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New breeding techniques

Hybrid breeding

Populations and seed legislation

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## Nachruf: Andreas Spanakakis (1943-2017)

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Andreas Spanakakis wurde am 5. April 1943 in Heraklion (Kreta) geboren wo er auch die Schule besuchte und das Abitur ablegte. Ab 1961 erfolgte seine Ausbildung in Weihestephana, zunächst eine Landwirtschaftslehre in Dornach, danach das Studium der Landwirtschaft an der TU München. 1972 promovierte A. Spanakakis bei Prof. Uwe Simon zum Thema Heterosisnutzung und Resistenzzüchtung bei Futterpflanzen. Nach Ableistung des Wehrdienstes in Griechenland trat Spanakakis 1975 die Stelle eines Saatzuchtleiters beim Uniplanta Partner Freiherr von Moreau Saatzucht in Schafhöfen bei Straubing an. Bis 1979 beschäftigte er sich dort mit der Züchtung von Mais, Weizen, Sommergerste und Hafer. 1979 wurde Andreas Spanakakis die deutsche Staatsbürgerschaft verliehen und 1980 wechselte er als Saatzuchtleiter zur Fr. Strube Saatzucht in Söllingen. Es wurden sehr erfolgreiche Jahre als Weizenzüchter bis 2007: seine Winterweizensorten 'Ares', 'Orestis', 'Astron', 'Batis', 'Pegassos', 'Xanthos', 'Asketis' und 'Akratos' prägten über Jahre hinweg die deutschen Vermehrungsflächen. Mit der Sorte 'Sorbas' gelang ein bedeutender Zuchtfortschritt in der Resistenz gegen Ährenfusarium. 1999 erfolgte in Österreich die Eintragung der Sorte 'Xenos', als erster Wechselweizen (WeW®), womit vor allem den Zückerrübenbauern eine Sorte mit exzellenter Saatzeitflexibilität bereitgestellt wurde. Weitere erfolgreiche Wechselweizensorten wie 'Thasos' oder 'Naxos' folgten. Andreas Spanakakis war ständig auf der Suche nach neuen, in der Praxis zur Selektion nutzbaren Methoden. Neben der systematischen Resistenzzüchtung gegen Ährenfusarium und der Saatzeitflexibilität realisierte er als Erster die Züchtung auf verbesserte Stickstoff-Effizienz durch Selektion auf den Kornstickstofftrag. Nicht zuletzt stand auch die Backqualität von Weizen im Mittelpunkt seiner Weizenzüchtung.

Andreas Spanakakis zeichnete sich durch eine engagierte Mitarbeit in vielen wichtigen Fachgremien aus, so zum Beispiel in der Arbeitsgemeinschaft Getreideforschung e.V. oder der AG Krankheitsbekämpfung und Resistenzzüchtung. Von 1976 bis 2007 war er auch ein regelmäßiger Besucher der Züchtertagung in Gumpenstein. Insgesamt 23 mal war er in Gumpenstein zugegen, viermal davon stand er als Vortragender selbst am Podium, zweimal war er als Mitautor an Vorträgen beteiligt. Viele Tagungsteilnehmer können sich noch an ihn als einen der lebhaftesten Diskutanten erinnern.

Andreas Spanakakis hinterlässt vier Kinder: Rainer, Alexis, Thalia und Marios.

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## Creating an enabling environment for agricultural innovation

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Innovation is a driver of economic growth and societal change. Since 1960 the landscape of global R&D investment changed significantly: UK, South Africa and Australia were replaced in the list of the ten most important countries with respect to public investment by France, Italy and Korea. Moreover, the ranking changed with China being first now, the United States second and India and Japan on third and fourth position in front of Brazil and Germany (Figure 1; Pardey *et al.* 2016, 2018).

Reflecting on traditional GM crops we can conclude that this innovation was a success story. By 2016, 185.1 million hectares were cultivated with GM crops, thereof 99.6 million hectares in developing countries (ISAAA 2016). The biggest exporter of GM seed, grain and food is Argentina with 49.2% of the market, followed by Brazil (22.9%), the USA (14.7%) and India (7.3%). The by far largest importer is Europe. The main growers of GM crops are the USA (38%), Brazil (27%), Argentina (13%), Canada and India (both 6%). The main crops are soybean, maize, cotton and rapeseed, the main traits herbicide and insect tolerance. Only two out of originally 560 nutritional and product quality crop biotech innovations are still on the market after 95% of genetic traits identified in the initial discovery phase are quickly eliminated from consideration, another 75% of the remaining traits fail in the proof-of-concept stage (Graff *et al.* 2010).

What is the future of precision breeding innovations? New breeding technologies allow a site directed genome editing or mutagenesis. Crops created by such technologies can't be differentiated from products of conventional breeding/mutagenesis as no foreign DNA is transferred. The question, therefore, is if such crops should be regulated under the scope of regulations for genetically engineered organisms. Examples of crops already developed by new breeding techniques are non-browning mushrooms, herbicide-tolerant rapeseed, waxy maize and high oleic acid soybean.

Argentina and Chile established a procedure to determine if an organism obtained with the aid of new plant breeding techniques is a GMO or not. A GMO is defined as an organism with novel combination of DNA obtained through the use of modern biotechnology. Therefore, a case by case consultation will be carried out. Israel decided that targeted mutagenesis and no insertion/incorporation of foreign DNA will be treated as not transgenic. In Australia, the Office of the Gene Technology Regulator prepared a discussion paper how new technologies could be regulated. Natural mutations, chemical and radiation mutagenesis is not gene technology. SDN-1, site-directed nucleases which involves the

unguided repair of a targeted double-strand break results in point mutations or deletions (no template is used), and should be, therefore, treated like point mutations. Contrariwise SDN-3, where a long template is used, is clearly within the scope of the current regulation (OGTR 2018).

In the United States a *National Strategy for Modernizing the Regulatory System for Biotechnology Products* was launched in 2017. Within this strategy USDA, EPA (Environmental Protection Agency) and FDA (Food and Drug Administration) examine their regulatory approach to genome edited products. Recently, APHIS (Animal and Plant Health Inspection Service) of USDA withdrew the proposed revision to its regulation. The current regulatory uncertainty has negative impact on R&D investment. The differing processes across countries could have negative implications for the trade in commodities, the movement of seed and research collaborations. International consistency and cooperation, and government to government engagements are necessary to define a clear pathway to market for genome edited crops based on consistency, transparency, predictability and functionality.

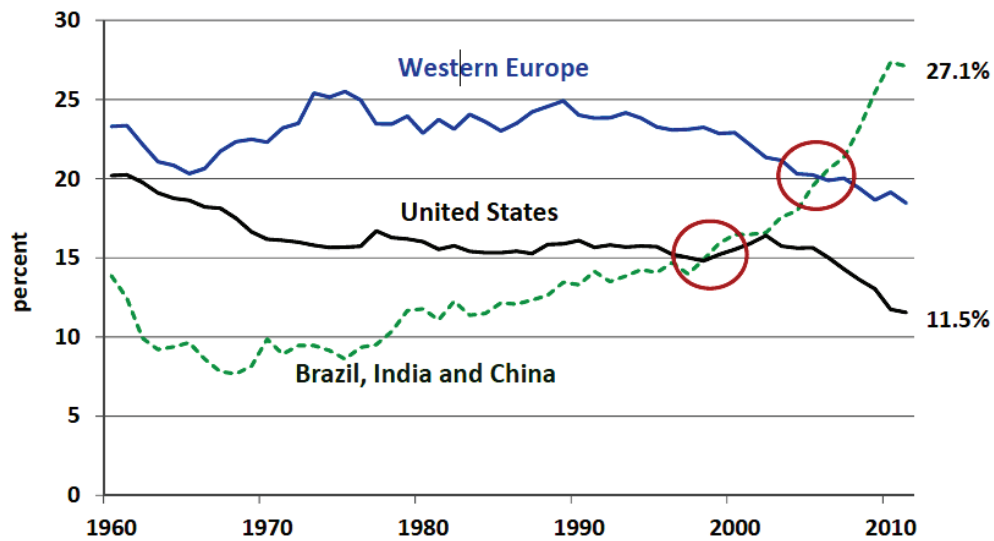
For 2018 not only a decision by the European Court of Justice is expected if New Breeding Techniques are within the scope of the current GMO regulation or not, but also two workshops on genome editing will be organised by the Inter-America Institute for Cooperation on Agriculture and the OECD, respectively.

### Keywords

Genetic engineering · genome editing · GMO · new breeding techniques · regulation · R&D investment

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**Figure 1:** Shifts in the global shares of public food and agriculture R&D from 1960 to 2011  
(Source: Pardey 2016)

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## Earning consumer trust in food and agriculture

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Every organization, no matter how large or small, operates with some level of social license, which is the privilege of operating with minimal formalized restrictions based on maintaining public trust by doing what's right. Social license is granted when you operate in a way that is consistent with the ethics, values and expectations of your stakeholders, which include customers, employees, the local community, non-governmental organizations, regulators, legislators and the media.

Once lost, either through a single event or a series of events that reduce or eliminate public trust, social license is replaced with social control, which includes regulation, legislation, litigation or market action designed to compel you to perform to the expectations of your stakeholders. Operating with a high degree of social control increases costs, reduces operational flexibility and increases bureaucratic compliance.

An US case in point - Arthur Anderson and Enron. Prior to the collapse of Enron, public accounting firms operated with fairly broad social license. The accounting industry had established the Financial Accounting Standards Board to regulate the implementation of Generally Accepted Accounting Principles by Certified Public Accountants.

Stakeholders relied on the industry to operate in a way that maintained public trust and in return the public was willing to grant accountants broad social license. The Enron debacle cost the accounting profession its social license. That single event was the tipping point that compelled Congress to adopt the Sarbanes-Oxley Act, a law that requires extensive reporting and verification of financial information by publicly-traded companies. Public company's now must spend millions to comply – costs that could have been returned to shareholders as dividends, or reinvested in research and development.

The question then becomes, what can be done to maintain public trust that grants the social license and protects freedom to operate? Transparency is the key. Anyone with a smartphone today is a cinematographer. Research clearly indicates that consumers increasingly go online to look for information to answer their questions about food. Growing skepticism about food safety and the use of technology fuel online communities that are raising issues and making their voices heard with increasing volume and frequency.

In this dynamic new environment, producers, processors and distributors are inextricably linked to their customers and NGOs interested in food issues. The question for food companies is no longer "will you be transparent," but rather, "how will you protect your social license in an age of radical transparency?"

The food system has an incredible challenge and opportunity ahead. By mid-century we must significantly increase food production to meet the needs of a burgeoning global population. To meet the challenge, we have to embrace new models of public engagement that build and maintain public trust and our social license to operate. We need stakeholders who control social license to understand that while our systems have changed and our use of technology has increased, our commitment to doing what's right has never been stronger. We need to be able to verify our claims with objective science and we have to be able to continue to operate profitably if we want to survive. We need to adopt systems and practices that are ethically grounded, scientifically verified and economically viable.

If food system practices are not ethically grounded they will not achieve broad-based societal acceptance and support. If they are not scientifically verified there is no way to evaluate and validate the claims of sustainability. If they are not economically viable they cannot be commercially sustained. If we can't operate a system that maintains this balance, it will collapse, subjecting producers, processors, restaurants or retailers to undue pressure that includes consumer protests or boycotts, unfavorable shareholder resolutions, uninformed supply chain mandates, regulation, legislation, litigation or bankruptcy.

As we increase both the distance most consumers have from farming, food processing and the level of technology we implement in food production we must dramatically improve our ability and commitment to build trust with our customers and other stakeholders who grant social license.

In 2006, we commissioned a meta-analysis of all the available research on the question of trust in the food system. Through that analysis, we were able to determine three primary elements that drive trust in the food system. Those three elements are influential others, confidence, and competence.

Influential others include family and friends as well as respected, credentialed individuals like doctors and veterinarians. The other two elements are the type of information the influential other shares. Confidence is related to perceived shared values and ethics and a belief that an individual or group will do the right thing. Competence is about skills, facts, science, ability and technical capacity. Historically competence is where we have focused our communication about food, under the assumption that stakeholders will make logical, data-based decisions if provided credible information.

A nationwide consumer survey in 2007 was conducted on behalf of The Center for Food Integrity to determine the role that confidence, competence and influential others play in creating and maintaining trust. Consumers were specifically asked to rate their level of confidence, competence and trust in various groups of influential others in the food system. Questions were asked related to food safety, environmental protection, nutrition, animal well-being and worker care.

The results of the survey were consistent and conclusive. On every single issue, confidence, or shared values, was three to five times more important than competence (facts or technical capacity) for consumers in determining who they will trust in the food system. That research has been peer reviewed and was published in 2009 in the journal *Rural Sociology* (Sapp *et al.* 2009). These results should serve as a call to action for the food system. No longer is it sufficient to rely solely on science or to attack the attackers as a means of protecting self-interest. This new environment requires new ways of engaging and new methods of communicating if the food industry and those in it want to build trust, earn and maintain social license and protect our freedom to operate.

## Keywords

Confidence · consumer survey · food system · food industry · meta analysis · social license · stakeholder · transparency

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## Telling the plant breeding innovation story

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Through advancements in agriculture and the development of new crop varieties, humans have historically strived to meet the needs of a growing population and to develop a safe, reliable and sustainable food supply. How will we continue to meet this challenge, while dealing with climate change and threats of new pests and diseases? Just like in other industries, continued innovation is paramount to the future of agriculture, and our quality of life.

The American Seed Trade Association (ASTA; [www.betterseed.org](http://www.betterseed.org), [www.seedinginnovation.org](http://www.seedinginnovation.org)) conducted consumer focus groups to better understand the attitudes, feelings and perceptions of key stakeholder groups around issues related to seed improvement and plant breeding innovation. This research has been used to inform the underlying themes of our public-facing communications on plant breeding innovations. ASTA in turn is working to spread these messages globally through partnerships with the International Seed Federation (ISF; [www.worldseed.org](http://www.worldseed.org)) and the European Seed Association (ESA, [www.euroseeds.eu](http://www.euroseeds.eu)).

Consumers want healthy and safe food for their families, their environment and their community – today and in the future. They want farmers to produce a variety of food choices, while wisely using natural resources, solving challenges locally, and reducing crop inputs. Plant breeding innovation, like gene editing, allows for this and more. Through evolving plant breeding methods that work within the genetic makeup of plants' own families, plant scientists and breeders provide farmers with seeds that can thrive despite challenges, such as changing weather, plant disease and pests, while reducing crop inputs.

As we talk about plant breeding innovation, it's important that we start with our history. Over the last several thousand years, humans have taken a more active role in improving plant characteristics by specifically selecting seed from plants that looked, smelled or tasted particularly interesting. The result was the domestication of plants, making them easier to grow and harvest and helping them to become better, more reliable and nutritious sources of food. This same process continues today, though in a more formalized way, using the science and art of plant breeding.

Newer plant breeding methods like gene editing work within the plant family's own genetic makeup. These methods build on what plant scientists and breeders have been doing for years, allowing us to reach the same endpoint as through traditional methods – but in a more precise way.

So how does gene editing work? In simple terms, gene editing allows plant scientists and breeders to precisely make specific changes to a plant's DNA using a plant's own internal processes.

The result can be:

- Activation of a beneficial characteristic – like drought tolerance or increased nutrition;
- Deactivation of an unfavorable characteristic – like disease sensitivity; or
- Small changes to the DNA that reproduce a characteristic found within the plant's family – like disease resistant characteristic found in a wild relative.

The seed industry has a phenomenal track record of safety. Most new agricultural plant varieties are introduced without a specific pre-market safety review, given the long history of safe use of the underlying varieties used for breeding. A key feature of the plant breeding process is extensive testing and evaluation starting early in the breeding process and continuing until the final product is commercially available. These tests are based on procedures breeders have used for many decades to create new plant varieties that are safe to grow and eat.

No matter the method used, the goal of today's plant breeders remains the same: producing better seed for a better quality of life. While most of the general public doesn't know very much about plant breeding, recent consumer focus groups have shown that when making purchase decisions at the grocery store, people want food that is: healthy and safe for their families safe for the environment, and they want a variety of options. Some examples of exciting research being done using gene editing can lead to fruits and vegetables with better taste, appearance and nutrition. In addition, research is being done to make wheat that can be consumed by people with gluten sensitivities.

In order to realize these exciting possibilities, we need a policy climate that allows, and encourages, continued innovation. ASTA's overarching position is this: plant varieties developed through the latest breeding methods should not be differentially regulated if they are similar to or indistinguishable from varieties that could have been produced through earlier breeding methods.

The seed industry and agriculture in general are global. Inconsistent global policies can negatively impact trade in agricultural products and research collaborations that cross country boundaries. Consistent and appropriate policies are needed for overall agriculture innovation and development. The ultimate goal is access to the full range of breeding tools in order for agriculture to continue to advance in the future.

### Keywords

Communication · gene editing · plant breeding innovation



## New breeding techniques and the EU

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In the European Union (EU), definitions with respect to genetically modified plants are laid down in the following legal documents:

(i) Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC (<http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32001L0018>);

(ii) Directive 2009/41/EC of the European Parliament and of the Council of 6 May 2009 on the contained use of genetically modified micro-organisms (<http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32009L0041>);

(iii) Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed (<http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32003R1829>).

In Article 2(2) of Directive 2001/18/EC a 'genetically modified organism' (GMO) is specified as an organism, with the exception of human beings, in which the genetic material has been altered in a way that doesn't occur naturally by mating and/or natural recombination. Techniques leading to genetic modifications are listed in Annex I A, part 1, whereas part 2 includes techniques which are not considered to result in genetic modification. In Annex I B, techniques are listed to which the Directive shall not apply.

