens, pests and weeds together with the maintenance of productivity and reduction of pesticide use. Varietal traits and agricultural practices can be explored to optimise the control of above ground pathogens and pests in mixtures (Boudreau 2013). Previous reports have shown that cereal / legume mixtures are likely to reduce grain legume pathogens epidemics (Fernandez-Aparicio et al. 2010, 2011; Schoeny et al. 2010). Aiming at identifying most efficient species assembling rules for the control of epidemics, we investigated the effects of plant and canopy architectural and phenological traits within winter pea / winter wheat mixtures on *Ascochyta* blight disease infection and development on pea.

In the present study, the antioxidant capacity of commonly consumed modern wheat cultivars were investigated in different human cancer cell lines in comparison to different minor cereals including spelt wheat, rye, oat and wild wheats. This knowledge is important for the selection of suitable material in future breeding programs.

## Material and Methods

Five winter pea cultivars were mixed to a single winter wheat cultivar, chosen for its precocity and resistance to wheat diseases. Mixture types were constructed using substitutive (50/50; 70/30) or additive (100/30) sowing densities (% of the recommended pea sowing density divided by % of the recommended wheat sowing density). The 50/0 and 100/0 pea plots were used as controls. Two split-plot designs with 3 blocks were set up, each comprising the five sowing densities as randomised subplots within blocks and randomised varieties within subplots. The first was sown alternating (1/1) pea and wheat rows, the second by fully mixing both species on each row. Both designs were carried out in 2015 and 2016.

Canopy closure and height were both monitored every two weeks from the end of the winter. Canopy closure was assessed from RGB image acquisition on each

plot, followed by automated analysis of % of aerial plant cover (leaves, stems and tendrils) using a modified ImageJ analysis module. Canopy height was assessed at the plot level using a graduated ruler.

Microclimatic conditions at low, medium and high levels of the canopy were followed on one cultivar  $\times$  two sowing conditions  $\times$  two sowing densities from the end of the winter during the remaining cropping season. Combinations of temperature and leaf wetness duration data allowed identifying periods conducive to pathogen infection using the Magarey model (Magarey et al. 2005). Disease severity was monitored at three dates assessing % of necrosis on aerial tissues at the plot level.

## Results and Discussion

In both years the control of weeds and pests was satisfactory, and the monitoring of architectural traits could be performed. The two years, however, differed very much in the balance between species since (i) in 2015 pea was very much more competitive than wheat (probably due to a late sowing date and a preceding cereal crop) and environmental conditions were not conducive to the disease, leading to very low epidemics. (ii) In 2016 species competition led to a more balanced canopy at maturity, and conditions were much more conducive to diseases, including bacterial blight, downy mildew and *Ascochyta* blight.

In both years canopy closure and canopy height did not differ between sowing methods, i.e. alternate rows or full mix on the row. Canopy closed however more rapidly in mixtures than in pure stands, and more rapidly for one of the pea varieties. Dynamics of canopy height differed between years, with delayed lodging in 50/0 and 50/50 mixtures (pea density effect) in 2015, and delayed lodging in 100/30 and 50/50 mixtures in 2016 (wheat tutoring effect on pea).

Contribution of wheat to biomass and overall yield was very low in 2015, and much more important in 2016. There was therefore no effect of wheat biomass and yield on pea biomass and yield in 2015, whereas wheat competition strongly reduced pea biomass and yield in 2016.

In 2016, there was no difference in disease severity between sowing densities and between varieties at the early stages of the epidemics, whereas slightly lower disease severity was observed in 50/50 sowing density and in two of the five cultivars after flowering. These differences between cultivars remained significant, but not the differences between sowing dates by the end of the vegetation at maturity. Monitoring of microclimatic conditions clearly identified that conducive conditions to the pathogen at the start of the epidemics did not differ between sowing densities and varieties, whereas mixtures showed less conducive conditions than pure stands by flowering.

We conclude that mixtures effects on the control of epidemics needs to be described depending on cultivars, sowing densities, actual balance between species biomass, and level of epidemics. Competition between species largely relies on varietal and management strategies, and infers the subsequent level of control in the epidemics. Exploring further the underlying processes (canopy microclimate, plant tissue receptivity and spore dispersion) will be a key issue to make epidemics control an operational level in the field.

## Acknowledgements

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## Effects of agrobiodiversity on pest control in *Vicia faba* L.

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### Introduction

Agrobiodiversity, achieved both in terms of crop diversification and diversity of surrounding spontaneous flora, provides a plethora of ecosystem services to cropping systems, among which sustainable pest control (Smith and McSorley 2000). Nevertheless, high-input farming practices directly affecting biodiversity, such as herbicide application or monocultures, may disrupt potential pest control services (Tschardt et al. 2016). Understanding how such practices affect crop-pest dynamics may help to evaluate their ecological drawbacks and to design diverse and sustainable cropping systems. Within this context, we used faba bean (*Vicia faba* L.) and its common pest, the bean beetle (*Bruchus rufimanus* Boh.) as model system to understand how farming practices affecting agrobiodiversity (herbicide application and monoculture) may influence pest damage. Agrobiodiversity plays a crucial role in this system, as adults of the beetle mainly feed on pollen and nectar from both crop and surrounding flora (Huignard et al. 1990). Hence, diverse cropping systems may provide a larger floral foraging habitat to the adults of the bean beetle, eventually scattering pest population and thus reduce pressure on the crop. This worked aimed to assess whether increasing agrobiodiversity, by means of crop diversification and low input weed management, might indirectly decrease bean beetle damage on faba bean.

### Material and Methods

A field experiment was laid out in Münster, NW Germany by sowing faba bean (cv. Fuego and Julia) and wheat (cv. Tybalt and Cornetto) in monocultures and mixtures, either with or without herbicide treatment. A split-plot design was applied with herbicide treatment assigned to sub-blocks and crop diversity (four monocultures and four mixtures) as plots. Faba bean seed set was assessed and damage from the bean beetle was monitored by counting damaged seeds by the beetle larvae. Spontaneously occurring weeds within plots were used to assess effects of herbicide application on weed species richness and pest damage. Statistical analysis was carried out using RStudio 1.1.383 (RStudio, Inc.).

## Results and Discussion

This work addressed whether increase in biodiversity through crop diversification and low weed management (i.e. no herbicide application) may reduce seed damage by the bean beetle on two faba bean varieties. We expected seed damage to decrease for faba bean mixed with wheat compare to when grown in monoculture, whereas low weed management was expected to increase weed species richness and indirectly reduce seed damage. Preliminary results show strong effect of weed species richness in reducing seed damage, which was interestingly higher in faba bean monocultures than in faba bean-wheat mixtures. Moreover, results also highlighted intraspecific differences in seed damage between faba bean varieties. These outcomes open the way to further questions on the dynamics between the bean beetle and surrounding flora and might lead to new options for cultural and breeding strategies.

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## Winter faba bean as target species in mixed-cropping with winter wheat

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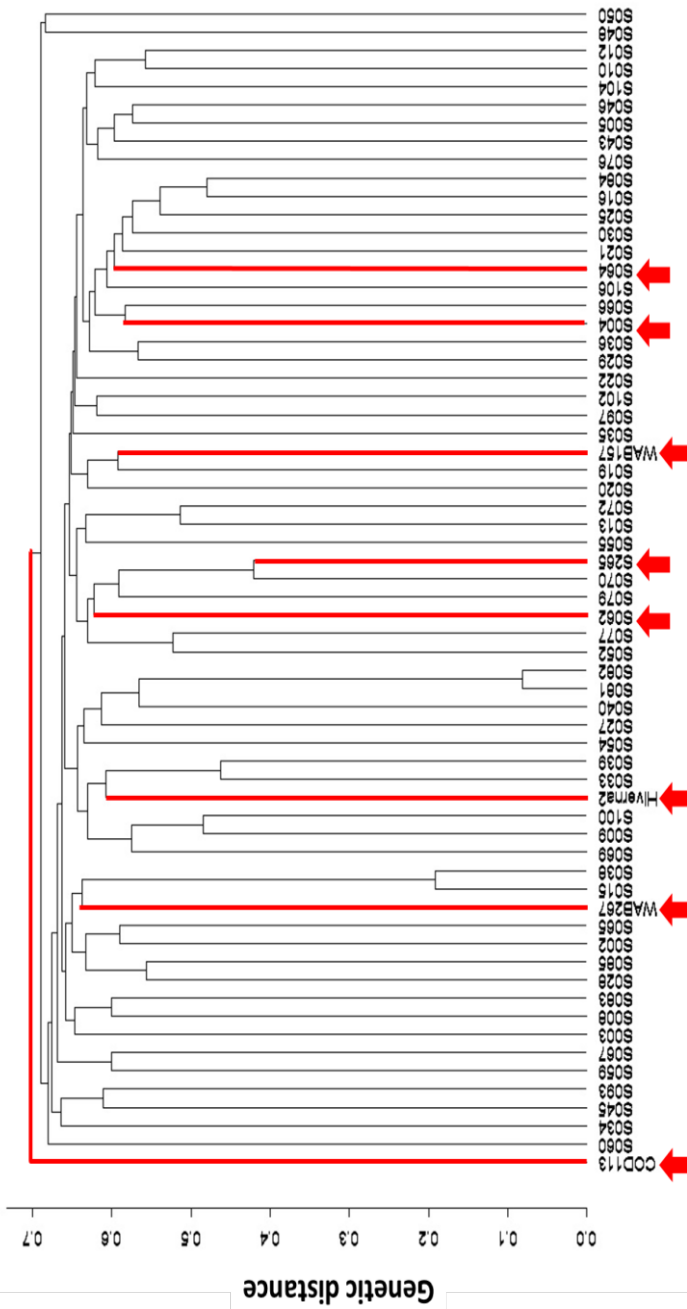
### Introduction

Usually, mixed stands yield higher than comparable pure stands. Such yield increment is assumed to result from complementarity between mixtures' component crops. Mixtures among pulses (such as faba bean) and non-legumes (such as wheat) promise increased efficiency, sustainability and resilience of food and feed production. Here we report on performance of winter faba beans (*Vicia faba*) and winter wheat (*Triticum aestivum*) as mixed-crop. Better understanding the genetic whys and wherefores of yield increment in this mixture would allow to more reliably exploit this phenomenon. (i) Which (type of) winter faba beans will achieve the highest grain yield performance when grown as mixed stands with wheat? (ii) Which breeding-relevant trait will co-vary with variation of yield and of yield increment? (iii) Will the novel winter faba beans show any kind of striking DNA-based diversity pattern?

### Material and Methods

To this end, mixed stands of  $N = 8$  novel winter faba bean lines and  $N = 3$  released winter wheat cultivars were compared with their corresponding pure stands within the frame of IMPAC<sup>3</sup>. In 2015, 2016 and 2017, field experiments at three locations ( $r = 4$ ) were implemented through the University of Göttingen. The pure and mixed stands were laid out in a row intercropping design over 360 plots; N-fertiliser application was zero. Developmental and yield parameters were observed in detail.

Additionally, 189 SNP and 1829 polymorphic AFLP markers were employed to compare the diversity pattern of these winter faba bean lines with that of the conventional, elite germplasm pool as currently used in breeding for pure stand performance of winter faba beans. Filtering for minor allele frequency (5%) yielded 175 SNP and 1147 AFLP markers, used to develop a similarity matrix. SNP-based similarities were calculated via Dice, AFLP based similarities via Jaccard. To calculate the combined genetic similarities, SNP and AFLP similarities were weighted according to their marker numbers. With this, an UPGMA cluster for a



**Figure P20.1:** UPGMA Cluster of the novel 8 winter bean genotypes (indicated with arrows) and 56 further non-IMPAC<sup>3</sup> genotypes (from the germplasm pool as used in current breeding and breeding research).



total of  $N = 64$  winter faba bean lines (including the  $N = 8$  novel IMPAC<sup>3</sup> lines) was created.

## **Results and Discussion**

Results showed significant yield increments of the mixtures compared to the pure stands. Moreover, there was a significant variation of this mixing effect caused by differences among the eight winter faba bean lines. Further, the DNA-based diversity pattern of our novel faba beans is obviously inconspicuous and shows no striking diversity pattern (fig. P20.1).

## **Acknowledgements**

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## Effect on durum wheat and quality parameters of mixed intercropping with faba bean

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### Introduction

In Central and Southern Italy durum wheat (*Triticum turgidum* subsp. *durum* (Desf.) Husn.) and faba bean (*Vicia faba* L.) are important crops and recently the need of increasing biodiversity to realise more sustainable agricultural systems has been widely suggested in several agricultural sectors (Bedoussac and Justes 2010; Hauggaard-Nielsen and Jensen 2001). This research aimed at testing the effect of mixed durum wheat and faba bean crops on durum wheat traits that are of utmost importance to drive farmers toward innovative production systems based on intercropping and breeding research to selection programs specifically aimed at increasing the performance of mixed cereal-legume crops.

### Material and Methods

Durum wheat (cv. Odisseo and cv. Aureo) and faba bean (cv. Chiaro di Torre Lama and cv. Prothabat69) were sown as sole crop and mixed crops (each variety sown at half of the sole crop density in the mixed crop) at two different nitrogen fertilisation levels: Conventional and Low Input (table P21.1). A Split-Plot in Randomised Complete Block design was applied with N fertilisation levels as main plots and Plant Teams (4 Sole and 4 Mixed crops) as sub-plots. Statistical analysis was carried out using the software JMP 11.0.0 (SAS Institute Inc.).

**Table P21.1:** Nitrogen fertilisation levels.

N fertilisation level	Sole crop		Mixed crop
	Wheat	Faba bean	
Conventional	160 N units	25 N units	80 N units
Low Input	80 N units	0 N units	40 N units

### Results and Discussion

Significant differences between nitrogen fertilisation levels were detected only for cereal seed protein content (16.3% and 15.0% for Conventional and Low Input

levels, respectively) and number of spikes per m<sup>2</sup> (390 and 325 for Conventional and Low Input levels, respectively). The lack of more evident effect of nitrogen fertilisation could be related to the extremely dry climatic conditions that characterised the 2017 growing season. Variance among Plant Teams was highly significant ( $P < 0.001$ ) for all variables but LER ( $P < 0.05$ ), as expected because of the main differences between sole and mixed crops, whereas a significant Plant Team  $\times$  Nitrogen fertilisation level interaction was significant only for Seed Protein content ( $P < 0.05$ ). Table 2 shows that cereal sole crops showed always a higher seed yield than the respective mixed crops but the cereal production of mixed crops was always

higher than half the yield of the corresponding cereal variety. This resulted in mean LER values that were higher than 0.60 for three out of four mixed crops (Table 2).

**Table P21.2:** Cereal crop: results of multiple comparisons (HSD test). Means with different letters are significantly different ( $P < 0.05$ ).

Plant Team	Seed Yield	LER	Seed Protein (%)	Plants (m <sup>-2</sup> )	Spikes (m <sup>-2</sup> )
ODISSEO sole	5.55 a	—	14.13 d	285.9 a	515.9 a
AUREO sole	4.36 b	—	16.44 b	265.5 a	460.7 a
ODISSEO (+Prothabat69)	3.50 c	0.63 ab	14.35 d	159.0 b	300.8 b
AUREO (+Prothabat69)	3.30 cd	0.76 a	16.56 b	145.4 b	288.5 b
ODISSEO (+Chiaro di Torre Lama)	2.85 cd	0.52 b	15.24 c	152.7 b	292.4 b
AUREO (+Chiaro di Torre Lama)	2.71 d	0.62 b	17.47 a	145.7 b	288.5 b

Mixed crops involving the combination of both durum wheat varieties with the faba bean Chiaro di Torre Lama always showed a higher seed protein content than both sole crops and mixed crops involving Prothabat69 (table P21.2). The Plant Team  $\times$  N Level interaction confirmed the effect of Chiaro di Torre Lama on cereal seed protein content in the mixed crop involving the durum wheat Aureo, especially at the Low Input level (table P21.3). This effect was less evident in the mixed crop involving Odisseo. These results suggest that investigation on cereal by legume interaction could be important to identify the optimal combination of varieties to combine more sustainable production systems (reduction of nitrogen fertilisation levels) with high quality of an important parameter in durum wheat such as protein content.