Techniques of genetic modification (Annex I A, part 1) are inter alia: (1) recombinant nucleic acid techniques involving the formation of new combinations of genetic material by the insertion of nucleic acid molecules produced by whatever means outside an organism, into any virus, bacterial plasmid or other vector system and their incorporation into a host organism in which they do not naturally occur but in which they are capable of continued propagation; (2) techniques involving the direct introduction into an organism of heritable material prepared outside the organism including micro-injection, macro-injection and micro-encapsulation; (3) cell fusion (including protoplast fusion) or hybridisation techniques where live cells with new combinations of heritable genetic material are formed through the fusion of two or more cells by means of methods that do not occur naturally.

Techniques which are not considered to result in genetic modification (Annex I A, part 2), on condition that they don't involve the use of recombinant nucleic acid molecules or GMOs made by techniques other than those excluded by Annex I B are: (1) *In vitro*

fertilisation; (2) natural processes such as conjugation, transduction, transformation; (3) polyploidy induction.

Annex I B specifies techniques/methods of genetic modification yielding organisms to be excluded from the Directive, on the condition that they do not involve the use of recombinant nucleic acid molecules or genetically modified organisms other than those produced by one or more of the techniques/methods listed below: (1) mutagenesis; (2) cell fusion (including protoplast fusion) of plant cells of organisms which can exchange genetic material through traditional breeding methods.

The annexes of Directive 2009/41/EC are similar, specified in this case with respect to genetically modified micro-organisms.

### The Austrian *Gentechnikgesetz*

In Austria, §3 of the *Gentechnikgesetz* (<https://www.ris.bka.gv.at/GeltendeFassung.wxe?Abfrage=Bundesnormen&Gesetzesnummer=10010826>) defines a GMO as 'organism, in which the genetic material has been altered in a way that does not occur naturally by mating or natural recombination or other conventional breeding techniques.' Techniques of genetic modification are in particular: (a) techniques of DNA recombination using vector systems; (b) direct introduction of genetic information prepared outside the organism including macro-injection, micro-injection, micro-encapsulation, electroporation and the use of micro-projectiles; (c) cell fusion or hybridisation techniques generating living cells with new combinations of genetic material that do not occur naturally, exempt techniques according to Article 2(2)(5) and 2(2)(6): On the condition that genetically modified organisms or genetically modified nucleic acids are not involved, this federal law exempt in particular the following activities which are not leading to genetically modified organisms: (1) *in-vitro*-fertilisation; (2) conjugation, transduction, transformation or every other naturally occurring process; (3) polyploidy-induction and elimination of chromosomes; (4) techniques of non-directional mutagenesis; (5) cell- and protoplast fusion of plant cells, as well as fusion of protoplasts of microorganism, under the condition that the resulting organisms could also be obtained by conventional breeding techniques; (6) generation of somatically-human or somatically-animal hybridoma cells, under the condition that this application deals not with deliberate release or placing on the market; (7) self-cloning of non pathogenic, naturally occurring micro-organisms, which fulfil the criteria for risk group 1, except for the purpose of deliberate release.

se and placing on the market. Self-cloning also applies for cloning with recipient and donor organisms of similar type, using defined and well characterised vectors.

### Progress of discussion in the EU

In 2006 a growing number of member states requested to the Commission, whether certain 'new breeding techniques' fall under the scope of Directives 2001/18/EC or 2009/41/EC. As a consequence the European Commission established an expert working group (New Techniques Working Group, NTWG) in October 2007 with the aim to analyse an inter alia list of these 'new breeding techniques'. The following techniques were under discussion: (i) oligonucleotide directed mutagenesis (ODM); (ii) zinc finger nuclease technology (ZFN) (comprising ZFN-1, ZFN-2 and ZFN-3); (iii) cisgenesis (comprising cisgenesis and intragenesis); (iv) grafting; (v) agro-infiltration; (vi) RNA-dependent DNA methylation (RdDM); and (vii) synthetic genomics.

Between 2008 and 2011 the NTWG had nine meetings and by November 2011 the 'Final Report' was forwarded to the Commission. Until today the 'Final Report' was neither officially published by the Commission nor discussed within the competent Standing Committees. In 2012 the Commission announced a legal interpretation of the directives, which did not happen up to day. In 2016: the Commission started the so called 'Scientific Advice Mechanism (SAM)' whose main tasks are (i) the elaboration of the 'key characteristics' of the different 'New Breeding Techniques', and (ii) the comparison with established ('old') techniques. A further task of the SAM is (iii) analysing expected trends to evaluate other 'New Techniques' like synthetic biology or gene drive for their potential use in plant breeding. The SAM report was published end of April 2017 (European Union 2017).

The SAM report is explanatory and does not take a position or make recommendations to policy makers. For the purpose of the report all breeding techniques applicable in agriculture were grouped as (a) conventional breeding methods (CBT), (b) established techniques of genetic modification (ETGM) and (c) new breeding techniques (NBT). However, the report summarized that

- there is heterogeneity within the NBT and some similarities between some NBT and some CBT and some ETGM;
- the genome editing subset of NBT can produce precisely located alterations to DNA sequences, ranging from point mutations to the insertion of (endogenous or exogenous) genes;
- the end products of NBT may or may not contain exogenous DNA. The development of an end product that involves the use of NBT may additionally use ETGM, and as a consequence, exogenous nucleic acids may be present in intermediate products but not necessarily in the end product;
- based on the variety and versatility of NBT grouping of these techniques together as NBT may not be optimal for scientific or other reasons;
- the employment of the NBT of gene editing does not exclude 'off-target' effects, where a precise change is made to a genetic sequence identical or similar to that in which the change is desired, however the frequency of unintended effects in NBT products is much lower than in products of CBT and ETGM;
- an assessment of safety can only realistically be made on a case-by-case basis;
- regardless of the technique used, the introduction of changes to genetic sequences and gene expression in an organism can induce unintended effects in the organism;

- detection is more challenging if no information concerning the introduced changes is available, but a significant attempt can be made through the application of whole genome sequencing in combination with bioinformatics, and in such cases detection depends on the availability of a suitable reference (baseline) genome. Nevertheless, it is generally impossible to distinguish the cause of such changes as natural or resulting from the employment of any breeding technique;
- the NBT can also be used for gene drives. Further research will be needed in relation to inter alia efficiency and safety before organisms to which this approach is applied can be considered for release into the environment.

With respect to recital 17 of Directive 2001/18/EC (This Directive should not apply to organisms obtained through certain techniques of genetic modification which have conventionally been used in a number of applications and have a long safety record) a problem arises with the definition of mutagenesis, e.g. conventional vs. new mutagenesis methods, directed vs. non-directed mutagenesis.

In October 2016 the French Conseil d'État requested a preliminary ruling of the European Court of Justice (ECJ) on four questions concerning the interpretation of Directive 2001/18/EC. Among others the ECJ is asked if only methods of non-directed mutagenesis (chemical mutagens or ionising radiation), which were used before Directive 2001/18/EC came into force, are exempted from the scope of the directive or if this exemption is also valid for the methods of directed mutagenesis which are discussed today.

The opinion of the Advocate General was delivered on 18 January 2018 (<http://curia.europa.eu/juris/document/document.jsf?docid=198532&doclang=EN>; <https://curia.europa.eu/jcms/upload/docs/application/pdf/2018-01/cp180004en.pdf>). The final judgment of the ECJ is expected for April 2018.

### Keywords

European Union · gene editing · genetic engineering · GMO · legal regulation · mutagenesis · new breeding techniques

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## Hybrid breeding in open field vegetables

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Bejo Zaden is a family owned vegetable breeding company, which is specialized in open field hybrid crops. We have more than 1700 employees worldwide, 50 different crops with 1200 varieties for conventional growing and an organic assortment of 40 crops and more than 150 varieties. Bejo has 30 daughter companies worldwide where we sell directly and in more than 100 other countries through our dealer network. We produce our commercial seeds on the Northern and Southern hemisphere.

Research and breeding is a joint effort with experts from the Phytopathology, Cell Biology, Marker and Breeding Departments. We developed more than 120 disease resistance tests in more than 15 vegetable species, *in vitro* techniques (*e.g.* embryo rescue method for interspecific crosses, doubled haploid method), molecular markers for various traits and try to implement the latest technologies to create the best possible varieties for each country and climate.

The main breeding goals are yield, earliness, colour and shape, taste, disease resistance, adaptability to climate, content of specific compounds (*i.e.* nutritional compounds) and health trends (*i.e.* phytochemicals).

To develop hybrid crops, each crop has its own challenges. Two different approaches for Brassica vegetables are discussed (*i.e.* self incompatibility and CMS). Spinach as an example of a wind pollinator and carrots as a crop which suffers a lot from inbreeding depression.

### Keywords

Brassica · carrot · cytoplasmic male sterility · hybrid breeding · spinach · vegetable





## Breeding progress of grain and quality traits in winter rye hybrid vs. population varieties and national on-farm progress in Germany over 26 years

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In Germany, winter rye (*Secale cereale* L.) is a minor cereal crop compared to winter wheat. Today about 6% of arable land is grown with rye. Two-thirds of the national harvest is fed to animals and 15% is used for human nutrition, mainly for bread baking. For animals, protein yield is the most important trait, but for bread baking the quality-determining compounds are starch, pentosans and alpha-amylase. Rye has a high dietary value because of its high fibre content and the good quality of rye protein for human nutrition. It is well known, however, that grain quality of rye is very volatile. On average 80% of the national harvest has baking quality. During 1992 to 2014 the harvested grain rye reaching baking quality according to a former EU-criterion was in the range of 25-100%. It is now for more than 30 years that the first hybrid varieties have been tested for their value of cultivation and use (VCU) and registered in Germany. The share of hybrid varieties in the total rye growing area in Germany rapidly increased until 2003 to about 80% and then ranged between 70-80% until 2014, whereas the share of population varieties continuously dropped to less than 20% in 2014.

We evaluated the progress of 13 traits (grain yield and its components, quality and disease related traits, see Table 1) achieved during 1989 and 2014 in VCU trials and on-farm. In the VCU data set 78 registered varieties were analysed, including 57 hybrid and 21 population varieties. Varieties which were not registered were dropped. Linear mixed models were applied to estimate gains and losses, derived from overall trends (comprising genetic and non-genetic influences), separately for hybrid and population varieties. Further, pooled phenotypic correlation coefficients based on pooled adjusted variety means were calculated for hybrid and population varieties.

Grain yield of hybrid varieties improved by 23.3% (18.9 dt ha<sup>-1</sup>) relative to the yield level in 1989 (80.9 dt ha<sup>-1</sup>), whereas grain yield for population varieties increased by 18.1% (13 dt ha<sup>-1</sup>) relative to 1989 (71.7 dt ha<sup>-1</sup>) as shown in Table 1. Our results further revealed that the yield gap between hybrid and population varieties widened over the 26 years. Despite of the rapid shift to the higher yielding hybrids, on-farm still a remarkable gap between trial and on-farm yields became apparent. The absolute on-farm yields gained less than half (8.7 dt ha<sup>-1</sup>) as compared to trial yields for

hybrids, but at a considerably lower level. Rye is grown mostly in the North-East of Germany. On the sandy low fertile soils of Brandenburg with frequent drought periods in spring 30% of rye is grown, followed by Niedersachsen with 20%. Less fertile soils and less water supply in rye growing regions, as compared to VCU trial sites, may be one reason for the observed lower on-farm yield level. Further, in Brandenburg still 32% of lower yielding population varieties are grown, whereas the share in most other federal states is less than 10%. A second reason for the large yield gap may be attributed to economic constraints in on-farm rye production, i.e. that not the full yield potential, but the economical yield optimum, is realized by farmers. For hybrid varieties, the increase in grain yield was mainly caused by a higher ear density of 14.4% (absolute 70.8 ears m<sup>-2</sup>) and for population varieties by higher single ear weight of 16.4% (absolute 0.24 g ear<sup>-1</sup>), which is about twice as high as for hybrids.

Falling number, amylogram viscosity and temperature are the most important quality related traits in winter rye. Higher values are more favourable for good bread quality. As Table 1 indicates, no significant changes for these quality traits were found in VCU trials and on-farm. Despite of a 40% increase of amylogram viscosity, this change is not significant due to the extremely large year-to-year variation of this trait. Further, hybrid and population varieties showed little difference in the observed pattern of changes. Only protein concentration of hybrids decreased significantly by 9.7% (absolute -1% \*\*) relative to 1989. We found a decrease of susceptibility for mildew, Rhynchosporium and leaf rust of hybrid and population varieties which was not significant, except for leaf rust, where susceptibility of hybrids was significantly reduced by 1.2 units on a 1 to 9 scale. The inclination for lodging increased for both types of varieties by 0.5 units on the 1 to 9 scale, but the increase was not significant. Grain yield correlated only weakly to moderately with quality traits (0.33 < r < 0.51), except for protein concentration, where a well-known strong negative association of r = -0.67 was observed. Protein concentration is only weak correlated with the other three quality traits (-0.29 < r < -0.44). Contrary to winter wheat where protein concentration is strongly positively associated with baking quality, for winter rye this is not the case. Hence breeding for higher yield had no hampering side effect on bread quality. Falling number shows a strong relation

with amylogram viscosity ( $r = 0.76$ ) and a very strong association with temperature ( $r = 0.90$ ).

Our results confirmed that during the last 26 years in Germany hybrid varieties accelerated yield progress in rye without loss in

disease susceptibility and in bread quality, but a remarkable large gap exists between VCU trials and on-farm yield levels.

## Keywords

Baking quality · breeding progress · *Secale cereale* · yield gap

**Table 1:** Gains and losses of yield and quality traits in VCU trials and on-farm calculated from overall trends. Percent values are based on 1989 absolute overall regression estimates. On-farm data for grain yield, protein concentration, falling number, amylogram viscosity and temperature were obtained from national statutory harvest survey as annual averages comprising results from all variety types. (Significance levels: \*,  $p \leq 0.05$ ; \*\*,  $p \leq 0.01$ ; \*\*\*,  $p \leq 0.001$ ; Hyb, hybrid varieties; Pop, population varieties; AU, amylogram units).

Traits <sup>1</sup>	Source	Type	Regression estimates <sup>2</sup>			
			1989	2014	Difference	%Diff
Grain yield (dt ha <sup>-1</sup> )	Trial	Hyb	80.9	99.8	18.9***	23.3
		Pop	71.7	84.7	13.0**	18.1
	On-farm		45.9	54.6	8.7*	18.9
Thousand grain mass (g)	Trial	Hyb	32.8	38.6	5.7***	17.4
		Pop	33.5	39.0	5.5**	16.5
Single ear density (m <sup>-2</sup> )		Hyb	493.4	564.2	70.8*	14.4
		Pop	505.1	515.7	10.6	2.1
Number of kernels per ear (ear <sup>-1</sup> )		Hyb	53.5	44.7	-8.8	-16.4
		Pop	45.3	41.6	-3.7	-8.2
Single ear weight (g ear <sup>-1</sup> )		Hyb	1.71	1.85	0.15	8.6
		Pop	1.47	1.71	0.24	16.4
Protein concentration (%)	Trial	Hyb	10.7	9.7	-1.0**	-9.7
		Pop	10.9	10.4	-0.5	-4.5
	On-farm		10.8	10.1	-0.7	-6.4
Falling number (s)	Trial	Hyb	226.1	234.8	8.7	3.8
		Pop	212.1	216.0	3.9	1.8
	On-farm		195.6	213.9	18.3	9.4
Amylogram viscosity (AU)	Trial	Hyb	801.7	881.2	79.4	9.9
		Pop	746.9	653.7	-93.2	-12.5
	On-farm		616.4	860.7	244.3	39.6
Amylogram temperature (°C)	Trial	Hyb	69.7	69.1	-0.6	-0.8
		Pop	69.3	68.3	-0.9	-1.4
	On-farm		68.0	68.4	0.4	0.5
Lodging (1-9)	Trial	Hyb	4.4	4.9	0.5	
		Pop	4.5	5.0	0.5	
Powdery mildew (1-9)		Hyb	3.1	2.4	-0.7	
		Pop	2.9	2.2	-0.7	
Rhynchosporium (1-9)		Hyb	3.9	3.5	-0.4	
		Pop	3.9	3.7	-0.2	
Leaf rust (1-9)		Hyb	5.0	3.7	-1.3*	
		Pop	4.1	3.4	-0.7	

<sup>1</sup> Assessed on treated (fertilizer, chemical treatments) plots; disease resistance assessed on untreated (no fungicide, no growth regulator) plots

<sup>2</sup> Mixed linear model for overall trend:  $y_{ijklk} = \mu_l + L_j + \phi_l t_k + U_{klj} + LY_{jk} + GY_{ijklk} + GLY_{ijklk}$ ; where  $y_{ijklk}$  is the mean yield of genotype  $i$  of group  $l$  in location  $j$  and year  $k$ ,  $\mu_l$  is the overall mean of the  $l^{th}$  variety group,  $L_j$  the main location effect,  $\phi_l$  a fixed regression coefficient for overall trend of group  $l$ ,  $t_k$  the continuous covariate for year,  $U_{klj}$  the random deviation of year  $k$  from linear overall regression line of fixed variety group  $l$  following a normal distribution ( $\mu=0$ ,  $\sigma^2_U$ ),  $LY_{jk}$  the location by year interaction,  $GY_{ijklk}$  the genotype by year interaction, and  $GLY_{ijklk}$  the residual including genotype by location by year interaction and sampling error arising from sampling the replications of group  $l$

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## Current status and perspectives of hybrid breeding in winter rye

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Hybrid breeding in winter rye (*Secale cereale* L.) is a great success story. As a cross-pollinating crop, rye shows a similarly high heterosis than maize and modern hybrid performance can reach more than 120 dt ha<sup>-1</sup> on better soils. Hybrid rye cultivars clearly show a superiority of 15-20% over population cultivars and a higher progress from breeding (see Laidig, this volume). In Germany, hybrid rye is cultivated on about 70% of the whole rye acreage. Prerequisites for hybrid breeding in the self-incompatible winter rye are self-fertility, divergent gene pools, cytoplasmic-male sterility (CMS) and pollen-fertility restoration. Their usage and their incorporation into breeding schemes have been reviewed extensively (Geiger & Miedaner 2009). Future perspectives of hybrid rye breeding in Central Europe are (i) improvement of pollen-fertility restoration, (ii) selection for disease resistances to ergot, Fusarium head blight (FHB) and stem rust, (iii) implementation of genomics-based breeding.