**Table P21.3:** Cereal crop: interaction between plant team and nitrogen fertilisation level on the seed protein content of the durum wheat crop. Means with different letters are significantly different ( $P < 0.05$ ).

Plant team	Conventional	Low Input
AUREO (+Chiaro di Torre Lama)	18,04 a	16,90 abc
AUREO sole	17,14 ab	15,74 cd
AUREO (+Prothabat69)	16,98 abc	16,14 bc
ODISSEO (+Chiaro di Torre Lama)	16,44 bc	14,04 e
ODISSEO sole	14,74 de	13,52 e
ODISSEO (+Prothabat69)	14,72 de	13,98 e

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## **Breeding for inter-species diversity perennial crops/agroforestry**



# Integrating diversification in short food and energy chains

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## Introduction

The middle of the nineteenth century saw, on the one hand, Charles Darwin's exposition of the basis of evolution through natural selection and, on the other, a step change towards the industrialisation of agriculture through invention of the process of production of superphosphate fertiliser. Subsequent research on evolution has underlined the importance of diversity and its positive relationship with both productivity and stability in ecosystems (Loreau 2010), which has major implications for farming systems. However, agricultural industrialisation pursued a path of reducing diversity at the levels of both species and variety, together with other developments with major downsides, summarised by Horton (2017). It is now essential to reverse these negative effects and to recognise not only the value of diversity at all levels but to integrate them into comprehensive systems that operate along whole food and energy chains from land manager to citizen consumer. In this presentation, I use examples from the Wakelyns system to help illustrate some of the breadth of applications of diversification that now need to be developed and integrated.

## Material and Methods

Diversification within species: following earlier work on variety mixtures, a series of projects funded in the UK, Germany and the EU enabled the development of composite cross populations in wheat (*Triticum aestivum* L.) which have been grown, observed and tested widely in the EU over recent years (e.g. Brumlop et al. 2017; Döring et al. 2015). One of these populations, ORC Wakelyns Population (YQ), is now being tested and marketed for bread and pastry production.

Diversification among species: many projects funded in the UK and the EU have been concerned with development of appropriate species mixtures including, for example, the UK DEFRA funded Legume LINK project (Döring 2013) which illustrates, for example, the beneficial effects of ley mixtures for pollinators, partly by extending the flowering season at both ends.

Recently, UK and EU funded projects have helped investigation and establishment of agroforestry alley cropping (particularly EU AGFORWARD) diversified among



trees, among crops and within understories. This contribution refers to two of these projects, the first of which is a comparison of apple (*Malus pumila* Mil.) performance (yield and prevalence of pests and diseases) between two organic apple plantations, one in the form of a standard orchard layout with a high density of apples per unit area and no species diversity, and the second in the form of alley cropping hedges with a low density of apple trees dispersed at random among seven other tree species (Smith et al. 2014). The second project is a study of willow (*Salix alba* L.) and hazel (*Corylus avellana* L.) coppice systems to compare the delivery of wood chip fuel from these two species grown in agroforestry hedges together with wheat as an arable crop grown in the alleys between the coppice hedges. This has allowed an estimation of the LER (Land Equivalent Ratio) comparison of the output of cereal and wood fuel from alley cropping compared with separate cropping of the cereal and coppice.

Part of the current activities are focussed on organisation of collaboration and co-operation among producers and processors within these food and energy chains both to improve the productivity of the chains and to engage with and expand local markets in different parts of the country.

## Results and Discussion

### Diversification within species

The most obvious benefit of cereal populations is their resilience under increasingly unpredictable environmental variation (Döring et al. 2015; Brumlop et al. 2017), illustrated further by farmer observations on positive sowing date effects, winter survival, weed competition and multiple disease resistance. This has created a need for more populations in wheat and development of appropriate populations in barley (*Hordeum vulgare* L.) and oats (*Avena sativa* L.).

Initially, it was thought that such a highly diverse crop as a wheat population would have little interest for the quality market. However, high hectolitre weights and straw yield indicate a useful outlet as animal feed, which is important for the stock farmer. More importantly, intensive efforts by an artisanal baker, Kimberley Bell (smallfood.org.uk), has led to methods for using organic stoneground wholemeal population flour to produce a range of sourdough breads and pastries of excellent taste and quality, which are now in high demand. The next phase of development is to develop local short-chain production systems where local farmers work with local millers and bakers to replicate the SmallFood system to satisfy local consumers; at least four such groupings are currently being developed across England. This is a form of decentralisation of food production, with many potential advantages for local businesses and consumers.

## Diversification among species

Organic systems demand temporal rotations among different crop species which helps to stimulate the introduction of new crops. There are also continuing attempts to develop spatially diversified inter-crops, the best established being the fertility-building ley crop. This has been improved through the DEFRA-funded Legume LINK project, such that the Wakelyns ley phase integrates different varieties of a range of five legume species together with one non-legume, chicory (*Cichorium intybus* L.), which has long tap-roots. Other, more recent developments include, for example, lentils mixed with 'Gold of Pleasure' (*Camelina sativa* (L.) Crantz), an initiative from Josiah Meldrum (hodmedods.co.uk) based on the experience of Austrian farmers.

The major species diversification, however, is among trees and crops in agroforestry alley cropping systems. Integrating crops and trees (and, ideally, livestock), offers the possibility of a wide range of highly diversified systems (for example AGFORWARD ([www.agforward.eu](http://www.agforward.eu)), EURAF ([www.agroforestry.eu](http://www.agroforestry.eu)), EIP.AGRI ([ec.europa.eu/eip/agriculture](http://ec.europa.eu/eip/agriculture))). The presence of trees in such cropping systems offers many advantages for cropping in the alleys between the hedges and within the hedges themselves. Most importantly, the production hedges can be used for production of food and fuel. For example, we have a wide range of apple varieties dispersed among seven other tree species. In the EU project COFREE, we were able to demonstrate large reductions in the levels of all of the common pests and diseases when compared with apples grown in a standard organic orchard containing apples alone (Smith et al., 2014).

Tree management at Wakelyns is now directed principally towards fuel production through coppicing and pollarding. Because of the north-south alley alignment which minimises competition for the trees on their west and east sides, they recover more rapidly than in random stands, with yields increased by as much as 50 percent, leading to high levels of LER (Land Equivalent Ratios). The fuel produced, in the form of wood chips, is currently used to provide hot water and central heating in the farmhouse. From next year, the boiler will be replaced by a small-scale CHP (combined heat and power) unit which will continue to provide the heating for the house but will also generate electricity sufficient to drive many other functions on the farm. This represents a form of decentralisation of renewable energy production which eliminates the power losses associated with energy transmission over longer distances in grid networks.

In the field, from our own observations and from others, it is clear that such highly diversified systems can be sustainable and productive because of the ways in which, fundamentally, they mimic nature. They can also provide the feedstocks for different ways of adding value to the numerous products that can be harvested. Creation of such highly diversified agricultural, horticultural and renewable energy systems also leads to large and obvious increases in natural biodiversity, from

microorganisms to birds and mammals. The additional end result is a healthy and attractive living environment for all of those involved.

The main downsides of these decentralised food and energy production systems are losses in simplicity and convenience and the associated need for more detailed management. However, the overarching value of integration of different forms and levels of diversity is to provide current and long-term security of food and energy production while diversifying the environment, jobs and diet.

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# Breeding for forage mixtures: Synthesis and prospects from theoretical and empirical approaches

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## Introduction

Breeding varieties adapted to multi-species forage mixtures is made difficult due to the large number of species interacting in sown grasslands. This presentation synthesises different breeding methodologies proposed for inter-specific crop mixtures. I also present a simple theoretical framework based on genetic-by-environment interactions (hereafter  $G \times E$ ) modelled through interspecific reaction norms. I provide an illustration of this approach with a simple case where genotypes are evaluated in pure stands in order to improve performance in mixture.