Pollen-fertility restoration is an important issue in winter rye not primarily for maximizing grain yield, but mainly for reducing ergot, caused by *Claviceps purpurea*, that infects foremost non-fertilized ovules. European restorer sources are oligogenically inherited, prone to environment and seed-parent line, and not very efficient (Miedaner *et al.* 2017). Better restorer-to-fertility (*Rf*) genes have been detected in those germplasm where the CMS-inducing P cytoplasm was derived from, in particular from Argentinian landraces (e.g. Pico Gentario), Iranian primitive rye (IRAN IX, Altevogt 14160) and Turkish weedy rye. Their pollen-fertility restoration is monogenically inherited and has a high efficacy. The *Rf* genes of Pico Gentario, IRAN IX, and Altevogt 14160 are located in the same short interval on chromosome 4RL (Hackauf *et al.* 2017). Unfortunately, all non-adapted restorer genes tested so far displayed a yield penalty (Miedaner *et al.* 2017). However, a large variation for yield penalty among different introgressed lines have been found in this study opening the possibility for developing superior restorer lines by marker-assisted selection (MAS).

Disease resistance is an important feature for modern hybrid cultivars although rye is rather resistant to abiotic stress factors. FHB epidemics occur not regularly, but the mycotoxin deoxynivalenol (DON) can be found in the harvest every year, although most often in lower concentrations than in wheat. The resistance is quantitatively inherited and can be efficiently improved by recurrent selection. However, the correlation between the performance of inbred lines and their corresponding hybrids is weak, thus requiring additional resistance tests of the latter. Stem rust resistance is a rather new breeding goal caused by frequently

occurring epidemics in Germany and Poland. The adapted German self-fertile breeding populations are highly susceptible, resistance sources have been found in Austrian landraces (Tiroler, Oberkärntner), Russian populations (e.g. Talovskaja), and US feeding rye (e.g. Wrens Abbruzzi, Gator, Elbon).

Genomics-based breeding became feasible in rye with the development of PCR-based marker techniques such as amplified fragment length polymorphisms (AFLPs), simple sequence repeats (SSRs), diversity array technology (DArT) and especially with the advent of next generation sequencing techniques. Their uses and achievements were recently reviewed (Miedaner *et al.* 2018). These techniques have been utilized in research for producing introgression libraries from plant genetic resources, for mapping qualitative traits like plant height,  $\alpha$ -amylase activity, and quantitative trait loci (QTL). The QTL studies revealed significant genetic variation for all analyzed agronomic and quality traits despite the need of using testcross progenies that reflect the commercially grown hybrids. Analyzed traits are inherited in a quantitative manner with many low-effect QTL. Even for very complex inherited traits, like grain yield or biomass yield, however, individual QTL with higher contribution and stably across a range of environments were found that might be a target for marker-assisted selection and candidate gene search in homoeologous genomes. In practical breeding, marker techniques are mainly used for assigning foreign breeding materials to the known gene pools, for introgressing non-adapted *Rf* alleles in breeding populations, and for testing CMS phenotypes and hybridity in hybrid seed multiplication.

Considerable progress has been achieved in the last years by adopting next generation sequencing techniques in rye enabled by public-private partnerships (Miedaner *et al.* 2018). Comprehensive resources of expressed sequence tags (ESTs) were established and a 5k, and later an extended custom 16k Illumina BeadChip were produced based on single-nucleotide polymorphisms (SNP). Both genotyping arrays were successfully used for mapping QTL and for evaluating the potentials of genomic prediction or genomic selection (GS) in rye. GS has the advantage of predicting the genomic breeding value (GEBV) of non-phenotyped entries and, thus, reducing cycle length and costs because in the meantime a genotyping array is cheaper than managing field plots. This is especially valid in hybrid breeding where every line has to be testcrossed before selection for grain yield, thus needing two years for only one stage of yield selection. GS generally resulted in higher prediction accuracies (PA) than MAS when applied to the quantitative traits

grain yield, plant height, starch and total pentosane content. Testing GS across breeding cycles revealed higher PA when data from all four selection cycles were combined. The design of crosses and familial structures is important, however, to ensure sufficient connectivity over breeding cycles via common ancestors. A simulation-based optimization of breeding plans in rye resulted in a combined GS and phenotypic procedure within a five-years cycle starting with a moderate phenotypic selection based on line per se performance, a sharp selection for grain yield by one step of GS followed by a conclusive phenotypic selection in the field (Marulanda *et al.* 2016). The annual selection gain of this procedure was 25% larger than the traditional seven-years pure phenotypic selection. In conclusion, these novel genome resources will allow a targeted introgression of monogenically inherited traits by MAS, make breeding cycles more efficient in terms of time and population size by GS and facilitate gene cloning and functional gene characterization in future. Both will help breeders to further improve hybrid rye and to compete with the effects of global climate change.

## Keywords

Disease resistance · genomic selection · QTL mapping · restorer genes · *Secale cereale*

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## Hybrid breeding in cereals: lessons from rye

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In rye (*Secale cereale* L.), hybrid breeding enables the systematic exploitation of heterosis since almost four decades and serves as a model to establish this cutting edge technology for the genetic improvement of barley and wheat. Breeding and commercial seed production of hybrid rye is mainly based on the cytoplasmic male sterility (CMS)-inducing Pampa (P) cytoplasm, which originated from an Argentinian landrace. Breeding of CMS-based cereal hybrids requires the use of effective restorer-of-fertility (*Rf*) genes, which are of central importance to minimize ergot contamination of the harvest. *Rf* genes from non-adapted rye populations have been mapped to a small segment on the long arm of chromosome 4R using conserved ortholog set (COS) markers (Hackauf *et al.* 2009, 2012). COS markers provide efficient tools for marker-assisted backcrossing (MABC) of *Rf* genes in the pollinator gene pool of rye. We report on the introgression of small donor chromosome segments (DCS) carrying *Rf* genes from three unadapted genetic resources of rye in two elite pollinator genotypes. The established genotypes were evaluated with respect to possible linkage drag effects associated with individual *Rf* genes using a highly diagnostic phenotyping platform.

Initial crosses were executed in 2011 and MABC of the restorer genes *Rfp1*, originating from a weedy rye population, as well as *Rfp2* and *Rfp4*, both originating from forage rye populations, has been conducted using the previously established flanking markers TC176835 and TC363404 (syn. TC135788), respectively (Hackauf *et al.* 2012). BC<sub>3</sub>S<sub>1</sub> single plants carrying either the elite (E) non-restorer allele or the donor (D) restorer allele from *Rfp1* ( $n = 3$  BC<sub>3</sub> lines), *Rfp2* ( $n = 4$ ) or *Rfp4* ( $n = 4$ ) have been selected to establish near isogenic homozygous BC<sub>3</sub>S<sub>2</sub> bulks (NIB), that were used as pollinator for crossing with two male sterile single-cross non-restorer genotypes between isolation walls. Evaluation of the established three-way cross hybrids in the field was done in 2016 and 2017 in 12 environments (locations  $\times$  year combinations). Entries were grown on drilled plots of 5 to 6 m<sup>2</sup> size. The experiment was laid out in a partially balanced incomplete block design (alpha design) with two replications per entry. All plots have been assessed for agronomic traits including grain yield (GYD, dt ha<sup>-1</sup>), thousand grain weight (TGW, g), and plant height (PHT, cm). Male fertility scores were visually assessed directly in the field on plot basis and used to calculate a restorer index (RI) as described by Miedaner *et al.* (2017). All statistical computations were performed with *R* software packages in a two-step procedure. Adjusted entry means were calculated for all traits in each environment separately and were used in a second step to estimate variance

components based on the following linear mixed model:  $y = G + E + G \times E$ , where *G* and *E* denote genotype and environment, respectively. Both factors as well as the interaction term were treated as random effects. Broad sense heritability ( $h^2$ ) on an entry-mean basis was estimated from the variance components as the ratio of genotypic to phenotypic variance. Linkage drag effects were calculated as the difference ( $\Delta E-D$ ) between the means of individual NIB partners, which either carry the E or the D at the 4RL DCS. Comparisons between individual NIB partners were performed with Welch's *t*-test.

All traits revealed significant genotypic and genotype by environment interaction variances. The estimates of broad-sense heritability were high ( $h^2 > 0.88$ ) for all traits. Non of the agronomic traits deviated from a normal distribution. On average, hybrids carrying *Rfp1* or *Rfp4* were significantly ( $p < 0.05$ ) taller compared to their near isogenic hybrids carrying the elite non-restorer allele. Likewise, *Rfp1*, *Rfp2* and *Rfp4* resulted in significantly ( $p < 0.05$ ) lower grain yield. Interestingly, the linkage drag effect of *Rfp2* (-3,73 dt ha<sup>-1</sup>) and *Rfp4* (-4,68 dt ha<sup>-1</sup>) were less pronounced compared to *Rfp1* (-8,41 dt ha<sup>-1</sup>). A significantly negative effect on TGW could only be observed for *Rfp2*. Hybrids carrying the restorer allele of the *Rfp1* and *Rfp4* gene revealed a high degree of pollen fertility restoration, while those with the corresponding non-restorer allele had a low pollen fertility. This could be observed for *Rfp2* as well except of one replicate in each genetic background, which both were characterized by a comparable low RI.

The described results demonstrate, that MABC of *Rfp* genes is superior to conventional backcrossing (Miedaner *et al.* 2017) in precision and efficiency. Furthermore, the yield penalty associated with *Rfp2* in the present study was less pronounced compared to a backcross programme, that was solely based on phenotypic selection (Miedaner *et al.* 2017). This observation may be attributed to the marker-assisted foreground selection on the short DCS in the present study.

A shared ancestry has recently been described between an Argentinian forage rye population and the population variety 'Carokurz' (Parat *et al.* 2016), the latter of which served as founder population of the pollinator gene pool in hybrid rye breeding. Thus, the 50% lesser linkage drag effect of *Rfp2* and *Rfp4* may be attributed to alleles located on the 4R DCS, that are more favourable for grain production in central European target environments than those alleles associated with *Rfp1* from weedy rye. The observed costs of restoration of *Rfp* genes can be counterbalanced *e.g.* by

using recently identified major QTL for grain yield (Hackauf *et al.* 2017). The comparable low RI in genotypes carrying the 4RL DCS from ‘Pico Gentario’ can be explained by epistatic interaction of *Rfp2* with modifier genes on chromosomes 1R and 6R (Hackauf *et al.* 2009). This obvious difference between *Rfp2* and *Rfp1* challenges the assumption of a single origin of the *Rf* mutation (Miedaner *et al.* 2017), as *Rfp1* is a gene acting independently from other genes (Hackauf *et al.* 2012).

In conclusion, nuclear-cytoplasmic gynodioecy in rye offers a natural, reliable, environmentally friendly and cost-effective production of hybrid seed. COS markers linked to *Rfp* genes increase the efficiency of hybrid rye breeding and provide efficient means to evaluate valuable gene variants from diverse genetic resources of rye. The genetic diversity at the *Rfp* locus opens novel options for the breeding of ergot resistant and toxin free rye hybrids.

## Keywords

Costs of restoration · cytoplasmic male sterility · marker assisted selection · restorer of fertility gene · *Secale cereale*

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## 30 years of hybrid wheat breeding in Europe: recent developments and future prospects

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### Abstract

Wheat is one of the world's most important cereals. This results in a high motivation to develop and market hybrid varieties. This paper describes how breeding of commercial hybrid varieties of winter wheat has been developed since the mid 1980s. When this development began over 30 years ago with relatively simple techniques and limited resources, almost all arguments militated against further investment in hybrid wheat breeding. Nevertheless, significant progress has been made in Western Europe since then. More than 30 registered hybrid varieties may be marketed throughout the EU. A chemical sterilization system, utilizing the gametocide Croisor®, is approved for seed production. Hybrid seed production takes place on several thousand hectares. It may now also be organized outside France, e.g. in Germany. Whereas commercial hybrid breeding programs provide an increasing number of varieties, accompanying scientific research projects support practical breeding. The article describes the important milestones of this development.

### Keywords

Chemical hybridization agent · gametocide · hybrid variety · pollen shedding · *Triticum aestivum*

### Introduction

There are countless publications on 'hybrid wheat breeding', but comparatively few on 'varieties of hybrid wheat'. Therefore, this report should focus only on the events and results that have significantly influenced the breeding of commercial hybrid varieties of winter wheat since the mid 1980s.

When all began more than 30 years ago with relatively simple means, almost everything spoke against investments in hybrid wheat breeding, above all:

- (i) the absence of an approved, reliable sterilization system,
- (ii) the absence of a male pool with high combining ability for grain yield and high pollination performance in  $F_1$  seed production,
- (iii) the low heterosis expected for a self-pollinated species,
- (iv) the great advances in line breeding, which still have to be surpassed by the long-lasting hybrid breeding.

Nevertheless, in contrary to the expectations, significant progress has been made until today. In  $F_1$  seed production of all hybrid varieties permitted today, male sterility on the female component

is achieved by the application of a Chemical Hybridization Agent (CHA), which is also referred to as gametocide because of its mechanism of action.

In Western Europe we have today

- (i) more than 30 registered hybrid varieties that may be marketed throughout the EU,
- (ii) the certified sterilization system, the gametocide Croisor®,
- (iii) hybrid seed production on several thousand hectares, which may now also be organized outside France, e.g. in Germany,
- (iv) the marketing, especially under the direction of the company Saaten-Union,
- (v) ambitious programs of hybrid wheat breeding with increasing output of commercial varieties, and
- (vi) the scientific research infrastructure that is expected to produce innovative results for breeding in the future.

In the following, we will outline the path that needs to be taken in order to develop hybrid wheat varieties for practical agriculture. Why did it continue, even after the big companies withdrew? What caused a relatively small company like Nordsaat not to share the general skepticism about hybrid wheat and to swim against the tide?

### Development and registration of gametocides

This process has been going on for over fifty years. According to von Rosenstiel (1970), an up-to-date breeding concept was discussed as early as 1968 in which chemical (CHA) and biological (cytoplasmic male sterility; CMS) means were used for pollination control to accelerate the development of efficient hybrids. After identifying particularly promising strains from line breeding as mother or father, these were used in so-called crossing blocks (with CHA). The resulting experimental hybrids were tested. The best cytoplasmic fertile lines were then transferred over several years into the gene pool of cytoplasmic sterile lines and used as 'mother lines' in the development of hybrid varieties.

In Europe, four companies initially participated in the development of gametocides as well as in hybrid wheat breeding (Table 1). The companies Rohm & Haas (later Dow) and Shell/Nickerson, however, withdrew in the late 1980s from the approval process for their respective gametocides, because obviously, there was no chance of a positive decision due to toxicological problems. The Monsanto Group, with its successful HybriTech joint venture, brought a number of hybrid varieties into the market by the year 2000, produced with the gametocide Genesis® and marketed their  $F_1$  seeds. Likewise, the DuPont Group, with its breeding company

**Table 1:** Gametocides for hybrid wheat in Europe (experimental and commercial use). Gametocides are reported since approx. 1968 (Rosenstiel, 25 Feb 1970, pers. commun).

Gametocide	Hybrex		Genesis®	Croisor®	Croisor®
active substance	RH 007	CHA SD 84811	MON 21200	Sintofen	Sintofen
right holders	Rohm & Haas	Shell/Nickerson	Monsanto	DuPont until 2004	SUR since 2005
preliminary permission	since 1984	no	since 1993	since 1993	since 1993
registration tests, approval status	stop ≈1988	stop 1987	stop 2000	tests since 1993	Registration 2011*
company	Rohm & Haas	Shell/Nickerson	HybriTech	Hybrinova	SUR
hybrid wheat breeding	from 1976 to 19??	from 1980 to 19??	from 1985 to 2000	from 1992 to 2002	since 2000
hybrid seed and sales	no	no	from 1994 to 2000	from 1994 to 2002	since 2000
Acquisition of plant variety rights			→ Nordsaat	→ Nordsaat	→ SUR

\* EU registration, national approval for use (e.g. in Germany from 2017 to 2022 and longer)

**Table 2:** Key companies and contributors in hybrid wheat breeding and seed production (Nordsaat took over business activities of Hybri tech and Hybrinova after their programs were stopped).

Company	Hybrid breeding	Breeders	F <sub>1</sub> seed marketing	CHA	Managing directors
SU Recherches	since 2000	Volker Lein	2000 - today	Croisor®	W. v. Rhade
SU France		2000 - 2015			G. de Castelbajac
Nordsaat	since 1985	Ralf Schachschneider		Genesis®/ Croisor®	C.-O. v. Rhade
		1986 - 2015			W. v. Rhade
Hybritech	1985 - 2000	Olivier Laudoyer	1994-2000	Genesis	
(Monsanto, Coop de Pau)		1985 - 1997	→ Nordsaat		
Hybrinova	1992 - 2002	?	1994-2002	Croisor	A. Gervais
(DuPont)		?	→ Nordsaat		

Hybrinova and the gametocide Croisor®, was active in breeding and marketing hybrid wheat in France until 2002. But then, Monsanto and DuPont gave up their hybrid wheat activities. The plant variety rights passed over to Nordsaat Saat-zucht GmbH, which, in turn, organized F<sub>1</sub> seed production and distribution in French companies (Table 2). The company Saaten Union Recherche (SUR) took over the gametocide Croisor® in 2005. SUR succeeded in registration of the active substance Sintofen in the EU in 2011 and until 2017, the permission to use Croisor® not only in France, but also in other countries. This is the temporary end of a ‘never ending story’!