## Material and Methods

I used basic quantitative genetics modelling extended it to a mixture of species. I reviewed the experimental literature by recording the reported genetic parameters and whether experimentalists refer or not to theoretical approaches and to notions previously developed for mixture improvement. Most of these experiments are species-centred approaches: they target a high value component of the mixture (typically legumes) and evaluate the potential gains in persistency and vigour in competition conditions with other species.

## Results and Discussion

Genetic gain in mixture for a given trait can be predicted from evaluations in pure stands only if one knows the slope of the genetic regression between the selected traits and its sensitivity to the average mixture effect. This relationship depends on the level of  $G \times E$  variation. Theory predicts that  $G \times E$  is non-zero if the genetic variances measured in two (or several) environments are not equal or if the pairwise correlation of breeding values between environments is not perfect. However, most of the published experiments allowing a quantification of  $G \times E$  caused by mixture (i. e. biotic) effects did not report all these parameters; neither a proper presentation of the components of phenotypic variance in pure vs. mixture conditions. This obscures our understanding of the quantitative genetics of species interactions.

More quantification and standardisation in reporting results from species-centred genetic experiments for mixtures is therefore recommended.

Although relevant quantitative knowledge can be obtained for simple cases (e. g. binary mixture), it is not sure that such ex situ experiments alone can cope with the huge diversity of mixtures used by farmers, especially when combined with a broad range of abiotic conditions. Thus, I discuss the potential strengths and limitations of evolutionary breeding strategies, considering variation in interspecific mixture compositions as one component of the local agro-environment. I suggest that species-centred, in situ evolutionary breeding approaches could represent a fruitful route to readily implement operational breeding schemes for mixtures in perennial crops. However, this would preliminarily require a careful examination of the determinants of fitness variation in the field and a clarification of the relationships linking individual fitness in perennial crops to the agro-ecological benefits targeted by breeders and users.

# **Traditional agroforestry systems and options of crop-tree combinations, with focus on conservation of native species**

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## **Introduction**

Although a number of studies and projects have analysed strategies of cultivation, structure and the role of species in agricultural systems, there is an urgent need for studies at local level that support small-scale and subsistence farmers in exchanging and improving knowledge on the design of their agroforestry systems (Cadena-González et al. 2013). Further, these studies could strongly contribute to conservation of local native species, enhancement of productivity of agroforestry systems, resilience of food production and nutrition, mitigation of impacts of global warming and to the local economy.

Our research is focused on analysing factors that determine whether farmers do cultivate native species in agroforestry systems in the tropics and temperate regions. A part of this research is a case study carried out in the Eastern Andean range in Colombia, in the municipality of La Vega in the province of Cundinamarca. This case study aims at identifying local factors that influence farmers' decisions to cultivate native species or not and, additionally, at studying the traditional structure that farmers currently give to their agroforestry systems.

The municipality of La Vega comprises a wide elevational range between 1100–2700 meters and, consequently, a marked climatic gradient and a diverse geographical structure, which allows for cultivation of a high diversity crop products and tree species. In spite of the benign characteristics of the natural environment and high quality of soils, many local farmers, especially subsistence farmers, struggle to gain some income to support their families. This situation is becoming worse due to effects of climate change. Thus, farmers need support to ensure food production, to diversify their income sources. Furthermore, effective programs are needed that motivate locals to look for options to improve and conserve local genetic material, e. g., via collection of seeds and seedlings found in the wild.

## Material and Methods

Five representative villages were selected in order to carry out the fieldwork. Data were obtained via qualitative and quantitative methods, namely community meetings, questionnaires, semi-structured and structured interviews with farmers, open talks, and visits to farms. A total of 70 farmers participated in the study.

## Results and Discussion

This study provides information on how the farmers structure their agroforestry systems and a detailed ecological and botanical characterisation of the crops and trees cultivated in the locality, including information on characteristics, such as origin of the species, drought tolerance, life cycle, red list status, and ecological requirements.

We could characterise a total of 152 species including crops and trees until now. One hundred three species of this list have been identified as perennials, and 20 species belong to the group of annuals and biennials. Among these species 50 are exotic and 71 natives, 22 are nitrogen-fixers, 17 species are resistant to drought, some of them with clear limits of resistance as they can only survive droughts of maximum three months.

The most popular agroforestry systems in the locality are home-gardens and intercropping systems.

We expect to contribute to: (i) the local improvement of design and structure of agroforestry systems based on the potentials of local plant species, (ii) the promotion of diversification, (iii) the study of options for combinations of crops and trees in agroforestry systems, (iv) local studies on genetic pools, and (v) the recovery of equilibrium of disturbed ecosystems due to the implementation of agriculture.

It is also expected that these results, would potentially help to develop plans focused on the conservation of local indigenous species and to improve community's life's conditions (Leakey 2014).

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## **Documentation of diversity breeding**





# Documentation of diversity breeding: The Information system ROBUSTUM

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## Introduction

The INSUSFAR project aims at analysing the effects of biodiversity within winter wheat and winter barley populations on their ability to adapt to low input farming conditions. The project furthermore analyses historical data on the breeding progress during the past two decades and conducts socio-economic and ecological studies of farming systems. In the course of INSUSFAR, the individual project partners deliver a very diverse array of data that have to be stored and have to be provided to all project partners for further analyses. This is achieved by the implementation of an information system that meets the specific needs of the INSUSFAR project.

## Material and Methods

A comprehensive demand analysis was conducted to identify the individual needs of the project partners regarding the information system. Based on the contributions of the partners, 11 use cases are described that define the functional requirements for the information system. The use cases cover all INSUSFAR aspects where data are generated, extracted, stored and retrieved, e. g. data on the origin of populations and pedigrees of varieties, data on cultivation conditions, genetic data, characterisation and evaluation data from field trials, climate and soil data, historical trial data, as well as economic data. In addition, non-functional and technical requirements are defined within the demand analysis.

A benchmarking with other information systems already existing or under development based on the requirements for the INSUSFAR project revealed that no other information system is actually capable of meeting all requirements of the INSUSFAR project. The information system thus needs to be newly designed for INSUSFAR. However, some individual components from other information systems may be incorporated. The information system was named ROBUSTUM. To meet the short-term need to store and retrieve historical trial data, a basic database was designed and implemented in a first step and 11 888 records from German variety trials between the years 2001 and 2015 representing 307 varieties tested at 60

locations were imported into the database. The data can be retrieved by the project partners using a web-based user interface and query tool.

## **Results and Discussion**

The structure of ROBUSTUM was designed based on the use cases defined previously. An entity-relationship diagram reflects the structure and dependencies of tables and attributes that are implemented within the database scheme running in the background. Activity diagrams were assembled to model the user-system interactions of ROBUSTUM with respect to breeding material import, editing, query, and export. The functionality for breeding material documentation and to record crosses as breeding actions was implemented. Exemplarily, an entire MAGIC crossing scheme that starts with 32 winter barley varieties and that comprises 716 crossing steps and lines was imported into ROBUSTUM. For optimal illustration of such complex structures, a module for graphical display of pedigrees was also implemented. Furthermore, a functionality was designed that enables adding of further attributes to specific tables. ROBUSTUM will provide access to the data generated within the project also beyond the duration of INSUSFAR, and will furthermore comprise a flexible structure to serve as an information system template for related future projects.

## **General concepts for breeding on improved diversity**



## Interspecific competition experiments aimed to define breeding strategies

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### Introduction

Intercropping usually implies the association of a legume and a non-legume species, owing to the several environmental and crop quality advantages provided by the presence of the legume. Breeding for intercropping has been given particular attention for forage crops, because of the long-standing cultivation of legume-grass mixtures. Legume companions in these mixtures or in annual legume-cereal ones often show a competitive disadvantage. Accordingly, compatible mixtures were defined as those that allow for a legume content large enough to optimise the benefits of its nitrogen fixation and superior forage or feed quality value (Rhodes I 1994). A typical feature of compatible mixtures relative to non-compatible ones is the occurrence of higher legume yield accompanied by similar yield of the non-legume companion (Annicchiarico and Piano 1994). That agrees with the general observation that the yield efficiency of a mixture depends mainly on the performance of its weaker partner (Harper 1977). In this situation, selecting the legume species for greater competitive ability may be the main avenue to achieve sufficient legume content and total yield of the mixture, as proved by research on white clover (Annicchiarico and Proietti 2010).