### Hybrid breeding and variety development

The CMS projects (about 1965 to 1985; Keydel 28 Oct 1981, pers. commun.) involved numerous breeders, including Nordsaat. Nordsaat committed itself still 1991 to 1993 for the CMS-program of the ex-GDR (Merfert *et al.* 1988, 1991). For several reasons no CMS-based hybrid wheat varieties have been yet launched in Europe.

At the beginning of the development, numerous breeders were involved in gametocide-based hybrid breeding through cooperations with chemical and breeding companies. This also resulted in the conference proceedings „Hybridgetreide“ (BAL Gumpenstein 1985). After the company Nickerson ended the registration procedure for its gametocide in 1987, almost all German wheat breeders stopped their hybrid wheat breeding programs.

Since the mid 1980s, the cooperation (contracted in 1988) developed between the companies Nordsaat and HybriTech and the successful joint venture between Monsanto and Coop de Pau. This was mainly due to the personal relationship between the breeders of Nordsaat and HybriTech (Olivier Laudoyer), by the interests of the breeder of Nordsaat and by the far-sighted willingness of the Nordsaat management to finance a breeding ‘playground’. All

quickly developed into a standalone hybrid breeding program in which CHA has been used since 1987. This program was supported by the successful and inspiring HybriTech cooperation (from 1989 to 2000). This was truly significant due to  
(i) the participation of 15 European breeders,  
(ii) the exchange of breeding material (130 to 250 lines, 530 to 950 hybrids),  
(iii) the annual research meetings at the partner companies,  
(iv) the helpful common database HYBRIBASE.

The cooperation was driven forward until 1997 mainly by Olivier Laudoyer. At the 48th annual meeting in Gumpenstein, Laudoyer (1997) and Schachschneider (1997) reported on the results of the practical hybrid wheat breeding.

In 1993, a decisive breakthrough was achieved and a true success story was initiated: the Nordsaat variety ‘Piko’ was discovered as the best pollinator (‘father’) for hybrid wheat breeding. With ‘Piko’, the highest heterosis (8 to 17%) and also the best pollination in commercial seed production was achieved. All eight hybrid varieties that were officially tested in 1997 in Western Europe included ‘Piko’ as male component. With ‘Hybnos 1’, the first hybrid variety was registered in Germany in 1999. As a father of eleven registered varieties so far and with a market share of 30%, ‘Piko’ has become the economically most significant male component to date (Table 3).

With the success of ‘Piko’ and the resulting optimism, some risky decisions of Nordsaat that followed can be explained. They include especially the takeover of the business activities of Monsanto (2000) and DuPont (2002) and the establishment of the companies Saaten Union Recherche and Saaten Union France for the breeding and marketing of hybrid wheat (Tables 1 and 2).

Since the beginning of the 2000s, successful hybrid wheat breeding and seed production has been established at SUR and the cooperation SUR-Nordsaat has been developed.



**Table 3:** Frequency of varieties/breeding lines used as male partners in hybrid varieties and market share of these hybrids (Sales: 1 unit = 500 000 grains).

Male <sup>1</sup>	Hybrid varieties (n)	Sales 1994-2014	Market share (%)
Piko	8	2 057 011	29.6
Apache	2	1 598 377	23.0
TP.506	4	1 156 177	16.7
Genial	3	412 605	5.9
Euro.88-06	2	367 773	5.3
Rival	3	257 151	3.7
Louvre	2	243 023	3.5
SUR.99934	2	236 304	3.4
Estica	1	111 563	1.6
Transit	1	86 657	1.2
Euro.88-06	1	83 550	1.2
Toni	1	80 136	1.2
Renan	1	71 082	1.0
Sum		6 941 549	100.0

<sup>1</sup> Alicante, Arlequin, Audace, CF.99007, EM.301, M.310, NH535, SUR.186 and SUR.99820 were used as male parents in hybrid varieties with less than 1% market share

In summary, it can be stated that until today, only a few companies and players have been involved in the development of commercial hybrid wheat (Tables 1 and 2). It is absolutely necessary to mention O. Laudoyer, whose enthusiasm for the subject continues even 20 years after his retirement. The early and far-sighted decisions of the Nordsaat management have made the described developments possible. It was of the utmost importance that the gametocide Croisor® was officially approved for hybrid seed production after more than 25 years of testing.

#### Development of practical hybrid breeding

How has practical hybrid breeding developed over the decades? What was the main driving factor? In the following, some meaningful examples are provided. Reference is also made to papers and publications by the author reporting on practical hybrid wheat breeding.

#### Practice of variety development

F<sub>1</sub> experimental hybrids are produced in numerous crossing blocks, each combining a specific father with a certain number of mothers. For this purpose, the mother plots will be sown next to the father stripes. The mothers are (male-)sterilized at tillering/

heading by spraying the gametocide. Thus, the pollen of the free-flowering, fertile father can pollinate the mother plots.

From very simple beginnings, SUR and Nordsaat have developed ambitious hybrid wheat breeding programs with increasing output of varieties. This is shown by the size of the Nordsaat breeding program since 1987 (Table 4) as well as by the SUR and Nordsaat programs in 2017 (Figure 1).

The methodical, organizational and technical improvement of the practical hybrid breeding and the increase of its efficiency (reduction of costs on duration of breeding period) were continuously evolved. Realistically, breeding of CHA hybrid varieties takes about four years longer than breeding line varieties; however, it is significantly shorter than the breeding of CMS hybrids.

For the breeding of competitive hybrid varieties, parent components with high *per se* performance and combining ability as well as a good pollination ability of the father are needed. Such lines are developed as a 'spin-off' of line breeding. The criteria/parameters for the selection of lines and their screening for suitability for hybrid breeding are still largely based on the experience of the breeder. Molecular tools validated by practical selection are not yet available.

The development of male components with high pollination performance in F<sub>1</sub> seed production has top priority. Only 2-7% of inbred lines have the potential for adequate pollination performance. This can only be reliably tested in costly crossing blocks. All indirect methods cannot adequately describe this characteristic.

Through cooperation between the breeding companies, the availability of parent components can / should be further improved.

In the beginning years, the gametocide application was carried out on the mother plots of crossing blocks still with a portable 'backpack sprayer'. Thus, only a few experimental hybrids could be produced. The selection options were correspondingly low. Over the decades, several gametocid sprayers 'built it yourself' have gradually evolved into GPS-controlled high-tech devices that can individually control and treat thousands of plots each day when the application date is reached. Thus, experimental hybrids can be produced in sufficient numbers, taken into evaluation and strongly selected.

The production of F<sub>1</sub> hybrid seeds for the market takes place in strip cultivation, where the stripes of father and mother are alter-

**Table 4:** Key figures of the hybrid wheat breeding program of Nordsaat (NL-2, NL-1: national list (VCU) trials minus two and one year, respectively; CB: crossing block = experimental design to produce hybrid seed; pilot production: number of F<sub>1</sub> productions for probable entry in official VCU trials; seed production: number of F<sub>1</sub> productions for varieties in official VCU trials).

Source	2012	2010	2005	2000	1995	1990	1987
Hybrids in NL-2	2367	509	490	218	123	97	
Hybrids in NL-1	94	48					
Hybrids in NL1	4	1					
Female lines in CBs and seed production	268	148	283	164	48	21	40
Males lines in CBs and seed production	86	64	42	13	22	33	2
Experimental F <sub>1</sub> hybrids seed production in CBs	2158	2160	1876	643	300	300	80
Pilot production	79	48	25	20	6		
Seed production	4	2	2	2			
Screening of inbred lines for use as parental lines in CBs	2000	500	2000	1500	1500		



**Figure 1:** Breeding and screening locations of the Nordsaat-SUR-SU France hybrid wheat program. Over the two breeding programs more than 1200 parental lines are tested each year in about 6000 combinations in crossing blocks from which 2000 are going forward into screening; 80 of them are kept for possible testing in NL-1 trials. Currently 12 new hybrids, 6 each from Nordsaat and SUR, are tested in National List (NL) trials for their registration (Source: SUR, Saaten Union Recherches).

natively allocated. The gametocide application on the mother strips is carried out with appropriately adapted, GPS controlled field sprayers. For decades, this strip cultivation has been the topic of much discussion and they have been repeated calls for mixed cultivation (as in hybrid rye). However, there are no developments in this direction yet.

The profitability of all hybrid wheat activities depends largely on the price of  $F_1$  seed production and that of  $F_1$  seed yield on the female-parent strip. This should amount to at least 40% of the grain yield of the untreated father's stripe. With pollinators like 'Piko', 85% can be achieved.

Since GPS technology has found its way into almost all stages of plant production and gametocide application, hybrid seed can also be produced with higher productivity and higher quality.

The chances of selection on the grain yield of hybrid wheat varieties have been discussed from time to time under different aspects. Since 1988, Nordsaat has been conducted yield tests on  $F_1$  hybrids and inbred lines as well as partially on  $F_2$  seeds. The most extensive experimental analyses were carried out on the basis of the data from the harvest years 2009 and 2012. In the following, a brief report of the results from 2012 is provided:

Figure 2 shows the results from NL-2 trials (2 years before the official national list trials). This evaluation included 2800 lines and 1200 experimental hybrids. Figure 3 shows the results from NL-1 trials (1 year before the official national list trials). In this evaluation, 126 inbred lines and 100 experimental hybrids were included. The results are very encouraging. They show the considerable selection potential that exists in a sufficiently large number of "well" planned experimental hybrids.

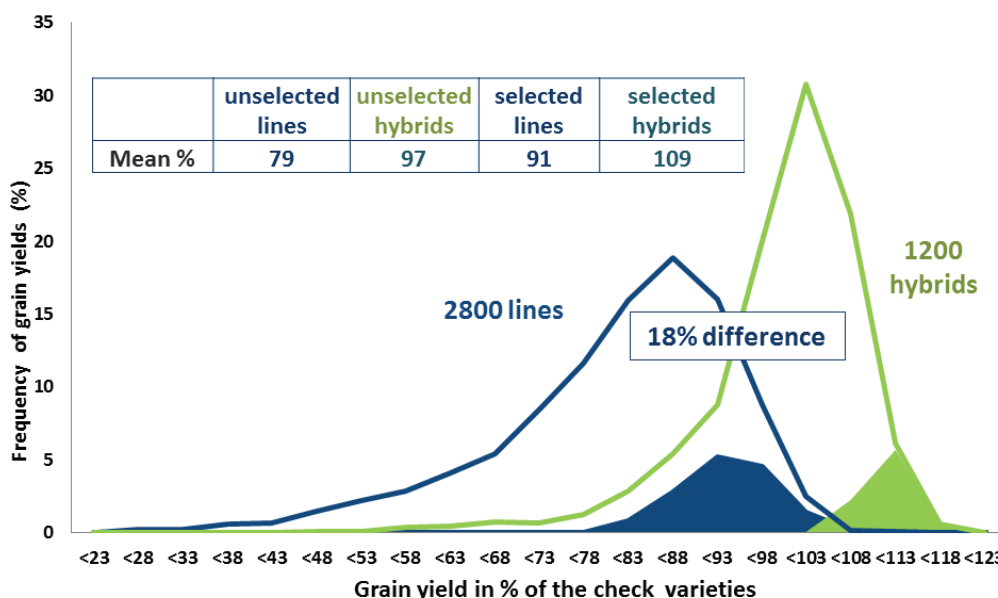
### Important research projects

Since 1995, numerous and sometimes very complex research projects have been carried out. Nevertheless, the practical hybrid wheat breeding has largely evolved through experience and technical optimization, but not with impulses from basic scientific research.

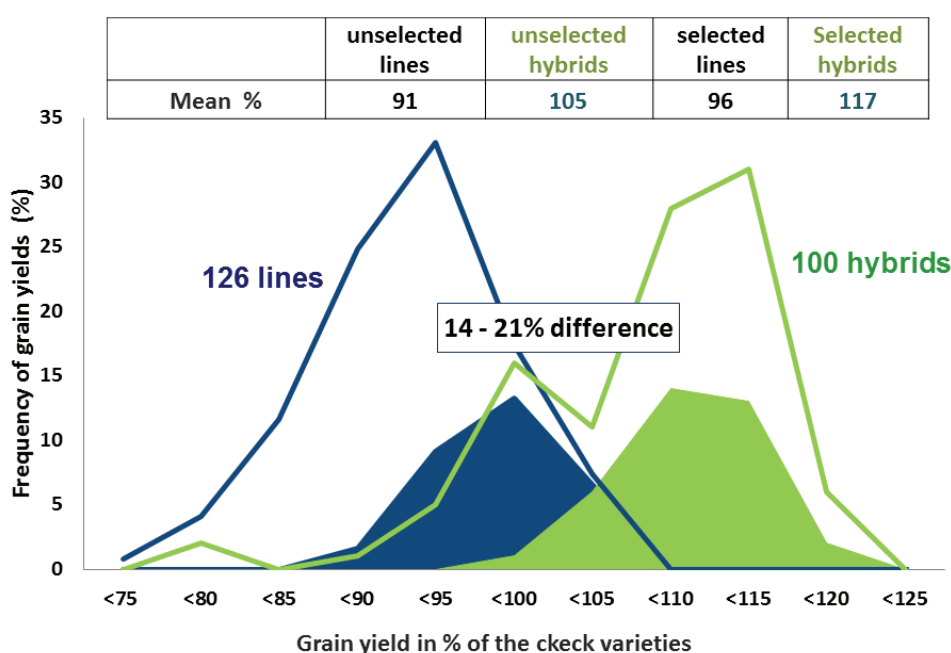
### Hybrid wheat varieties, market and value characteristics

#### Hybrid wheat varieties and market

The following information is based on figures from SUR. Hybrid varieties that entered the market have been authorized in Europe since 1994 (Table 5). By 2016, there were 74 varieties, with 37 from the group Saaten-Union. The seeds of all varieties are cur-



**Figure 2:** Frequency distribution of grain yield of winter wheat lines in NL-2 (2 years before National List trials) in 2012. The distribution curves show results before selection, the colored areas after selection, especially on grain yield. The mean grain yield of selected hybrids is 18% higher than the mean grain yield of selected inbred lines.



**Figure 3:** Frequency distribution of grain yields of winter wheat lines in NL-1 (1 year before National List trials) in 2012. The continuous distribution curves show the result before selection and colored areas after selection, especially on grain yield. The grain yield of the selected hybrids (110-117%) is 14-21% above the mean grain yield of the selected inbred lines.

For several years, large, publicly funded collaborative projects between scientists and breeders have focused on hybrid wheat breeding research (HYWHEAT 2011-2014; ZUCHTWERT 2014-2019). A central goal remains the prediction of the suitability of parent components as well as the breeding value of technically possible hybrid combinations, for the benefit to optimize the planning of the crossing blocks and to obtain a higher frequency of high-performing hybrids.

#### Discussions of ethical issues

Several questions were occasionally discussed in small circles. They include such as:

- (i) is it socially responsible action to practice hybrid breeding? Will farmers become dependent on multinational companies?
- (ii) will line breeding be jeopardized in favor of hybrid breeding? Does this reduce its competitiveness?
- (iii) are there any efforts to develop so-called 'terminator genes' and to use them in hybrids?

**Table 5:** Registered hybrid wheat varieties in Europe (EU Common Plant Variety Database)

Period	Breeding/applying companies	Varieties (n)	Production/marketing/sales companies
1999 - 2016	SaatenUnion/Nordsaat	37	Saaten Union (SU)
1994 - 2001	HybriTech (Monsanto)	7	HybriTech → SU since 2001
1994 - 2002	Hybrinova (Dupont)	21	Hybrinova → SU since 2003
2002 - 2016	Limagrain	5	SU & Limagrain
2002	Sejet	1	SU & Sejet
≈1990	INRA + Shell	3	-
1994 - 2016	Total	74	

**Table 6:** Frequency of female and male lines in relation to the number of registered hybrid wheat varieties

Partner line frequency in n hybrid varieties	Female lines		Male lines	
	n	%	n	%
1	39	75	16	46
2	9	17	13	37
3	3	3	2	6
4	1	1	3	9
11			1 (Piko)	3
Total	52		35	

**Table 7:** Frequency of varieties/breeding lines used as female partners in hybrid varieties and market share of these hybrids (Sales: 1 unit = 500 000 grains).

Female <sup>1</sup>	Hybrid varieties (n)	Sales 1994-2014	Market share (%)
QH.529	1	1 596 330	23.3
Isengrain	1	924 610	13.5
Tilburi	1	630 681	9.2
Eole	2	578 906	8.4
Euro.97-15	1	379 758	5.5
Trintella	1	327 455	4.8
Malabar	2	320 997	4.7
MH.98-16	1	263 128	3.8
Phobos	1	241 205	3.5
CF.99007	2	202 973	3.0
VM.517	1	200 304	2.9
SUR.99820	2	199 251	2.9
Soisson	2	195 222	2.8
Audace	3	156 569	2.3
Buenno	2	104 531	1.5
SUR.2001-166	2	98 694	1.4
Picard	1	86 657	1.3
Euro.91-10	1	83 550	1.2
F.078	1	80 136	1.2
Sum		6 857 999	100.0

<sup>1</sup> Adhoc, AJ536, BA701, Grisby, RE001, RE.04022, RE.204, Shango, SUR.748, TM.007, TP.538 and Virtuoso were used as female parents in hybrid varieties with less than 1% market share

rently produced and marketed by Saaten Union. There are business-agreements with the company Limagrain.

Every breeder has an economic interest in using parental components as often as possible in hybrid varieties. The evaluation shows (Table 6) that a total of 52 different mothers and 35 different fathers in hybrid varieties were used. 75% of mothers and 46% of fathers were used in a single hybrid variety. The mothers were rarely used in several varieties. Only one mother appeared in four hybrid varieties. Three fathers appeared in each four hybrids. Notably, the variety 'Piko' was used in eleven hybrid varieties.

From the marketing figures of the hybrids and knowledge of the parents, the market shares of the parent components can be calculated. The three best mothers (Table 7) achieved a market share of 46%. Many mothers had less than 1% market share. The three best fathers (Table 3) had a market share of 69%. Among the fathers, 'Piko' and 'Apache' lead the ranking of varieties with 30% and 23%, respectively, followed by some other varieties, which also contribute to sales. Some fathers had less than 1%. The numbers show that particularly the 'best' fathers have great economic potential. However, the development of such fathers is much more challenging.

Table 8 shows that the market for hybrid wheat has developed mainly in France. So far, 80% of total hybrid seed production has been sold there, consisting of 38 hybrids. After France, Germany, Hungary and the Czech Republic were important markets. The developments of international markets are, however, dynamic, therefore, the figures are only a snapshot.

With the beginning of the variety approvals from the end of the 1990s, (national and European) marketing strategies were developed. Public relations and marketing activities required attention and possibly considerable time commitment of the breeders.

#### **Value of Cultivation and Use**

The economic success of a variety depends not only on 'marketing', but above all on the 'Value of Cultivation and Use' (VCU). This terminus includes numerous value properties and is usually subdivided into the complexes grain yield, agronomy, quality and resistance.

#### **Baking quality marketing**

The hybrid varieties were VCU tested in Germany and after successful release included in the German list of cultivated varieties. However, this was accompanied by a 'blemish' that ultimately limited marketing in Germany.



**Table 8:** Seed sales of hybrid wheat varieties in European countries (Sales: 1 unit = 500 000 grains; Source: Saaten Union Recherches).

Country	1994-1999	2000-2004	2005-2009	2010-2014	1994-2014	Share (%)	Varieties (n)
France	791 580	1 288 241	1 343 859	2 060 968	5 484 647	80.0	38
Germany		343 644	10 572	161 112	815 328	11.9	27
Hungary			9 411	180 050	189 461	2.8	17
Italy			2 295	111 704	113 999	1.7	9
Czech Republic			14 823	51 533	66 356	1.0	16
United Kingdom		35 415	1 407	9 349	56 171	0.8	12
Slovakia				37 846	37 846	0.6	11
Denmark			26 468	7 415	33 883	0.5	6
Latvia				14 477	14 477	0.2	4
Portugal		1 203	3 825	7 751	12 779	0.2	7
Poland			765	8 929	9 694	0.1	11
Belgium		3 255	1 176	5 116	9 547	0.1	7
Switzerland		339	1 992	6 978	9 309	0.1	9
Ireland		1 620			1 620	0.0	5
Romania				1 380	1 380	0.0	6
Slovenia				805	805	0.0	2
Austria			132	183	315	0.0	3
Finland				165	165	0.0	2
Bulgaria				162	162	0.0	1
Spain				60	60	0.0	2
Total	791 580	1 673 717	1 716 725	2 675 978	6 857 999	100.0	

When classifying the baking quality, our hybrid wheat initially achieved only the official predicate ‘feed wheat’, *i.e.* ‘C quality’ (Table 9). This was caused by the slightly lower crude protein content in the official tests. In these tests a uniform nitrogen fertilization is carried out. Varieties with a very high grain yield are thus disadvantaged in the official procedure because they cause a protein dilution. For ‘Hybnos 1’ and ‘Hybnos 2’, the classification as B wheat (bread quality) was missed only 0.3% crude protein. However, this phenomenon did not play a role in practical cultivation, because due to variety-specific treatments mostly good B quality was produced. This was also the positive experience of many farmers with such hybrids. Nevertheless, because of their official classification as C wheat, they often had to accept price deductions. Thus, the marketing of hybrid wheat, especially in Germany, had clear limits.

Only 12 years after the registration of ‘Hybnos 1’, in connection with the official registration of the inbred line ‘Tobak’, it became clear (Table 9) that very high grain yields should not be punished by deductions in the official quality classification. For bread wheat, the expression level 2 in crude protein content has now also been accepted. Thereafter, the classifications of all registered varieties were corrected, if necessary. According to the ‘Descriptive list of varieties’ in 2012, all hybrids were also ‘bread wheat’, that means, they had ‘B quality’. Obviously, the marketing of hybrids from 1999 onwards would have been significantly better and their market share would have been higher today if they had already been initially classified as ‘bread wheat’.

#### **Biological and socioeconomic benefits of hybrid**

The value of cultivation and use of the hybrid varieties have been analyzed over the years in numerous official and private experiments, including complex multi-factorial field trials at numerous sites. Therefore, hundreds of results are available. At many locations, maximum yields were achieved with hybrid varieties. There is data from millers, bakers, brewers, from the starch and animal feed industry. Added to this are business analyses and customer surveys. In general, they show that even today's hybrid varieties often have advantages over the line varieties, which initially result from their biological and socio-economic benefits. The biological advantages are:

- (1) Higher grain yield, especially under stress conditions,
- (2) ‘Heterosis of vegetative life processes’:
  - (i) faster growth in autumn and spring,
  - (ii) much deeper rooting of the plough land, thus, greater use of the water resources of the soil,
  - (iii) weaker wilting symptoms; tolerance to water stress and heat,
  - (iv) longer duration of function of the photosynthetic biomass after flowering, thus, resulting in a prolonged grain filling period,
  - (v) slower growth of fungal infection; better field resistance due to heterozygous basis of resistance.

The socio-economic benefits exist from the point of view of

- (i) agriculture in the improvement of the value of cultivation and use, above all the grain yield, the energy yield and the stress tolerance;

**Table 9:** Baking quality classification (of hybrid (H) and line (L) varieties according to the German descriptive variety list (*BSL, Beschreibende Sortenliste*) based on crude protein content (PROT). QG, quality group: E, 'improver wheat' (*Eliteweizen*), A, 'quality wheat' (*Qualitätsweizen*), B, 'bread wheat' (*Brotweizen*), C, 'feed wheat' (*Sonstiger Weizen*).

Variety	Type	BSL at registration			Underrating (years)	BSL 2012		BSL 2017	
		Year	PROT	QG		PROT	QG	PROT	QG
Hybnos 1	H	1999	2 <sup>#</sup>	C	12	2	B		
Hybnos 2	H	2001	2 <sup>#</sup>	C	11	2	B		
Hybred	H	2003	4	B			B		
Hycory	H	2007	5	B			B		
Hyland	H	2009	2 <sup>#</sup>	C	3	2	B	2	B
Amplly	H	2002	2 <sup>#</sup>	C	10	2	B		
HYFI	H	2016	4	B				4	B
Hyvento	H	2016	4	A				4	A
LG Alpha	H	2016	2	C				2	C
Tobak	L	2011	2 <sup>§</sup>	B		2	B	2	B
Minimum score for PROT for QG 'B'			3			2			

<sup>#</sup> classification as 'bread wheat' (QG B) was missed by 0.3% PROT; <sup>§</sup> missed the minimum requirements for PROT for QG B in variety tests due to very high grain yield at uniform N fertilization levels

- (ii) global development in increased food production;
- (iii) breeding in the protection of intellectual property and return of investment;
- (iv) environment and sustainable development in the increased efficiency of inputs and energy used and in adaptation to climate change.

Hybrid wheat is still a niche market today. This can change with a new generation of hybrid varieties.

## Outlook

What is needed to successfully continue the practical breeding of hybrid wheat?

- (1) Long-term use of the 'no-alternative' gametocide Croisor® in F<sub>1</sub> seed production. There is a political and administrative responsibility to extend the approval of the Croisor® and its components.
- (2) Powerful (GPS-controlled) technology for breeding fields, in order to be able to process a very high number of plots at comparatively low cost per unit.
- (3) Access to the best strains of line breeding, also through cooperation between the breeders.
- (4) Further optimization of the selection of genetic components from line breeding, especially through experience ('breeder's view') and useful molecular tools.
- (5) Systematic development of pools for male and female parent components.
- (6) Planning of crossing blocks using practically usable, reliable methods for evaluating potential hybrid combinations (e.g. ZUCHTWERT project).

## Note

The author has been involved in the development of hybrid wheat breeding and development of hybrid wheat varieties since the mid 1980s as wheat breeder and head of breeding of the company Nordsaat. Unless reference is made to other authors and sources, all information in this report is, therefore, supported by extensive documents as well as own projects, evaluations and experiences.

## Acknowledgements

My special thanks go to my colleagues in hybrid wheat breeding Olivier Laudoyer and Volker Lein. Through their dedicated work, even in difficult phases, they have given hybrid wheat breeding important impulses. I also thank the management of Nordsaat Saatzzucht GmbH for the continuous support and financing of this difficult breeding project.

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## Status quo and prospect of hybrid breeding in wheat

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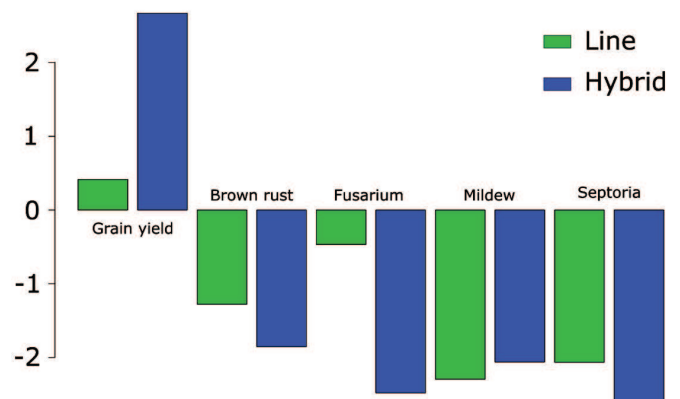
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Commercial heterosis for grain yield is present in hybrid wheat but long-term competitiveness of hybrid versus line breeding depends on the development of heterotic groups to improve hybrid prediction. Further, yield stability, nutrition use efficiency and resistance traits are important selection criteria and criteria for the evaluation of the performance of lines and hybrids. Detailed knowledge of the amount of heterosis and quantitative genetic parameters are of paramount importance to assess the potential of hybrid breeding. Our objectives were to (1) examine the extent of midparent, better-parent and commercial heterosis in a vast population of 1604 wheat (*Triticum aestivum* L.) hybrids and their parental elite inbred lines and (2) to compare quality related parameters such as resistance traits and nutrition use efficiency.

Fifteen male lines were crossed in a factorial mating design with 120 female lines, resulting in 1604 of the 1800 potential single-cross hybrid combinations in the framework of the HYWHEAT project. Additionally we used data from the ZUCHTWERT project, where 41 male lines were crossed with 200 female lines also in a incomplete factorial mating design, resulting in 1815 hybrids. The hybrids, their parents, and ten/eleven commercial wheat varieties were evaluated in multi-location field experiments for grain yield, plant height, heading time and susceptibility to frost, lodging, *Septoria tritici* blotch, yellow rust, leaf rust, and powdery mildew at up to five locations in HYWHEAT and up to 12 locations in ZUCHTWERT.

We observed that hybrids were superior to the mean of their parents for grain yield (10.7%), show in tendency a higher nutrient use efficiency and comparable resistance traits to the lines (Figure 1). Moreover, 69 hybrids significantly ( $p < 0.05$ ) outyielded the best commercial inbred line variety of HYWHEAT underlining the potential of hybrid wheat breeding. The estimated quantitative genetic parameters suggest that the establishment of reciprocal recurrent selection programs is pivotal for a successful long-term hybrid wheat breeding.



**Figure 1:** Comparison of line and hybrid performance for grain yield and four disease resistance traits using the ZUCHTWERT data set. Traits are standardized; values close to zero indicate healthier plants for the disease resistance traits.

### Keywords

Disease resistance · grain yield · heterosis · *Triticum aestivum*





## Epistatic interactions and distribution of the fertility-restoring locus *Rf1* in common wheat

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Hybrid wheat breeding is a promising method to increase wheat productivity. Currently, hybrid wheat seed production in Europe is accomplished by chemical hybridising agents (CHAs). As the success of CHAs depends on the treated genotype, the time of application and environmental conditions, genetic hybridising mechanisms such as cytoplasmic male sterility based on the plasmon of *Triticum timopheevii* are promising alternatives. Although several fertility-restoring loci were identified for this cytoplasm, fertility restoration is often not stable. Previous studies suggested stacking of major restorer loci as a solution to overcome this barrier. A suitable locus for this approach is the restorer locus *Rf1*. However, molecular markers for the selection of *Rf1* were unavailable, and previous studies were inconsistent regarding epistatic interactions. Furthermore, it was not clear whether this locus is exclusively derived from *T. timopheevii*. In the present study, we mapped *Rf1* in three populations using quantitative trait locus (QTL) analysis and analysed the target region in lines of *T. timopheevii* and common wheat.

Three BC<sub>1</sub> mapping populations, comprising 197, 201 and 230 individuals, were developed using the recurrent parent CMS-Sperber and the restorer accessions R3, R113 and L19, respectively. We further analysed 507 winter wheat breeding lines and nine accessions of *T. timopheevii*. Fertility restoration was determined under greenhouse conditions by isolating the spikes of the three populations using glassine bags. The seed set was defined as the number of kernels divided by the number of spikelets. The plant material was genotyped with a 15K + 5K Infinium® iSelect® single nucleotide polymorphism (SNP) array. Linkage maps were constructed using JoinMap® 4.0. QTL analyses of the seed set were performed using the R package R/qtl and the two-part model. Effects of QTL and possible interactions were estimated using the Haley-Knott regression method. Markers located in the *Rf1* support interval were used for the analysis of the target region in common wheat and *T. timopheevii*. Hierarchical cluster analysis was performed using the complete linkage method.

In the population derived from R3, the number of sterile plants exceeded the number of fertile individuals, indicating incomplete penetrance of *Rf1*. In the populations involving R113 and L19, the segregation of fertility restoration did not deviate from the patterns assumed for a digenic and a monogenic model, respectively. In the populations derived from R3 and L19, a one-dimensional scan detected *Rf1* on chromosome 1AS and a modifi-

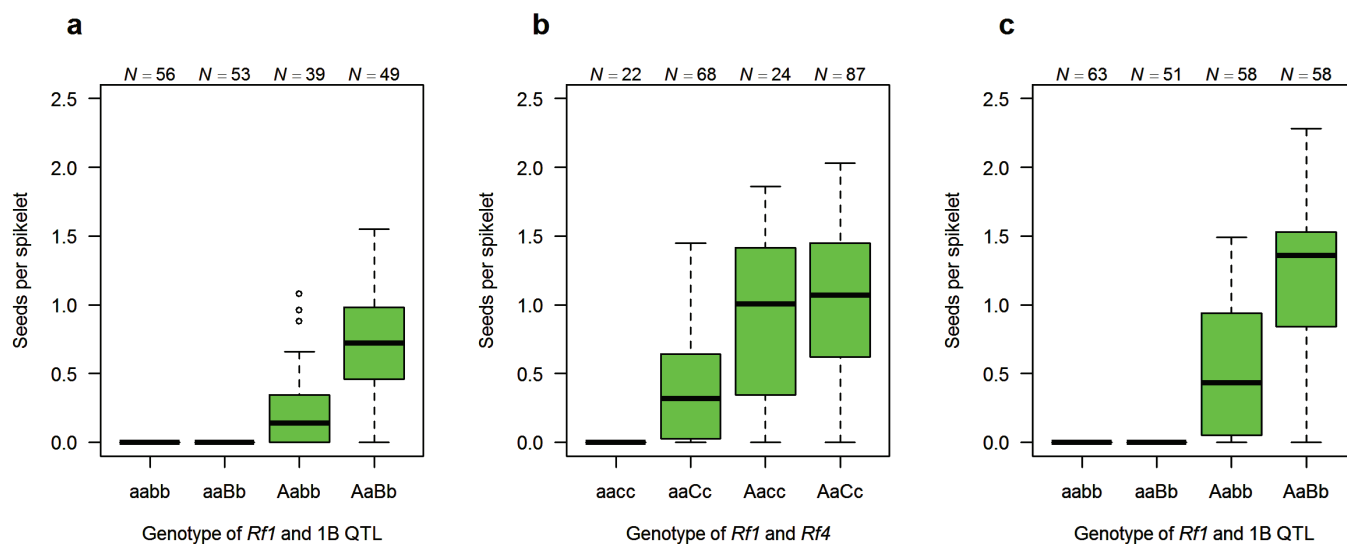
er locus on chromosome 1BS. In both populations, the modifier locus affected only the quantitative phenotype of the fertile individuals. According to linear models considering the two loci and their significant interaction, the modifier alleles derived from R3 and L19 had an additive effect of 0.51 and 0.72 seeds per spikelet. In the population involving R113, we detected *Rf1* and a QTL on chromosome 6B, probably representing the restorer locus *Rf4*. Considering the significant interaction between the two restorer loci, *Rf1* had an additive effect of 0.90 or 0.60 seeds per spikelet, depending on the homozygosity or heterozygosity of *Rf4*. Whereas *Rf1* showed an expressivity higher than that of *Rf4*, the effects of the two restorer loci were not additive (Figure 1). The QTL analyses revealed 17 SNP markers common to any of the *Rf1* support intervals. Minor allele frequencies of these markers in the common wheat lines ranged from 0.19 to 0.49. Hierarchical cluster analysis of the marker haplotypes demonstrated that the R3 haplotype was identical to those of seven *T. timopheevii* accessions. Furthermore, the haplotypes of R113 and L19 were more similar to the *T. timopheevii* haplotypes than to the common wheat haplotypes, indicating that *Rf1* may be derived exclusively from *T. timopheevii*. The present study demonstrates the importance of epistatic interactions between fertility-restoring loci and modifier loci. Identified SNP markers can facilitate the exploitation of *Rf1* and the modifier locus on chromosome 1BS in hybrid breeding programs.

### Keywords

Cytoplasmic male sterility · hybrid wheat · *Triticum timopheevii*

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**Figure 1:** Seed set of crosses involving R3 (a), R113 (b) and L19 (c) ordered by genotype classes. The letters A, B and C at the bottom of the figure represent *Rf1*, the modifier locus on chromosome 1BS and *Rf4*, respectively. Lower case and capital letters refer to the alleles of ‘Sperber’ and the restorer parents, respectively.