Specific-compatibility effects leading to better yield of specific pairs of genotypes or varieties may occur, especially for material with a history of co-evolution (e.g. Evans et al. 1989). However, these effects need be large to justify their commercial exploitation. Estimating their size and pattern is important also when selecting for general compatibility, given the large number of potential companions and the impossibility to devise more than a few ones as testers in selection schemes.

### Key questions for defining breeding strategies

There are a few key questions that need elucidation via interspecific competition experiments, to define efficient selection procedures for one or both species targeted to be intercropped, such as: (i) What is the loss of efficiency when selecting in pure stand (PS) for mixed stand (MS) target cropping (considering that PS selection is usually less laborious and costly than MS selection)? (ii) What is the relationship between compatibility in MS, competitive ability in MS and performance in PS,

for genotypes of a target species? (iii) What is the relative extent of specific-compatibility vs general-compatibility effects? (iv) Which traits may provide greater compatibility and competitive ability of genotypes (with possible implications on indirect selection procedures in PS)?

## Types of experiments and generated information

The type of experiment depends on the key questions into focus and the available budget. Budget constraints usually impose a replacement design with 50 % density reduction of each companion in MS relative to PS. Relatively low-budget experiments may help identify optimal plant ideotypes for MS cropping, by studying PS and binary mixtures of few contrasting plant types of legume and non-legume species. For example, taller pea stature reduced the pea competitive gap and tended to result in higher total yield of different pea-cereal intercrops (Annicchiarico et al. 2017). Likewise, a climbing type of cowpea displayed better compatibility and ability to withstand competition by pearl millet than a bushy type adapted to PS cropping (Nelson and Robichaux 1997).

Larger experiments imply the evaluation of  $m \times n$  crops resulting from the factorial combination of  $m$  legume by  $n$  non-legume genotypes. These experiment should also include the PS of legume and non-legume companions. The size of general and specific compatibility effects can be assessed by components of variance for total yield in MS relative to legume and cereal genotype main effects and legume  $\times$  cereal interaction, respectively. Results from intra-specific interference studies (Zannone 1985) suggest to verify whether the total yield of forages or measures of MS performance such as the Relative Yield Total or the Land Equivalent Ratio can be maximised by pairs of legume and non-legume companions that feature similar yield in PS, or pairs that exhibit higher mean yield in PS. For the same experiment lay-out, investigating genotype yield responses across PS and MS conditions for one species by models developed for the analysis of genotype  $\times$  environment interaction may provide crucial information. In white clover, joint regression modelling revealed that the inconsistency of genotype yield responses between PS and MS increased as a function of the grass vigour and competitive ability, irrespective of the grass species (Annicchiarico and Piano 1994). Thus, clover yield responses reflected the clover variation in general competitive ability, whose exploitation was essential to breed for compatibility with high-yielding grass companions.

Results from the previous experiments may provide guidance for other types of large experiments that include a high number of genotypes of a target species in just a few condition. These experiments can be crucial to thoroughly assess the consistency of genotype yield responses across different conditions in terms of genetic correlation, to compare the efficiency of selection in PS vs MS based on genetic gains predicted from selection theory, and to identify traits associated with general compatibility and/or competitive ability that could be exploited by an index

of selection in PS. For white clover, one such experiment indicated for PS selection about 50 % lower efficiency when based on genotype yield, and about one-third lower efficiency when based on three traits contributing to greater competitive ability, compared with selection for yield in MS (Annicchiarico 2003). A further step may be the comparison of PS vs MS selection in terms of actual gains in MS.

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## Crop diversity for sustainability

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Most current research on crop diversity has focused on increasing resource utilisation to increase yields or reduce inputs through the 'complementarity effect' of biodiversity, or increasing yield stability via the 'selection effect'. Here, I will try to address another, very different question, which has not been given sufficient attention: What can crop diversity contribute to long-term agricultural sustainability? I have argued that there is a 'limiting tradeoff' between short-term yield and long-term sustainability. If this is the case, we cannot maximise both of these simultaneously. Agricultural practices should have two objectives, which we should balance consciously: The short-term production of yield and a long-term improvement in soil fertility. Biomass density, especially below ground, will play an important role here. Crop diversity can contribute to soil fertility by (1) increasing biomass input to the soil, and (2) increasing the chemical, structural, and therefore biological diversity of the soil, and therefore to ecosystem services supported by this biomass. Genotypes that produce some high C:N plant residues with good structure are important here, because if decomposition is too fast, soil organic matter will decrease. Composite cross populations do not evolve to maximise yield, but they may be very good for maintaining and increasing sustainability, especially if resource levels are not too high. Increased plant diversity in combination with reduced chemical inputs can make a major contribution to increased sustainability for a small, transient price in short-term yield. Agricultural science need to change its objective from short-term optimisation to bet hedging and sustainability, which together correspond to food security.

# The relation of intragenotypic, intra- and inter-specific competition and related canopy architecture traits

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## Introduction

Competitive interaction is omnipresent in every crop stand, between plants of the main crop, between crop plants and weeds, and – in multicropping systems – between the different crop species. Competition is one of the main driving forces in evolution and the reduction in competitive behaviour has been one of the main aspects of crop domestication and plant breeding. A general concept in studying competition is the differentiation into competitive effect, the effect of a species or genotype on the competing species or genotype, and competitive response, which is the response of a species or genotype to the presence of a competing species or genotype. In order to understand and manipulate the manifold interactions in crop stands – between crop plants, weeds, or different crop species – it is crucial to study the competitive effects and responses and the relations to responsible canopy traits.

Therefore, the objective of this study was to investigate competitive effects and responses on different levels of interactions in winter wheat pure stands of different densities, a variety mixture and with two undersown model weed species. In order to relate effects and responses to explanatory traits, extensive assessments of canopy architecture traits have been carried out.

## Material and Methods

**Field trial:** In field trials near Freising (South-West Germany) nine winter wheat varieties (4 tall varieties which are famous under organic growers, 3 short varieties famous in conventional farming, one hybrid, and one variety with red kernels) were grown under five different competition treatments, representing three different scenarios of competition:

1. Interspecific competition: two different species undersown in spring: vetch and ryegrass
2. Intergenotypic competition: 50:50 mixture with Rosso, a variety with red kernels
3. Intragenotypic competition: pure stand under normal and half sowing density

The experimental design was a split-plot design with competition treatment as main plot factor and variety as sub plot factor, with three replicates. The trials were grown over two years (2016 and 2017) under organic and conventional conditions, resulting in overall four trials. Leaf and canopy traits (height, leaf angle, leaf geometry, and leaf colour as surrogate for N content) were assessed around anthesis in the pure stand under normal density treatment.

Interspecific competitive effect was assessed by sampling vetch biomass and indirectly by NDVI two weeks after harvest in the ryegrass treatment. Intergenotypic competition effect was calculated after separating red (Rosso) and white kernels with a single seed sorter as the ratio of Rosso in mixture to Rosso in pure stand under normal density. Response to increased density (intra-genotypic competition) was calculated as the ratio of grain yield under normal density to grain yield under half density. Competitive responses were calculated as the ratio of grain yield in the respective competition treatment to grain yield in pure stand. Relations between competitive responses and effects, and architecture traits were assessed by Pearson correlations of variety means over all trials.

## Results and Discussion

**Seed Sorting:** Sorting took about 5 min per sample, including preparing and packing of samples. The unclassified fraction was negligible and sufficient purity was achieved after one run.

**Competitive Effect:** Competitive effects were significantly correlated between the interspecific and Intergenotypic level (table T16.1), indicating that varieties which suppress the undersown species vetch or ryegrass also suppress the wheat variety with red kernels (Rosso). Therefore, the variety mixture with Rosso in combination with single seed sorting could be used as a high-throughput screening system for the competitive effect on weeds or partner species in mixed cropping systems. The competitive effect on vetch and Rosso was positively correlated to the response to increased density, which means that varieties that are better adapted to high density are weaker competitors. This observation shows a trade-off between adaptation to dense stands and weed suppression.