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## Hybrid barley - the journey to change an inbred into a hybrid crop

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Hybrids in field crops show increased performance over inbred lines and heterosis potential in many crops. Following the development of corn as the first major agricultural hybrid crop, hybrids were developed in different species with varying success. A prominent example in cereals is the success of rye where yields boosted from around 35 dt in the 1980s to 60 dt in the 2000s. Critical factors for such success have been genome structure, flower biology, availability of a suitable pollination control and the economics of seed production. Those requirements were investigated for barley by the Syngenta researchers. Genome structure in barley is diploid without major duplications. In barley a stable *cms* system with monogenic restoration has been described already in the 1970s by Ahokas (1978). Looking at the opportunities, six-rowed winter barley turned out to be the best target for hybridization due to outcrossing ability yield potential and growing acreage. The main constraint for developing barley hybrids on a commercial scale turned out to be the reliability of seed production. In winter barley we could identify genotypes with the required flower biology to allow efficient cross pollination. This was the starting point of the development of an interplanting seed production system which made hybrid seed production commercially viable. Recent investigations led to the cloning of the restorer gene in the hybrid system (Rizolatti *et al.* 2017). After starting a first backcrossing program in the early 1990s the breeders developed the parental material and in 2000 the first two hybrids were entered into UK national list trials. Main achievements in the program were the development of distinct gene pools, implementation of molecular markers in purity as well as for tracking the sterility factors and a consistent on farm yield advantage of more than 0.5 t/ha in general. Detailed analysis showed mid parent heterosis in small plot trials averaged 11.3% (Mühleisen *et al.* 2013) as well as an increased yield stability (Mühleisen *et al.* 2014). Specific growing protocols developed for hybrid barley supported yield advantage and stability. Today hybrids are grown in many countries across Europe. The biggest acreage is reached in France with approx 130 000 ha, Germany 115 000 ha and UK with 95 000 ha. The potential for further commercial development is expected to be much higher than the current figures.

### Keywords

Cytoplasmic male sterility · heterosis · *Hordeum vulgare*

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## Current challenges of barley hybrid breeding

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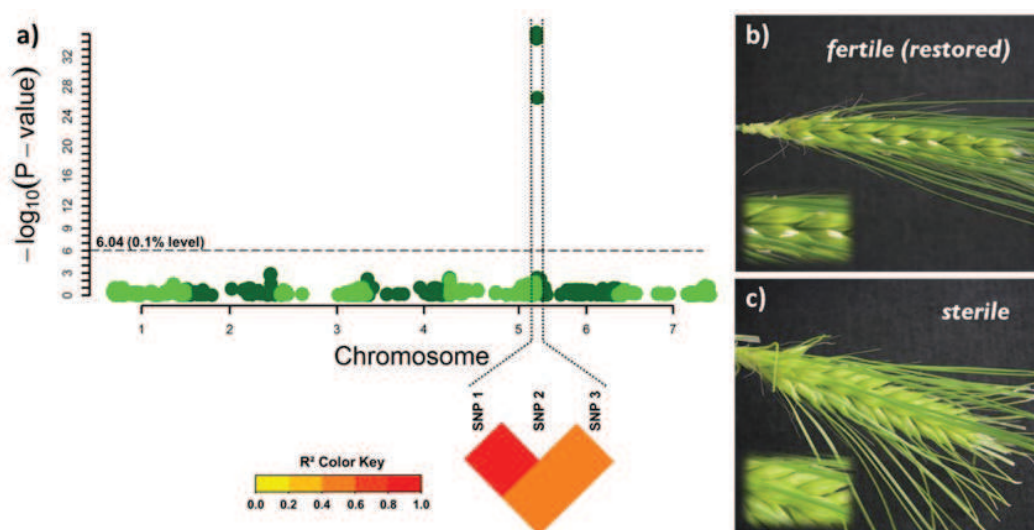
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Winter barley (*Hordeum vulgare* L.) is the third most important crop in Germany, mainly used for animal fodder. As an auto-gamous cereal, the majority of registered barley varieties are true breeding inbred lines. However, breeding efforts are today tending towards the incorporation of hybrid varieties, which combine higher yields with a better yield stability and stress tolerance. In our study, we tested a factorial cross of 96 test hybrids (8 males × 12 females) and their parental lines for grain yield (8 environments, 1 replication) and dry matter yield performance (4 environments, 2 replications). In addition to these major yield traits, we also recorded plant height, number of spikes per m<sup>2</sup>, and dry matter content as dry matter yield affecting traits and hectoliter weight, thousand grain weight, number of grains per spike, and number of spikes per m<sup>2</sup> as grain yield affecting traits.

We found that both grain yield and dry matter yield show mid-parent heterosis (MPH) of 9.19% and 11.69%, respectively. Due to similar results for hectoliter weight, thousand grain weight and spikes per m<sup>2</sup>, the higher grain yield of hybrids compared to parental lines seems to be based on higher numbers of grains per spike (MPH: 9.65%). In contrast, the higher dry matter yield performance seems to be due to a higher whole plant productivity of hybrids compared to their parental lines, since hybrids were 7.63% taller and showed 14.53% more spikes per m<sup>2</sup>.

We further investigated the predictability of heterosis and hybrid performance and found that neither the parental performance nor the general combining ability (GCA) are adequately correlated with these traits. This suggests that hybrid and line breeding programs have to be planned and conducted separately, which might decrease the impact of dominance effects (specific combining ability, SCA) and thereby increase the effect of additive effects (GCA). As we found in our data, a certain genetic distance between males and females is already existing. However, the genetic distances between the individual lines within the respective pools are only slightly smaller, suggesting an insufficient pool development. Due to the known correlation between the parental genetic distance and the amount of heterosis, one major aim of current hybrid barley breeding is the development of more distinct parental pools leading to increased heterotic effects and performance of winter barley hybrids.

For an effective and cost-efficient seed production of hybrid barley a cytoplasmic male sterility (CMS) system is needed to ensure high hybridity. The common CMS system (*msm1*; *Rfm1*) found in *H. spontaneum* is known to be temperature-dependent and photo-sensitive, which currently represents the second major challenge for the production of homogeneous hybrid seeds. To investigate the impact of elevated temperature on undesired fertility restora-



**Figure 1:** Manhattan plot describing marker trait associations for undesired fertility restoration in absence of the known *Rfm1* restorer gene. The dashed line indicates the Bonferroni corrected 0.05 threshold level (a). Unstable (restored) CMS mother line (b) and a stable (sterile) CMS mother line (c).

tion, we tested three CMS mother lines under two temperature regimes (Control: 16°C, 16h; 12°C, 8h and treatment: 30°C, 16h; 24°C, 8h). Plants were exposed to elevated temperatures at different developmental stages, whereby each treatment comprised one of the three most important stages in pollen development: Pollen mother cell stage, meiosis and tetrad stage, and pollen mitosis. We found, that two of the three CMS mother lines reacted with a significantly higher fertility when exposed to elevated temperatures during spike emergence and flowering. Thus we concluded, that anther and pollen development are somehow reactivated at later stages, since CMS-based sterility induction already takes place during meiosis to tetrad stage. Nevertheless, the complete sterile genotypes principally enable the selection for stable CMS mother lines.

Besides these environmental factors, the genetic background of potential female lines also affects the reversion of sterility, causing seed set in selfed “sterile” mother lines (Figure 1b and 1c). In previous experiments, both a CMS mother line and maintainer dependent effect on the genetic background of this particularly trait could be observed. Investigations using BC<sub>1</sub>S<sub>1</sub> populations, derived from crosses between two CMS mother lines with various maintainer lines, revealed a clear segregation of the CMS instability trait following a 3:1 ratio (fertile:sterile plants) suggesting a monogenic inheritance. The high range of fertility, ranging from very little grain setting to nearly complete fertility, might thereby be due to heterozygote genotypes. We conducted a genome-wide association study (Illumina iSelect 9k SNP array) using the percentage of fertile flowers per spike as phenotypic trait. For this, the mentioned segregating BC<sub>1</sub>S<sub>1</sub> populations were grown under semi-controlled greenhouse conditions and three spikes per plant were selfed to avoid outcrossing. We found three SNPs on the short arm of chromosome 6H to be highly significantly correlated with

the trait of interest (Figure 1a). No other SNPs exceeded the Bonferroni corrected 0.05 threshold level, confirming the expected monogenic inheritance of undesired fertility restoration in absence of the known restorer gene *Rfm1*. As already suggested by the high range of fertility, heterozygous plants showed an on average intermediate fertility of 34% (130 fertile, 3 sterile), while homozygous A-alleles of the first two SNPs combined with the B-allele of the third SNP cause an average fertility of 51% (59 fertile, 1 sterile). In the reverse case, plants showed an average fertility of only 11% (13 fertile, 62 sterile). A marker assisted selection of stable CMS mother lines thus is feasible, facilitating an effective and cost efficient parental pool development, prerequisite for successful hybrid breeding programs.

## Keywords

Combining ability · cytoplasmic male sterility · fertility restoration · *Hordeum vulgare* · temperature · yield

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## Experiences with hybrid barley seed production in Switzerland

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Swiss seed multiplication organizations started hybrid barley seed production in 2013. Their aim was to compensate decreasing seed sales of barley line varieties by reducing the quantity of imported hybrid barley seed. In 2014, Swiss seed companies purchased 223 tons hybrid barley seed from domestic production, whereas in 2017 more than 500 tons of the hybrid varieties 'Hobbit' and 'Wootan' were overtaken. On average, hybrid seed contributed 8.8% to the total barley seed sales over the last four years. Seed quality was comparable to line varieties. Close collaboration, open knowledge exchange and readiness to assume risk within the whole seed production chain were a prerequisite for the realization of the added value in seed sales. A further increase in hybrid seed production is expected.

The production surface of fodder cereals like barley decreased considerably in the last decades due to low financial returns (e.g. 1990: >50 000 ha; Ø 2014-2016: 26 770 ha) and inexpensive fodder cereal imports. Positive results in the official variety trials and breeders' efforts in marketing provoked an increasing demand for hybrid varieties. Seed provision from abroad was not always guaranteed. Challenges were missing knowledge in hybrid seed production with an autogamous species and in seed certification, respectively, combined with high production costs and royalties. All stakeholders made efforts in establishing a multiplication contract, to provide individual growers with technical knowledge along the growing season and to fulfill the certification procedure in order to assure seed quality.

Our experiences revealed that the hybrid barley seed production under Swiss conditions is quite resource-intensive (e.g. applications of insecticides and growth regulators coupled with a high, unique N dose at an early stage). Two field inspections assured field isolation, varietal identity and occurrence of other species. Average seed yields of 45 dt ha<sup>-1</sup> were realistic, despite considerable differences from field to field. Reasons for yield variability were not always obvious. A pre-harvest yield estimation with stalk and kernel counts was laborious, but based on averages across several fields it was quite reliable for the estimation of import requirements. Additional observations in control plots showed that the parental inbred lines were authentic and that ears of female plants which were protected from cross-pollination with bags developed no kernels. Our seed testing results showed that the germination capacity of hybrid seeds is high and comparable with seeds of line varieties. Technical purity and the number of seeds from other species were not critical. However, ergot sclerotias occurred in some lots. Additional efforts were necessary during seed cleaning to reject the sclerotias by optical sensors. Additional payments are necessary to balance seed growers' income.

### Keywords

*Hordeum vulgare* · seed multiplication · Switzerland

**Table 1:** Key figures of hybrid barley seed production in Switzerland

Year	2014	2015	2016	2017
Total winter barley seed production surface (ha)	992	929	916	887
Total winter hybrid barley seed production surface <sup>1</sup> (ha)	48	68	99	101
Total winter barley seed sales (t)	3858	3868	3596	3784
Total winter hybrid barley seed sales <sup>1</sup> (t)	223	293	301	524
Ø-Germination capacity of line varieties (%)	93	93	92	94
Ø-Germination capacity of hybrid varieties <sup>1</sup> (%)	95	95	90	94

<sup>1</sup> hybrid barley cvs. 'Hobbit' and 'Wootan' only in 2016 and 2017; seed production in Switzerland; Ø production surface of barley from 2014-2016: 26 770 ha (Source: Agristat, swissem)





## Six-row winter barley: comparison of hybrid and line cultivars

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### Abstract

The aim of this study was to compare the grain yield potential and other characters of six-row hybrid winter barleys with those of line barleys on the basis of results obtained from the Austrian VCU trials. The tested cultivars were 'Hedy', 'Mercurioo', 'Oodin', 'SY Leoo', 'Tatoo' and 'Wootan' as hybrid cultivars, and 'Henriette', 'KWS Meridian' and 'KWS Tonic' as line cultivars. Using a conventional crop management system (110 trials run from 2010/11 to 2016/17, 12 to 75 comparisons), the hybrid barleys yielded -6.4 to +0.9 dt ha<sup>-1</sup> of grain on average as compared to 'Henriette', -6.9 to +0.7 dt ha<sup>-1</sup> as compared to 'KWS Meridian' and -8.8 to -0.7 dt ha<sup>-1</sup> as compared to 'KWS Tonic'. Even in the "hybrid-optimized" trial series (19 trials from 2012/13 to 2016/17, 4 to 19 comparisons), no significantly higher yields were obtained. The hybrids yielded between -7.4 and +1.6 dt ha<sup>-1</sup> of grain compared to the three line cultivars. Hybrid barleys can be tested in the normal VCU trials to achieve meaningful results. The hybrid barley grain yield would need to be 3.4 to 6.1 dt ha<sup>-1</sup> (at a feed barley price of 120 to 160 € t<sup>-1</sup>, excluding VAT) higher than that of the line cultivars, depending on the sowing density and thousand grain weight of the line cultivars, to cover the extra cost of the seed. At this time, it is only occasionally possible to achieve this yield. Marketing hybrid barleys promises to be more profitable for breeders and seed companies. However, the higher costs of the hybrid seed reduce the farmers' profit margins.

### Keywords

Austria · economy · *Hordeum vulgare* · hybrid breeding

### Introduction

Hybrids of many crops (rye, maize, sorghum, oilseed rape, sunflower, oil pumpkin, sugar beet, some vegetable species) have become established in recent decades. In addition to the higher yield potential, increased stability of the yield, especially in marginal and drought-prone environments, is considered beneficial. Another advantage is the possibility to combine complementary dominant inherited characteristics of the male and female parental components in the hybrid (Longin *et al.* 2012, Mühleisen *et al.* 2013, 2014). For breeders and seed companies, the annual seed exchange by the farmers represents a special economic incentive. Today the biggest acreage is reached in France, Germany and the United Kingdom (Stiewe & Spiller 2018). Hybrid winter barleys

(*Hordeum vulgare* L.) have been tested in Austria since 2005/06 and, in December 2008, the cultivar 'Yoole' was registered as first hybrid barley cultivar. The four six-row cultivars 'Hedy', 'Mercurioo', 'SY Leoo' and 'Wootan' are currently registered and have been described in detail (AGES 2018). Due to the weaker heterosis in autogamous species, the economic aspects of cultivation are often viewed critically (Lehrke 2013, Nickl *et al.* 2014a).

### Material and methods

#### Field trials

110 mono-factorial winter barley trials in series with "conventional crop management" (in part with the application of a growth regulator or fungicides) were carried out from 2010/11 to 2016/17. Furthermore, 19 results obtained from trials conducted from 2012/13 to 2016/17 with a cultivation system adapted to hybrid barleys were also available. Most of the experiments were conducted as part of the Austrian official testing of the value for cultivation and use (VCU). The test sites were located in the production areas in the north-eastern plains and hills (Fuchsenbigl, Probstdorf, Prellenkirchen, Großnondorf, Mistelbach, Tulln), alpine foothills (Pyhra, Prinzersdorf, Grabenegg, Gießhübl near Amstetten, Ritzlhof, Marchtrenk, Bad Wimsbach-Neydharting, Reichersberg), south-eastern plains and hills (Gleisdorf) and the Carinthian basin (Hörzendorf, St. Donat).

The field trials were arranged as completely randomized block designs with 8 and/or lattice designs with 9 to 42 genotypes with 3 to 4 replicates per trial. The plot size ranged from 7.4 to 13.5 m<sup>2</sup> in the individual environments; plots consisted of 8 to 12 rows.

#### Plant material

The hybrid barleys (H) 'Hedy' (released in Austria in 2015), 'Mercurioo' (2015), 'Oodin' (2015, removed 2016), 'SY Leoo' (2013), 'Tatoo' (EU variety, no registration in Austria) and 'Wootan' (2014) were compared with the six-row line cultivars (L) 'Henriette' (2011), 'KWS Meridian' (2010), and 'KWS Tonic' (2013). 'Hedy' is a cultivar of W. von Borries-Eckendorf GmbH & Co KG, Germany, the other hybrids come from the barley breeding program of Syngenta Seeds Ltd., UK. 'Henriette' (Nordsaat Saatzeugesellschaft mbH), 'KWS Meridian' and 'KWS Tonic' (KWS Lochow GmbH) are of German origin. In the conventional trial series there were 12 to 18 and 63 to 75 comparisons to the three-line cultivars for 'Tatoo' and 'Wootan', respectively. For the "hybrid-optimized

**Table 1:** Total grain yield, yield of grains >2.2 mm and grain protein yield for six-row winter barley: differences in yield (dt ha<sup>-1</sup>) between hybrid cultivars and check line cultivars (HE, 'Henriette'; KM, 'KWS Meridian'; KT, 'KWS Tonic'), trials from 2011-2017

Trial series	Testing period	Trials/ Comparisons	Grain yield (dt ha <sup>-1</sup> )			Grain yield sieved (dt ha <sup>-1</sup> >2.2 mm)			Grain protein yield (dt ha <sup>-1</sup> )		
			HE	KM	KT	HE	KM	KT	HE	KM	KT
Conventional cultivation system (reduced use of fungicides)											
Hedy	2015-2017	25-33	+0.9	+0.7	-2.3	-3.5	-1.2	-3.8	-0.10	-0.11	±0.00
Mercurioo	2013-2015	29-35	+0.6	-1.0	-0.7	-5.6	-6.4	-5.8	-0.44	-0.48	-0.07
Oodin	2013-2016	41-47	+0.3	-1.5	-3.3	-6.2	-5.9	-7.5	-0.18	-0.25	-0.01
SY Leoo	2011-2013, 2015, 2017	33-41	-1.8	-2.2	-4.8	-5.0	-4.0	-6.2	-0.35	-0.28	-0.16
Tatoo	2011-2012, 2014	12-18	-6.4	-6.9	-8.8	-6.8	-7.3	-9.2	-0.77	-0.60	-0.23
Wootan	2012-2017	63-75	+0.3	-1.1	-2.5	-5.8	-5.6	-7.1	-0.19	-0.26	+0.07
Hybrid-optimized cultivation system <sup>1</sup>											
Hedy	2016-2017	7	-5.4	-2.9	-7.0	-8.7	-4.3	-9.8	-0.67	-0.79	-0.34
Mercurioo	2014-2017	15	-1.4	-0.9	-3.9	-8.8	-7.3	-11.1	-0.60	-0.68	-0.32
Oodin	2014-2016	12	-0.1	-0.3	-2.4	-4.7	-4.0	-6.6	-0.46	-0.59	-0.13
SY Leoo	2013, 2015, 2017	11	+1.6	-1.1	-4.1	-0.3	-2.1	-5.4	+0.09	-0.05	+0.06
Tatoo	2014	4	-6.6	-6.5	-7.4	-8.4	-8.3	-9.5	-0.75	-0.55	+0.10
Wootan	2013-2017	19	-0.1	-1.3	-3.7	-3.5	-3.7	-6.6	-0.30	-0.41	-0.03

<sup>1</sup> tendency towards later sowing date, 75% of the normal seed rate, a higher number of trials with applied growth regulator, partially less first N fertilization, but later higher rates, fungicide treatment in all trials

cultivation system", 4 to 19 comparisons for 'Tatoo' and 'Wootan', respectively, were available (Table 1). An environment in which all nine barley cultivars were examined together did not exist.

#### Agronomic traits

Grain yield (dt ha<sup>-1</sup>) was normalized to 14% moisture content. The grain size fractions of <2.2, 2.2-2.5, 2.5-2.8 and >2.8 mm were measured using a slotted sieve. The grain yield >2.2 mm (dt ha<sup>-1</sup>) was calculated by multiplying the grain yield and sieve fraction above 2.2 mm (%). The protein yield (dt ha<sup>-1</sup>) is the product of grain yield (dt ha<sup>-1</sup>, d.m.) and crude protein content (%). Furthermore, agronomic data, evidence of disease infestation, the thousand grain weight and specific weight were recorded.

#### Cultivation system

The 129 trials were sown primarily between 18<sup>th</sup> September and 18<sup>th</sup> October, two trials were drilled on 27<sup>th</sup> October. The six-row line cultivars were sown with 270 to 330 viable seeds m<sup>-2</sup> depending on the site location. The seed rate of the hybrid barleys was reduced by 25% to 200 to 250 germinated seeds m<sup>-2</sup>. The use of fertilizers, growth regulators, herbicides and insecticides was adapted to the local conditions. The plants received on average 106 kg ha<sup>-1</sup> nitrogen in total (32 to 212 kg N ha<sup>-1</sup> in the individual environments), with the application usually performed in 2 or 3 doses. In some of the conventional experiments, diseases were controlled with fungicides. In the "hybrid-optimized series", diseases were always controlled. 1.0 L ha<sup>-1</sup> Aviator Xpro® (active substances: prothioconazole, bixafen) was usually used. Because fungicide-resistant *Ramularia* strains had spread in 2016 and 2017, Aviator Xpro® was supplemented with 1.0 L ha<sup>-1</sup> Balear 720 SC® (active substance chlorothalonil).

#### Results and discussion

In order to take advantage of the yield potential, the breeding company Syngenta Seeds GmbH, Germany, recommends using a cultivation system for their hybrid barleys that deviates from that of line cultivars and which is referred to as a "hybrid-optimized crop management system". The company claims that this takes

the special growth habits of the hybrid cultivars into account. It is essential to reduce the seed rate, use a modified nitrogen distribution method, consistently use a growth regulator and apply an adapted fungicide strategy (Albers 2011).

Some claim that hybrid barleys are more flexible in terms of their sowing time than conventional line cultivars. In most locations, late sowing should be possible well into October without major losses of yield. Hybrid cultivars of winter barley produce more tillers and are more vital and vigorous in spring than line cultivars. This must be taken into account when applying nitrogen fertilizer. It is essential to adapt the first nitrogen dose to the number of tillers per plant. The early application supports the early development of hybrid barley. According to the company, if the plant develops a superior root system, it can efficiently utilize the available nitrogen and water in the soil. Treating the plants with the growth regulator Moddus® (active substance trinexapac) should further increase the root mass and root length as well as maintain stem strength. In order to keep the leaves healthy, two applications of fungicides are recommended, depending on the incidence of disease (Syngenta 2017).

#### Grain yield, protein yield and further traits

As in other countries, the hybrid barleys did not consistently outperform the six-row line cultivars in terms of grain yield. A significant yield advantage would be necessary to compensate for the higher seed costs of hybrids compared to line cultivars.

Using a conventional crop management system, the six hybrid barleys yielded an average of -6.4 to +0.9 dt ha<sup>-1</sup> of grain as compared to 'Henriette', -6.9 to +0.7 dt ha<sup>-1</sup> compared to 'KWS Meridian' and -8.8 to -0.7 dt ha<sup>-1</sup> compared to 'KWS Tonic'. Even in the "hybrid-optimized" trial series, no significantly better yields could be obtained. 'Hedy', 'Mercurioo', 'Oodin', 'SY Leoo', 'Tatoo' and 'Wootan' yielded between -6.6 and +1.6 dt ha<sup>-1</sup> grain as compared to 'Henriette', -6.5 and -0.3 dt ha<sup>-1</sup> compared to 'KWS Meridian' and -7.4 to -2.4 dt ha<sup>-1</sup> compared to 'KWS Tonic' (Table 1).

The grain size (>2.2 or >2.5 mm) of the hybrid barleys were medium to below average. Accordingly, hybrid barleys produced grain

**Table 2:** Characteristics of selected hybrid and line cultivars of six-row winter barley (Austrian descriptive variety list 2018; AGES 2018). (Characteristic score: 1, very early heading/ripening, very short plant height, very low susceptibility to lodging/brackling/necking/diseases, very high yield potential/sieving/test weight/protein content; 9, very late heading/ripening, very tall, very susceptible to lodging/brackling/necking/diseases, very low yield potential/sieving/test weight/protein content)

Cultivar	Heading	Ripening	Plant height	Lodging	Brackling	Necking	Powdery mildew	Leaf rust	Net blotch	Rhynchosporium	Ramularia leaf spot	Grain yield (semi-arid area)	Grain yield (humid area)	Sieving >2.2 mm	Sieving >2.5 mm	Thousand grain weight	Test weight	Crude protein content
<b>Hybrid barleys</b>																		
Hedy	5	6	7	6	5	3	5	3	5	3	6	2	3	5	6	5	7	8
Mercurioo	4	6	6	7	8	6	3	6	5	3	5	2	3	6	8	7	6	8
Oodin <sup>1</sup>	5	6	7	6	7	7	3	5	5	3	5	2	3	6	7	7	6	7
SY Leoo	4	5	5	5	6	6	3	8	6	3	5	3	3	5	6	7	5	7
Tatoo <sup>2</sup>	4	5	5	5	7	7	4	-	5	3	5	4	4	4	6	7	5	7
Wootan	5	6	6	6	7	6	4	7	5	3	5	3	3	6	7	7	5	7
<b>Line barleys</b>																		
Azrah	3	6	6	3	3	6	6	5	3	4	5	3	2	2	3	3	7	7
Belinda	4	4	6	3	5	4	3	5	4	5	5	2	2	1	2	5	5	7
Finola	2	4	5	4.5	2	2	5	5	5	5	6	1	2	1	3	4	6	8
Henriette	3	4	5	5	5	5	6	4	3	4	6	4	3	2	3	4	6	7
KWS Higgins	5	5	6	5	5	3	4	5	4	3	6	1	1	3	4	4	6	8
KWS Meridian	5	5	6	5.5	5	4	5	3	4	3	5	2	2	3	4	5	7	7
KWS Tonic	5	6	6	4	5	4	6	4	4	4	6	3	1	3	4	4	7	8
Michaela	5	6	4	3	5	4	5	4	4	3	6	3	3	4	5	5	8	7

<sup>1</sup> deleted from the variety list in 2016

<sup>2</sup> EU variety (not registered in Austria)

yields of -9.2 to -1.2% (conventional trial series) and -11.1 to -0.3% (hybrid-optimized series) compared to the three line cultivars. The grain protein yield of hybrid barleys, a key parameter for nitrogen efficiency, was also below that of the majority of ‘Henriette’, ‘KWS Meridian’ and ‘KWS Tonic’ yields (-0.79 to +0.10 dt ha<sup>-1</sup>).

The closely related hybrids ‘Mercurioo’, ‘Oodin’, ‘SY Leoo’, ‘Tatoo’ and ‘Wootan’ displayed medium to high plant growth and medium to low lodging tolerance. The resistance to powdery mildew and *Rhynchosporium* was above average, but a susceptibility to leaf rust was observed. An observed shortcoming was the breaking of stems along their length (brackling) or under the ears (necking) at full and over-ripening. The energetic feed value was not affected by the smaller grain size. The tall cultivar ‘Hedy’ demonstrated a high resistance to leaf rust and did not display as much necking (Table 2).

In terms of grain yield, the hybrid barley did not reach the same levels as would be expected with best-parent heterosis and commercial heterosis, as is the case in hybrid rye. Accordingly, one of the current, main goals of hybrid barley breeding is the development of more distinct parental pools that lead to increased heterotic effects and performance of hybrids (Bernhard *et al.* 2018).

#### Yield stability

Mühleisen *et al.* (2014) reported that hybrid barley usually showed significantly higher dynamic yield stability and, consequently, lower genotype by environmental interactions than line barleys.

However, conducting a reliable assessment of this cultivar trait is difficult and would require a large number of environments (Piepho 1998). Furthermore, yield stability also varies depending on the production area and cultivation system. The low heritability of dynamic yield stability makes selection difficult. According to the results of the Austrian VCU tests, the hybrid barleys were not significantly better in this regard. In terms of stability variance (Shukla 1972), ‘SY Leoo’ proved to be fairly stable in the area of the alpine foothills. For ‘Wootan’, an average yield stability was obtained in the north-eastern plains and hills and a below average stability in the alpine foothills. In response to a serious water shortage, ‘Wootan’ responded more sensitively regarding its grain quality and grain yield than all other tested line barleys (Table 3). ‘Hedy’ and ‘Oodin’ also did not prove to be particularly environmentally stable (AGES 2018).

#### Studies conducted in other countries

The experimental data suggest that, with the exception of the reduced seed rate, a specific cultivation system is not required for hybrid barleys. In experiments conducted in the UK from 2003 to 2005, the hybrid barley ‘Colossus’ behaved in a similar way as line cultivar ‘Siberia’ with variable nitrogen fertilization. Although in some environments delayed nitrogen fertilization lowered the yield, in some experiments, the opposite results were obtained. In principle, hybrid barley must not be treated differently than a conventional six-row line cultivar (Freer 2006). In Baden-Württemberg, a “hybrid-optimized nitrogen fertilization strategy”

**Table 3:** Total grain yield (GY), yield of grains >2.2 (GY2.2) and >2.5 mm (GY2.5), grain protein yield (GPY) and grain quality (G2.2, G2.5, L2.2, sieving >2.2, >2.5 and <2.2 mm, respectively; TGW, 1000 grain weight (d.m.); TW, test weight; PC, crude protein content) for six-row line (L) cultivars and the hybrid (H) barley 'Wootan' under conditions of drought stress (means from two trials in the north-eastern plains and hills in 2017)

Cultivar	GY (rel%)	GY2.2 (rel%)	GY2.5 (rel%)	GPY (rel%)	G2.2 (%)	G2.5 (%)	L2.2 (%)	TGW (g)	TW (kg hL <sup>-1</sup> )	PC (%)
Finola (L)	111	138	170	104	86.2	44.4	13.8	36.5	65.6	13.8
SU Ellen (L)	107	131	176	105	84.0	47.4	16.0	36.3	61.0	14.3
KWS Meridian (L)	100	101	106	101	69.6	30.9	30.4	31.4	58.6	14.8
KWS Tonic (L)	98	94	83	97	66.5	26.3	33.5	32.8	60.2	14.5
Michaela (L)	97	84	53	101	58.6	15.9	41.4	30.8	57.0	15.3
Azrah (L)	95	94	72	95	68.9	22.7	31.1	34.3	60.7	14.8
Wootan (H)	93	58	39	98	41.7	12.3	58.3	27.7	59.1	15.5
Mean (dt ha <sup>-1</sup> )	72.8	50.5	21.5	9.2						

**Table 4:** Comparison of hybrid and line cultivars of winter barley with regard to seed, seed costs and the additional yield of hybrid s compared to line cultivars required for economic efficiency

Seed rate/ cultivar type	Seed price per unit (€) <sup>1</sup>	Content per unit (Seed)	TGW (g)	Seed costs (€ ha <sup>-1</sup> ) <sup>1</sup>	Costs difference (€ ha <sup>-1</sup> )	Additional needed grain yield (dt ha <sup>-1</sup> ) at price for feeding barley (€ t <sup>-1</sup> )		
						120	140	160
Seed rate A <sup>2</sup>								
Hybrid cultivar	45.0	700 000 s	–	127	–	–	–	–
Line cultivar	53.3	100 kg	42	59	-68	5.7	4.9	4.2
Line cultivar	53.3	100 kg	47	66	-61	5.1	4.4	3.8
Line cultivar	53.3	100 kg	52	73	-54	4.5	3.9	3.4
Seed rate B								
Hybrid cultivar	45.0	700 000 s	–	137	–	–	–	–
Line cultivar	53.3	100 kg	42	64	-73	6.1	5.2	4.6
Line cultivar	53.3	100 kg	47	71	-66	5.5	4.7	4.1
Line cultivar	53.3	100 kg	52	79	-58	4.8	4.1	3.6

<sup>1</sup> exclusive of value-added tax (VAT)

<sup>2</sup> seed rate A: 188 and 250 seeds m<sup>-2</sup> for hybrid and line cultivars, respectively; seed rate B: 202 and 270 seeds m<sup>-2</sup>, respectively

that was carried out from 2011 to 2013 for 'Hobbit', 'SY Leo', 'Yoole' and 'Zzoom', yielded no benefits when compared to line cultivars 'Fridericus', 'Kathleen' and 'Lomerit' (Mokry & Ott 2014). In experiments of the Bavarian State Research Center for Agriculture in 2013 and 2014, it was not clear whether 'Hobbit' or 'SY Leo' responded more positively to "hybrid-optimized nitrogen fertilization" than line cultivars 'KWS Meridian' and 'Souleyka'. When seeds were sown late, the yield losses averaged 4 dt ha<sup>-1</sup>, and hybrid cultivars tended to perform worse than line cultivars (Nickl *et al.* 2014a, b). Experimental data collected in Lower Saxony also did not reveal that hybrid barleys are more suitable for late sowing (Lehrke 2013). Similarly, results obtained in experiments conducted in Mecklenburg-West Pomerania from 2014 to 2016 did not reveal any benefit of the "hybrid-oriented cultivation system". The hybrids 'Hobbit', 'Galation' and 'Wootan' did not tolerate late sowing or short growing seasons in the autumn better than the line cultivars 'Lomerit' and 'Antonella'. Sufficient plant development before winter is also desirable in hybrid barley. Cultivars did not demonstrate any specific suitability regarding the nitrogen fertilization regime (Pienz 2017, Pienz & Ziesemer 2017).

The data collected in the normal VCU trials for hybrid barley are meaningful. It does not seem necessary to test hybrid barleys in separate experiments using a specially modified cultivation system. For this reason, the "hybrid-optimized trial series" carried out from 2012/13 to 2016/17 was discontinued.

### Economic evaluation

The competitiveness of the cultivation of hybrid barleys is assessed by comparing them with six-row line cultivars. In this case, the seed costs, achieved grain yield and market value of feed barley are relevant. The expenses for tillage, sowing, fertilization, crop protection and harvesting are identical for hybrid and line cultivars and can be disregarded in the economic analysis. For reasons of simplification, differences in grain quality (sorting, weight per hectolitre) have not been assumed to have an effect on revenue. For a unit of hybrid seed (700 000 seeds), 45 € were invested, for line cultivar seed 53.3 € for 100 kg (each excl. VAT). These are the average prices for the period 2015 to 2017. The calculation is based on seed rates of 188 and 202 germinated seeds m<sup>-2</sup> for hybrid barley and 250 or 270 germinated seeds m<sup>-2</sup>, as well as the exclusive use of certified seed for the line cultivar. Accordingly, the seed costs differ by 54 to 73 € ha<sup>-1</sup>. Depending on the thousand grain weight (TGW) of the line barley (42, 47 and 52 g) and the feed barley price (120, 140 and 160 € t<sup>-1</sup>, excl. VAT), the hybrid barley would need to yield at least 3.4 to 6.1 dt ha<sup>-1</sup> more than the line cultivar (Table 4). At this time, it is only occasionally possible to reach this yield with the hybrid cultivars, and it is not possible to achieve it on average. At low producer prices for feed barley, it is difficult or even impossible for current hybrid barleys to fully offset the additional seed costs.