Contrary to the correlation of competitive effects, there was no correlation between the competitive responses, although competitive response measures showed significant variety effects in ANOVA. This absence of correlation could indicate that the response to the presence of different competing species or varieties is dependent on the respective competitor. The ryegrass treatment produced the highest yield among all treatments in all four trials (overall 72.7 dt ha<sup>-1</sup> vs 71.1 dt ha<sup>-1</sup> in pure stand). The reason for this positive effect on wheat yield is still unclear.

**Table T16.1:** Significant ( $P < 0.05$ ) Pearson correlation coefficients of variety means between different competitive effects and responses and selected explanatory traits.

Trait	EffV	EffG	EffR	ResV	ResG	ResR	Dens	Yield
Effect on vetch biomass (EffV)		0.81**	0.88**	-0.70*		-0.72*	0.88**	
Effect on ryegrass NDVI (EffG)	0.81**		0.77*	-0.75*		-0.71*		
Effect on red wheat (Rosso) (EffR)	0.88**	0.77*				-0.88**	0.84**	
Response to vetch (ResV)	-0.70*	-0.75*					-0.74*	
Response to ryegrass (ResG)								
Response to Rosso (ResR)	-0.72*	-0.71*	-0.88**					
Resp. to increased density (Dens)	0.88**		0.84**	-0.74*				
Overall grain yield (Yield)								
Date of heading				0.72*			-0.77*	
Canopy height	-0.73*							-0.79*
Hue of flag leaf								0.86**
Leaf area of flag leaf		-0.74*		0.81**	0.68*		-0.73*	
Leaf area of second leaf					0.74*			0.71*
Leaf length of flag leaf				0.74*			-0.92***	
Leaf length of second leaf	-0.90***	-0.73*	-0.80**	0.70*	0.70*		-0.71*	
Leaf width of flag leaf				0.81**	0.72*			
Leaf width of second leaf					0.71*			
Relative light extinction (PAR)						-0.68*		

Overall grain yield was not correlated to any of the competitive effect or response measures. This independence could allow for selection for competitive traits in plant breeding, without reduction in grain yield.

While there was a negative correlation between canopy height and grain yield, canopy was correlated to the competitive effect on vetch biomass (taller varieties showing stronger suppression), showing that tall varieties are not necessarily the best strategy for weed suppression. N or chlorophyll content (measured by Hue) was only related to grain yield and not to competitive measures. Among the leaf geometry traits, greater leaf length is the most related to increased competitive effect and decreased competitive response.

It can be concluded, that the assessment of competitive effect and response and the direct manipulation of competition related traits offers potential for the inclusion in breeding programs.

# The cropping system matters—contrasting responses of winter faba bean genotypes to drought stress

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## Introduction

In the context of global change, the frequency and intensity of summer droughts are expected to increase as regional precipitation patterns are predicted to undergo drastic changes (IPCC 2014). Thus in future, there is a growing demand for improved concepts to ensure high productivity, sustainability, resource use efficiency and stress tolerance of agricultural systems.

While still being challenged for a number of reasons, it is a promising option for arable systems to develop alternative cropping systems such as intercropping systems, where complementary stand architecture and rooting patterns may increase the efficiency of light and water use as well as nutrient uptake. Intercropping of cereals with legumes is particularly common and introducing N<sub>2</sub>-fixing legumes into cereal-based crop rotations may additionally reduce needs for mineral N fertiliser and lower the dependency on external inputs.

A major goal of the present study was therefore to identify genotypes that show superior performance under drought conditions. Also, selected genotypes grown in intercropped stands may lead to improved water use efficiency. Therefore we took a closer look at the physiological responses of plants in pure stands and intercropping under water deficit. Implications of genotype-specific responses and species interactions in intercropping under water deficit on canopy water use efficiency and drought tolerance were studied.

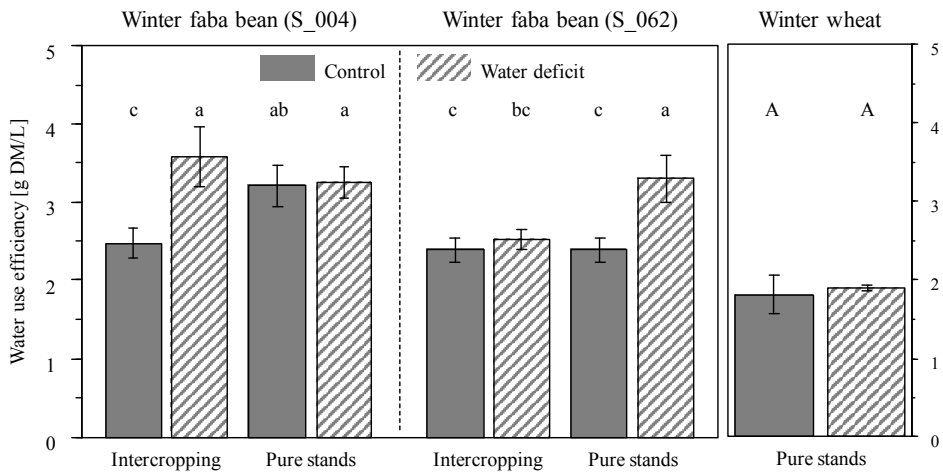
## Material and Methods

In order to identify genotype-specific properties of stress adaptation in more detail, a pot experiment was conducted under controlled environmental conditions in the greenhouse. Two genotypes (S\_004 and S\_062) of winter faba bean (*Vicia faba* L.), differing in growth habitus and maturity, were cultivated to study the tolerance

to temporary water deficit in pure stands as well as in intercropping with winter wheat (*Triticum aestivum* L.; Genius).

Plants were grown in low-N soil substrate and drought stress was induced by deficit irrigation, while the control plants always received sufficient irrigation depending on plant growth. Water consumption was monitored by a balance system (TQ30, ATP Messtechnik, Germany).

At three stages of water deficit (beginning of water deficit, end of water deficit and re-watering), canopy gas exchange, leaf relative water content (ratio of water content in fresh leaves to water content in fully turgid leaves), proline content of leaves and canopy surface temperature were evaluated. Additionally, plant biomass production and water use efficiency (WUE; calculated as total aboveground biomass per water consumption during the growth period) were investigated.



**Figure T17.1:** Water use efficiency of winter faba bean genotypes S\_004 and S\_062 as well as winter wheat in intercropped and pure stands. Intercropping considers the canopy of both species together. Error bars display the standard error. Different letters indicate significant differences. Duncan-test,  $P < 0.05$ ,  $n = 4$ .

## Results and Discussion

Surprisingly, water deficit did not cause impaired productivity in all treatments. Instead, winter faba bean genotypes showed different abilities in drought stress tolerance in pure stands or in intercropping. With water deficit, biomass was reduced in genotype S\_004 only in pure stands while genotype S\_062 had impaired biomass production only in intercropping. The same effect has been observed for CO<sub>2</sub> assimilation and transpiration, indicating the maintenance of photosynthetic processes of genotype S\_004 in intercropping under water deficit. Moreover, these physiological properties lead to increased WUE of intercropped stands with winter

faba bean genotype S\_004 as well as pure stands of winter faba bean S\_062 (fig. T17.1). Water re-supply to the control level (sufficient water supply) led to adjustment of all measured parameters to control levels, showing reversible drought stress induced responses of all treatments.

In summary, winter faba bean showed little interference of water deficit for genotype S\_062 in pure stands while genotype S\_004 exhibited little interference of water deficit when grown in intercropping. Therefore, it can be concluded that the performance and drought tolerance of intercropping systems can be improved by the right choice of the winter faba bean genotype.

## Acknowledgements

We thank the team of the Division of Plant Breeding, University of Göttingen for providing the germplasm used in these experiments. Funded by the Federal Ministry of Education and Research (FKZ 031A351A,B,C), IMPAC<sup>3</sup> is a project of the Centre of Biodiversity and Sustainable Land Use at the University of Göttingen.

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# HealthyMinorCereals: An integrated approach to diversify the genetic base, improve stress resistance, agronomic management and nutritional / processing quality of minor cereal crops for human nutrition in Europe

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## Introduction

The productivity of European and global agriculture has been vastly improved through focussing on a relatively small number of crop species. Cereals grown in Europe such as common wheat and barley have been mainly bred for high yields, and are dependent on large inputs of artificial fertilisers, pesticides and energy. Other cereal species, including rye, oat, spelt, einkorn and emmer were important in the early development of agriculture. Many of them were widely grown in Europe prior to the Second World War. However, they are no longer widely grown in Europe and are now classified as 'minor cereals' due to small areas of cultivation. Many minor cereal species retain characteristics that have been lost, to a large

extent in modern, major cereals. They often have higher resistance to crop diseases and are less dependent on fertiliser and pesticide inputs.

There is a renewed interest in these minor crop species to increase the diversity, sustainability and resilience of cereal production. They are also increasingly popular as nutritious alternatives to major cereals. However, minor cereals have not received the same attention as the major cereals in modern agricultural and food research. There are typically only a few varieties of each species available for cultivation in each country and the best farming conditions are not always properly investigated. Many historic minor cereal varieties, conserved in genebanks, have hardly been studied. These could provide useful genetic material for breeding improved minor cereal crop varieties.

## Material and Methods

Objectives of the project are thematically divided to the following topics:

- Identify minor cereal varieties with promising genetic characteristics for yield, resistance to important fungal diseases, more efficient use of fertilisers, nutritional quality, suitability for food processing and other beneficial traits
- Apply new breeding strategies to generate new improved minor cereal varieties
- Optimise cultivation conditions of minor cereal varieties in four European countries with different climates and soils, with a focus on organic fertilisation and advanced agronomic management tools
- Investigate the genetic diversity of minor cereals grain for micronutrient and antioxidant content, and for any potential harmful / anti-nutritive components, and analyse possible beneficial effects of minor cereal grains on human health through cell biological studies on human cell cultures

## Results and Discussion

### Genetic characterisation of minor cereals

Genetic characterisation of oat, rye, spelt and wheat wild relatives has been completed for 262 oat genotypes from 26 countries, 218 rye genotypes from 13 countries, and 265 spelt genotypes from 14 countries. For the characterisation of wheat wild relatives, seeds of 190 accessions (incl. *Aegilops* sp., *Triticum araraticum*, *T. dicoccoides*, *T. boeoticum*, *T. dicoccon*, *T. monococcon*) were provided by CRI, of which 39 accessions were selected to perform analysis of interspecific genetic diversity.

## Phenotypic characterisation of minor cereals in field trials

Three seasons of phenotyping data of minor cereals genotypes have been obtained to date by partners BOKU, ETKI, Selgen and GZPK, focusing in the last two seasons on 80 spelt, 112 oat and 100 rye genotypes, and with CRI evaluating 344 wild wheat relatives from their own collection. The four partners have also been developing new crossbred varieties of spelt, rye and oat, crossing between genotypes studied in HealthyMinorCereals and various breeding lines, and aiming to improve their cultivation performance. F<sub>1</sub> and F<sub>2</sub> generations have so far been obtained and over 400 crosses are currently being evaluated by partners.

## Resistance to diseases and drought

Resistance to diseases in minor cereals has been studied in field trials in the Czech Republic, Switzerland, Austria and Estonia, employing either natural or artificial infections. These included rusts in spelt and oats, common bunt in spelt, *Fusarium* head blight in spelt, and *Microdochium nivale* in rye. Of the 80 spelt genotypes studied in HealthyMinorCereals, several show a higher level of resistance to yellow rust, but with only one a few showing a high resistance to common bunt, stem rust or leaf rust. One genotype is notable for being highly resistant to several crop diseases. For oat, several genotypes are identified with higher resistance to stem rust or crown rust. The *Fusarium* trials in spelt, using artificial infection and analysis of fungal toxin DON, show relatively large differences in susceptibility among the 80 spelt genotypes. Drought resistance trials by our partner Geokomi in Crete with four spelt varieties have demonstrated the value of investing in supplementary irrigation, and significant differences in yield due to the type of fertiliser used, especially with low natural rainfall.

## Effect of agronomic management practices on the performance of minor cereals

Field trials to evaluate the effects of agronomic practices on the performance of four varieties each of spelt, rye and oat have been carried out in the UK (spelt and rye), Czech Republic (spelt and oat) and Estonia (rye and oat). Despite problems at some sites with weather and pests, data from two seasons of trials is providing some interesting conclusions. There are some clear variety differences in yield, probably due to differences in resistance to the yellow rust epidemic currently affecting almost all European countries. The yield obtained with biogas digestate is looking comparable to that from conventional fertiliser, especially in the UK trials.

## Nutritional analysis

A comprehensive analysis of the nutritional diversity of 54 rye, 200 oat, 300 spelt and 64 wild wheat genotypes was done. A large amount of data has been obtained on seed mineral nutrients, antioxidants, total phenols, dietary fibres, beta-glucan,

and other important compounds, compared also the respective nutrient content in 12 modern wheat cultivars. In addition, seed extracts from 40 genotypes with contrasting higher and lower nutrient content were subjected to cell biological tests by using human cell cultures. In these tests, the effects of seed extracts were analysed for their impact on cellular levels of reactive oxygen species, mitochondrial health, DNA damage and cell survival. Results clearly showed that, with a few exceptions, minor cereal species had a higher antioxidative activity in human cell cultures than the modern wheat cultivars.

## **Acknowledgements**

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# Marketing of a genetically diverse wheat (ORC Wakelyns population): Lessons learnt and routes forward

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## Introduction

Genetically diverse populations are an alternative to pure line cereal breeding to bring genetic diversity back into agricultural systems and enable greater control of genetic resources by the farmer (Döring et al. 2011). The ORC Wakelyns Population (OWP) is a winter wheat (*Triticum aestivum* L.) composite cross population (CCP) that was generated in 2002 following this approach. Eight parents selected for high quality and 12 selected for high yield were crossed with each other, following a half-diallel scheme. The progeny was reproduced under field conditions on organic farms since the  $F_3$  generation. It has undergone a range of experimental analyses as part of past UK and EU projects (WheatLINK, WheatBreeding LINK, SOLIBAM and COBRA). This research supports the resilience of OWP, wheat CCPs, and heterogenous material in general, in variable organic systems and the potential for wider application of this approach. In fact, OWP has shown competitive yield and quality performance in comparison to conventionally bred varieties, and better performance stability over time (Döring et al. 2015).

However, it is not possible to market seeds of genetically diverse wheat under current seed legislation. The DUS (Distinctness, Uniformity and Stability) and VCU (Value for Cultivation and Use) regulations both present challenges to the certification of heterogenous material. Development of protocols for organic VCU testing, and the production of guidelines to better characterise heterogenous material, have been a debated issue for years, are an ongoing research focus within organic breeding and part of the LIVESEED H2020 project. In 2014, the EU granted a temporary experiment allowing 'certain derogations for the marketing of populations of the plant species wheat, barley, oats and maize' (EC 2014) and we are now reflecting on the success of this experiment for the OWP.

## Material and Methods

We have worked closely with Defra (Department for Environment, Food and Rural Affairs, UK), APHA (Animal and Plant Health Agency, UK) and NIAB (National Institute of Agricultural Botany, UK) to ensure the successful certification of OWP

seed under the EU temporary experiment. Each year of the temporary experiment, we have recruited farmers to grow a seed crop and engaged with different seed companies to collaborate on the marketing as follows:

- Y1 of the experiment (2014 / 2015): Seed crop grown on one farm (Shimpling Park Farm, UK), no crop certified due to bunt infection.
- Y2 of the experiment (2015 / 2016): Seed crop grown on two farms (Greenacres Farm, UK and Wakelyns Agroforestry, UK), both crops certified as seed (7.75 tonnes in total) and marketed with a small organic UK seed company (Organic Seed Producers).
- Y3 of the experiment (2016 / 2017): Seed crop grown on the same two farms, a proportion of one crop certified as seed (13 tonnes) and being marketed by a small U.K. seed company (Walnes Seeds).

In collaboration with Walnes Seeds, we intend to establish a seed crop for 2017/2018. The OWP has also been included in on-station and on-farm organic winter wheat field trials (2016 / 2017 and 2017 / 2018) to ensure that comparisons to heritage, modern and emerging varieties are made, and it remains of interest to farmers in fluctuating seed markets. Engaging with other end-users (e. g. grain traders, millers and bakers) has also been key to the success of marketing the OWP seed.

## Results and Discussion

There is a detailed process to certify a seed crop that includes steps where heterogenous material fails to comply which will be outlined in the presentation. Ongoing communication of what to expect from heterogenous material (e. g. that it will not produce a uniform crop according to the DUS protocol) with both farmers and the government seed agencies has been key to ensuring that OWP seed was certified and entered the market. The temporary experiment mostly relies on traceability and accurate record keeping which may be challenging depending on the actors involved and their interactions.

In the case of OWP, seed sales have increased from year-to-year. The growing popularity of OWP is based around the evidence of its field resilience as well as the emerging market for its grain / flour. Individual innovators within the food industry, who have historical links with the Organic Research Centre and Wakelyns Agroforestry (one of the farms where the OWP was bred and grown) and / or who have taken an interest in the concept, have led this development through market pull especially for artisanal bread-making. We now have arrangements with a seed company, grain traders, millers and a baker who all wish to continue trading in and working with OWP.

Development of both the grain and seed market may be key to the development of small-medium scale initiatives working with heterogenous material. We hope to engage such chain players in an inclusive breeding process to ensure smooth marketing of future genetically diverse populations encouraging research and development in the application of heterogenous material to specific end-uses.

## Acknowledgements

Ongoing work on the certification and marketing of heterogenous material, and the OWP, is part of the LIVESEED project and funded by the European Union's Horizon 2020 research and innovation programme under grant agreement No 727230.

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# High-throughput sorting of coloured wheat grains

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## Introduction

Studying weed suppression or interaction within variety mixtures needs separate sampling of weeds or mixture components, which is costly and laborious. Coloured varieties in combination with fast sorting could serve as screening system. Here, we test the application of a high-throughput seed sorting device in combination with the red wheat variety Rosso to assess the suppression ability of wheat varieties.

## Material and Methods

**Field trial:** In 2 years, under conventional and organic management, eight winter wheat varieties were grown in 50:50 mixture with variety Rosso (red kernels) and in normal pure stand. The design was a split-plot design, with mixture treatment as main plot-factor and 3 replicates.

**Seed Sorting:** From the plot harvests of the mixture treatment, a sample of around 180g was sorted by color based on Hue with the QSorter Explorer by Qualysense, and the weight of both fractions recorded.

**Analysis:** Using the weight of the fractions and the plot yield, the yield of Rosso in each plot was derived. In order to determine the suppression of Rosso, the competitive effect was calculated as  $CE(\%) = \frac{\bar{Y}_p - 2Y_m}{\bar{Y}_p}$ , where  $Y_m$  is the yield of Rosso in the mixture plot, and  $\bar{Y}_p$  is the mean yield of Rosso in pure stand in the respective experiment. While  $CE = 0$  indicates no interaction between the mixture components, the higher the value the stronger the suppression of Rosso.

## Results and Discussion

**Seed Sorting:** Sorting took about 5 min per sample, including preparing and packing of samples. The unclassified fraction was negligible and sufficient purity was achieved after one run.



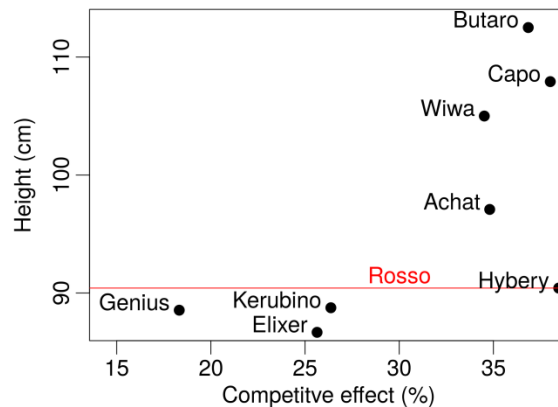
**Competitive Effect:** There was a significant difference between trials in the overall level of suppression of Rosso (table P23.1). In all four trials, varieties differed significantly in their suppression of Rosso. Heritability in single trials was higher where overall suppression was lower. The overall heritability over all trials was  $h^2 = 0.80$ . The relation of canopy height and CE reveals an interesting grouping of the varieties (fig. P23.1):

- Taller line varieties than Rosso are more but equally competitive.
- Shorter line varieties are less competitive.
- The hybrid Hybery is as competitive as the tall varieties, but with same height as the shorter line varieties.

**Table P23.1:** Mean competitive effect (CE), variety effect based on ANOVA and heritability ( $h^2$ ).

Year	Management	Mean CE* (%)	Variety (F)	$h^2$
2016	Conventional	10.6 d	4.9 **	0.80
	Organic	21.0 c	7.5 ***	0.87
2017	Conventional	53.5 a	2.9 *	0.66
	Organic	41.4 b	3.0 *	0.67

\* Comparison between trials, Tukey-Test (5%)



**Figure P23.1:** Relation of competitive effect to canopy height. Red line indicates the canopy height of Rosso.

### Acknowledgements

The hybrid Hybery is as competitive as the tall varieties, but with same height as the shorter line varieties.

# Modulation of three-dimensional structure and research of folding-analogues of AMB A 6 allergen of *Ambrosia artemisiifolia*

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## Introduction

Amb A 6 allergen of *Ambrosia artemisiifolia* is a ragweed allergen (a principle cause of late summer hayfever in North America and Europe) (Hiller et al. 1998). The weed has recently become spreading as a neophyte in Europe, while climate change may also affect the growth of the plant and additionally may also influence pollen allergenicity (Kelish et al. 2014). In Ukraine, the number of diseases caused by this allergen has recently increases. The three-dimensional structure of Amb A 6 allergen is undescribed.

The aim of our study was to modulate of three-dimensional structure and search of folding-analogues of AMB A 6 allergen of *A. artemisiifolia*.

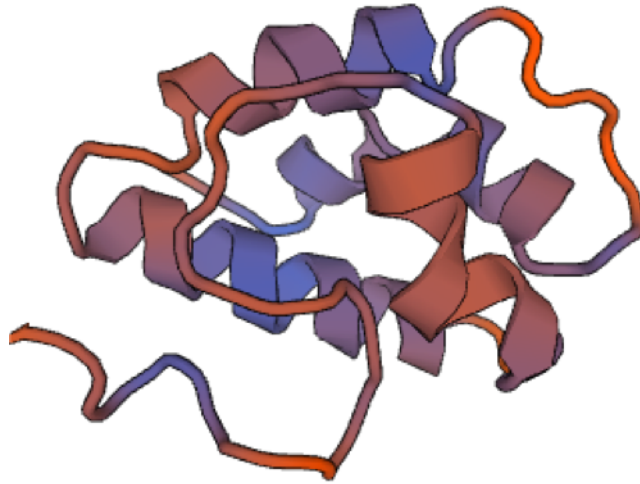
## Material and Methods

Template search with Blast and HHBlits has been performed by the SWISS-MODEL template library. Models are built based on the target-template alignment using ProMod3. Coordinates which are conserved between the target and the template are copied from the template to the model. Insertions and deletions are remodelled using a fragment library. Side chains are then rebuilt. Finally, the geometry of the resulting model is regularised by a force field. In case loop modelling with ProMod3 fails, an alternative model is built with PROMOD-II (Guex and Peitsch 1997).

## Results and Discussion

Three-dimensional structure of Amb A 6 allergen has been built (fig. P24.1).

As folding-analogues of AMB A 6 allergen the maize nonspecific-lipid transfer protein was found.



**Figure P24.1:** Three-dimensional structure of Amb A 6 allergen.

Information about three-dimensional structure and partial analogy with nonspecific-lipid transfer protein can help to understand properties and spatial configuration of Amb A 6 allergen.

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