## Market significance

The hybrid barleys have been established in Austria, but they have only been able to achieve a modest market share so far. In the autumns of 2014, 2015 and 2016, seed from hybrid cultivars accounted for 2.4 to 3.2% of the winter barley seed quantities sold in Austria. In terms of the six-row barleys, these quantities ranged from 7.6 to 9% (Saatgut Austria 2015-2017). Because the amount of seed obtained from hybrid barley per hectare is lower and, on the other hand, partially farm-saved seed is used for line cultivars, currently about 3% of the Austrian winter barley area is sown with hybrids. As long as the hybrid barley has no significant yield advantage, their importance in Austria is expected to remain low. Farmers rate the possibly good yield stability as much lower than the yield.

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## Hybrid triticale: achievements and challenges for the future

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Hybrid breeding relies on the systematic exploitation of heterosis and hybrid vigour. It should boost yield and yield stability in marginal environments and allow to stack dominant genes easily. Research on hybrid triticale is done since 1998. Two hybrid systems are available, i.e. hybrids using Genesis® as CHA (chemical hybridizing agent) from Monsanto and CMS hybrids with the cytoplasm from *Triticum timopheevi* and other cytoplasm.

About two decades ago, Saatzzucht Dr. Hege GbR and the State Plant Breeding Institute of the University of Hohenheim started to investigate triticale hybrid breeding based on a CMS system. The *timopheevi* cytoplasm showed some effect on plant height (-4.4 cm) and earliness (+0.46 BBCH) (Boeven *et al.* 2016). After more than 5000 test crosses it can also be summarized that the frequency of partial restorers in the triticale germplasm is >95% and that strong selection pressure for the development of complete maintainer and restorer lines results in a poor *per-se* performance and high susceptibility to diseases. For CMS hybrid triticale a mid-parent heterosis of 2-3% was reported (Gowda *et al.* 2013, Mühleisen *et al.* 2015), whereas for CHA hybrids 10% were reported (Oettler *et al.* 2005).

In a recent study, Losert *et al.* (2016) reported an average mid-parent heterosis of biomass yield of 4.8% and a maximum commercial heterosis of 9.1% but no positive commercial heterosis for grain yield.

With respect to dynamic yield stability Mühleisen *et al.* (2015) reported significantly lower stability as measured by Shukla's stability variance compared to lines and no correlation between grain yield and yield stability. The reasons were obvious and complex: (i) on the female side mainly for maintainer lines and due to a long breeding cycle only low yields were realized; (ii) on the male side selection was for restorers; (iii) no selection for grain yield was carried out in experimental hybrids, whereas line varieties were strongly selected for grain yield.

From the work hitherto carried out it can be summarized that hybrid triticale breeding is a resource- and time-intensive work compared with line breeding but an attractive niche market as commercial heterosis is feasible and a working hybrid mechanism for stacking dominant genes is available. However, further re-

search is needed to understand and optimize the CMS-cytoplasm and to identify heterotic groups. Joint efforts between private and public partners to promote hybrid triticale breeding is necessary in the future.

### Keywords

Biomass yield · CHA · CMS · commercial heterosis · grain yield · *Triticosecale* · *Triticum timopheevi* · yield stability

### Acknowledgements

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## Hybrid triticale in Switzerland: past experience

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In Switzerland, E. Oehler and M. Ingold started research on triticale during the 1950s. The main goals at that time were spike fertility improvements and octoploid triticale chromosome number stability. In 1976, after a visit to INRA Clermont Ferrand, A. Fossati decided to shift the triticale breeding programme from octoploid to hexaploid triticale. Many genetic exchanges and collaborations took place with Poland (T. Wolski), the USA (R. Metzger, M.F. Kolding), CIMMYT (G. Varughese, W. Pfeiffer) and France (M. Bernard). For cultivar development in France, a cooperation started with a company called Claeys-Luck (later changed to Orsem and then to DuPont Hybrinova). One of the main goals of this programme was the improvement of lodging resistance by reducing plant height. Indeed, towards the end of the 1980s, all registered triticale cultivars were tall (135-150 cm). Our nursery contained several significantly short lines (<100 cm), likely with the dwarfing gene *Rht3*. However, these lines, despite highly fertile ears, had a low yield caused by insufficient tillering and a bad grain filling. Since 1987, with the opportunity to use chemical hybridising agents (CHA) for experimental purposes, F<sub>1</sub> hybrids were produced, with the idea to combine the productivity of tall genotypes with the ear fertility of the shortest genotypes. Since 1994, other kind of combinations have been produced.

The hybrids were produced by Delley Seeds and Plants Ltd. using three CHAs provided by Ciba-Geigy, Hybrinova (Croisor®) and Hybritech (Genesis®). The level of sterility of the treated plants was controlled by bagging some ears at heading. The following year, growing-on tests were used to assess the identity and purity of hybrid seeds. The material was evaluated in a lattice design, with two or three replications in three to nine locations, each plot measuring 7 m<sup>2</sup>. To compare the results of the hybrids with the mean of their parents, we calculated the relative values compared to two standards (cvs. 'Brio' and 'Tridel'). Among all produced hybrids, we only analysed the results of those tested together with these two standard cultivars.

The CHA from Ciba-Geigy was highly efficient, but quite expensive, and its use on triticale was only on a small experimental scale. The two other CHAs sometimes had a phytotoxic effect (reducing plant height, producing red coloration or discoloration on the leaves) depending on the genotypes, but the male sterility was almost complete. During the first years of experiments (1987–1994), significantly short lines (likely with the dwarfing gene *Rht3*) were combined with taller lines (Table 1). The F<sub>1</sub> hybrids were in general

shorter than the mid-parent values. The thousand kernel weight (TKW) was higher, but not the yield due to weak tillering. The specific weight tended to be equivalent to that of the worst parent. The cytological instability of the *Rht3* gene caused a lack of uniformity in the hybrids. In conclusion, the negative characters related to this gene were not compensated by the heterosis effect. In the following years (1995–1998), genotypes of a medium to short plant height, such as the cv. 'Tridel' (≈101 cm), were combined with tall lines. In general, the produced hybrids had good uniformity. Hybrids confirmed that the heterosis effect, as observed in wheat, was most important on grain weight. However, strong parental influences on the TKW and number of grains per square metre were observed. On average, plant height increased only slightly (≈5%). Yield increased as well, sometimes considerably. The effect of heterosis on earliness and specific weight was not important. The average results masked a great variability, but the best hybrids were agronomically highly promising. The highest yielding hybrid exceeded 120 dt ha<sup>-1</sup>. One of our hybrids was the highest yielding variety (114% of the standards) in the first year in official French trials. After 1996, we also produced some spring triticale hybrids (results not presented). In 1998–1999, four triticale F<sub>1</sub> hybrids based on 'Tridel' were registered, two in France (cvs. 'Kador' and 'Clint') and two in Switzerland (cvs. 'Hybridel' and 'Delrac'). As the CHA owner did not obtain the authorisation for its utilisation in triticale, no commercial development followed the registrations. Combining tall male lines with not-too-short female lines was successful from an agronomical viewpoint. Compared to wheat, F<sub>1</sub> hybrid production in triticale is easy thanks to a huge pollen production. As triticale is a forage cereal with a relatively low price, it is more challenging to compensate for the higher F<sub>1</sub> seed cost using the higher yield.

### Keywords

F<sub>1</sub> hybrid · gametocide · heterosis · × *Triticosecale*

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We would like to thank the companies DuPont Hybrinova and Hybritech for their past collaboration.

**Table 1:** Performance of F<sub>1</sub> triticales hybrids compared to the mean of both parents, expressed as the difference between the relative values (%) to two check varieties (cvs. 'Brio' and 'Tidel').

Period		Grain yield	Thousand kernel weight	Plant Height	Test Weight	Heading
1987–1994 (n = 38)	Mean	-8.5	8.6	-3.7	-2.3	-0.04
	Minimum	-29.4	-7.3	-18.1	-10.5	-1.9
	Maximum	30.9	29.3	10.1	5.3	2.5
1995–1998 (n = 235)	Mean	7.5	9.7	5.3	0.3	0.04
	Minimum	-28.4	-23.4	-17.5	-6.6	-7.4
	Maximum	36.4	45.5	21.4	7.1	8.2

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## Development and performance of new maize populations: selection method and progress

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A frightening loss of diversity is increasingly observed in general, and an alarming loss of diversity of different variety types in particular. For major agricultural crops like maize, sunflower, and sugar beet no open pollinated varieties (OPV) are available in Germany. For rye, 50% of the variety types are already hybrids. In the vegetable sector where hybrids are dominating in the open pollinating species, it is even worse. Open pollinated varieties are heterogeneous, genetically diverse and have a high adaptation capacity. Additionally, there is an increasingly demand for appropriate varieties for low input and organic farming.

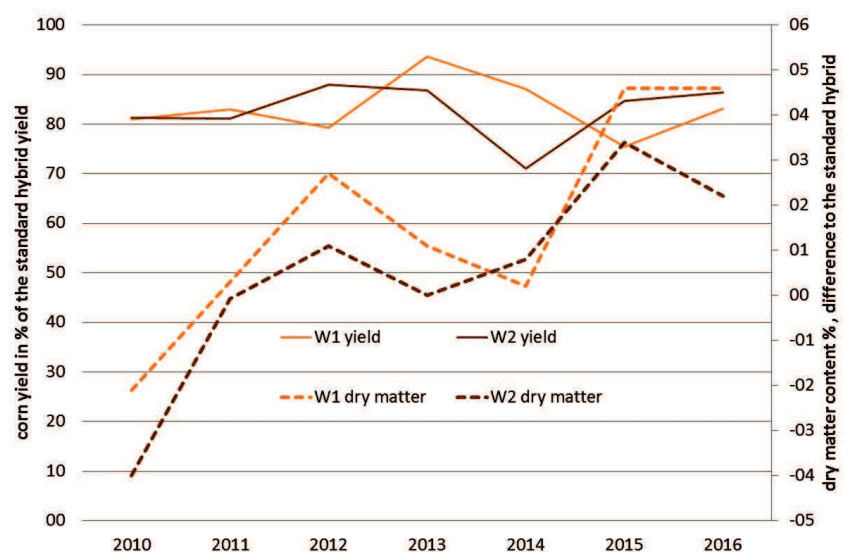
We started to develop new OPVs and used - due to the lack of alternative breeding material - the best performing hybrids. OPV W1 was set up 10 years ago and consists of 10 different hybrids from 5 different seed companies (representing 20 different genotypes). OPV W2 was developed in the same way using 8 different hybrids from 6 different seed companies. The hybrid varieties were sown randomly in isolation fields (2000 plants) to ensure cross pollination of all possible combinations. The selection method was recurrent mass selection. The results presented in this study are from the 7<sup>th</sup> selection cycle. Performance testing of the selected material was done in standardized field trials at one site for 7 years. The single cross hybrid 'Torres' was used as check cultivar.

'Torres' achieved on average 130 dt ha<sup>-1</sup> over seven years. The population varieties realized about 80% of the hybrid yield. Results from other studies supported our results and showed that OPVs could even achieve up to 105% of the yield compared to hybrids (Chidzuza *et al.* 1994). Thus, with further breeding efforts, OPVs could be competitive to hybrids.

In the cropping season 2013 with severe weather conditions, both OPVs reached 90% of the yield of 'Torres' indicating that populations can compensate adverse weather conditions better than hybrids. This could be due to the higher genetic diversity of population varieties. Having climate change in mind, this could become an important feature for the future.

The most important selection goals were increased dry matter yield and dry matter content. However, these traits are negatively correlated since early ripening plants have lower yield due to a shorter growing period than late ripening plants. Thus, it is difficult to increase dry matter yield and content simultaneously. Figure 1 displays the dry matter yield and the difference of the dry matter content of the OPVs compared to 'Torres'.

**Figure 1:** Dry matter yield relative to 'Torres' and the difference of the dry matter content between the OPVs and 'Torres'. A negative difference indicates later ripening than 'Torres'.



The aim would be to increase yield and the difference of the dry matter content to enhance the security for an appropriate harvest of corn. The results show that the relative yield was more or less stable across years and the dry matter content could be increased by 8% in seven years. Thus, the recurrent mass selection applied for developing the OPVs, provided satisfying results.

Future research questions are: (i) How do OPVs perform at other environments and other cropping systems such as low input: Are they better than hybrids? (ii) Can we measure the adaptation capacity and is there an influence of the number of genotypes a population variety is derived from? (iii) How to set up OPVs? Is there an influence of controlled crosses versus open pollinated crosses? (iv) Can we achieve better or faster results with marker assisted breeding methods? (Eder *et al.* 2017); and (v) What about the approval of varieties? Populations of some crops will hardly meet the requirements of the distinctiveness criteria.

### Keywords

Cross pollination · grain yield · recurrent mass selection · *Zea mays*

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## Composite cross populations: legal considerations and their value for plant breeding

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Until recently, it has not been possible to market heterogeneous plant material in the European Union since cultivars have to be distinct, uniform and stable (DUS test) as a prerequisite for official variety registration. The legal basis for the introduction of heterogeneous plant material in the EU has been established in 2014 with decision no. 150 of the European Commission, which demands the organization of a temporary experiment. This experiment will expire at the end of 2018 and is restricted to four cereal species: wheat (*Triticum* spp.), barley (*Hordeum* spp.), oats (*Avena* spp.) and maize (*Zea mays* L.). Note that both composite cross populations (CCPs) of the predominantly self-pollinating species wheat (Figure 1), barley and oats as well as random mating maize populations are referred to as ‘populations’ in the experiment. According to the regulation a population is defined as a plant grouping which (a) results from heterogeneous source material, (b) can be reproduced unchanged once it has adapted to the specific agro-climatic conditions of a given region of production, and (c) emerges from pairwise crosses of at least five genotypes in all possible combinations or a different crossing protocol, which produces a similarly diverse population. The crossings of the genotypes are followed by bulking of the progeny and exposing the stock to natural selection and supervised selection by breeders in successive generations.

Clause (b) of the new regulation requires a careful interpretation since heterogeneous populations will certainly always change in their composition and characteristics even after they have adapted to a given region of production. What clause (b) really means is that it should be possible to specify certain defining characteristics of a population, which do not change after the stock has adapted to the location. With respect to clause (c) of the regulation, let us note that an example of an alternative crossing protocol to the (full) diallel cross would be, for instance, the so-called MAGIC crossing scheme. The MAGIC scheme has been recently used by the Julius Kühn-Institut (JKI) to produce a barley CCP. Finally, it is important to emphasize that a CCP is something qualitatively different from a mixture of varieties or genotypes. In the case of a variety mixture the seeds are physically mixed, while in a CCP the genotypes are combined using crosses. A beautiful experiment, which illustrates qualitative differences between CCPs and variety mixtures has been published by Allard & Adams (1969). Today, there is a large body of scientific research showing that composite cross as well as random mating populations offer tremendous potential for the improvement of biodiversity in agriculture, which

is associated with numerous agronomic and ecological advantages. These advantages include higher yield stability, increased and more durable disease and pest resistances and an ability to adapt to different cultivation sites. Due to their resilience to adverse environmental impacts and changing environmental conditions, populations have been proposed as a strategy for climate change adaptation. Allard & Hansche (1969) have proposed to utilize CCPs as dynamic mass reservoirs for the conservation of genetic diversity. This type of *in situ* conservation offers many advantages including high cost efficiency as well as the ability to adapt the material to changing biotic and abiotic environmental conditions and new locations. From these mass reservoirs breeders may extract individual plants to develop exceptional pure line cultivars. Thus, we expect population breeding programs to supplement pure line and hybrid breeding programs rather than replace them.

Until today, a number of European countries, including Denmark, France, the Netherlands, the United Kingdom and Germany, have implemented the new European legislation at a national level. In Germany, the first populations were approved by the German Federal Plant Variety Office in 2016. The leading German and Swiss organic breeding initiatives, Forschung & Züchtung Dottenfelderhof (FZD) and Getreidezüchtung Peter Kunz (GZPK), have developed 7 winter wheat and 8 summer wheat CCPs. Moreover, the Bavarian State Institute for Agriculture (LfL) and the breeding initiatives FZD and GZPK have released 5 maize populations. In preliminary field trials, these populations have demonstrated their value for agriculture and plant breeding. It is essential that both CCPs as well as maize populations are tested more extensively in field trials and practical agriculture. Constraints to consumer and farmer acceptance of CCPs throughout the value chain are studied in a project of the European Innovation Partnership for Agricultural Productivity and Sustainability (EIP-Agri) in the federal state of Hessen. A second important goal of the project is to collect information in order to evaluate the value of the new European regulations for farmers, the whole value chain and end-consumers. It is essential that more funding for projects with a focus on heterogeneous populations is made available. In fact, it seems likely that when the population experiment will expire at the end of 2018, there will not be enough information available for a robust assessment of the legislation. Thus, the leading breeding initiatives and universities involved in the development of populations demand that the regulations on heterogeneous plant material should be





**Figure 1:** Genetic diversity in a winter wheat Composite Cross Population (CCP), Dottenfelderhof, Bad Vilbel, Germany.

extended beyond 2018. However, due to legal restrictions, the experiment can be maximally prolonged until 2022. Therefore, the breeding initiatives demand that the scope of the regulations will be extended to more species than the four cereal crops mentioned above and incorporated in national and European seed legislation.

#### Keywords

Biodiversity · breeding method · climate change · maize · wheat

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## High-throughput sorting of colored wheat grains to determine competitive effect and response

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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 purple wheat variety ‘Rosso’ to assess the suppression ability of wheat varieties.

In 2 years, under conventional and organic management, eight winter wheat varieties were grown in 50:50 mixture with ‘Rosso’ (purple grains) and in normal pure stand. The design was a split-plot design, with mixture treatment as mainplot-factor and 3 replicates.

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  $Y_p$  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’.

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. There was a significant difference between trials in the overall level of suppression of ‘Rosso’ (Table 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. 1), i.e. 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 the same height as the shorter line varieties.

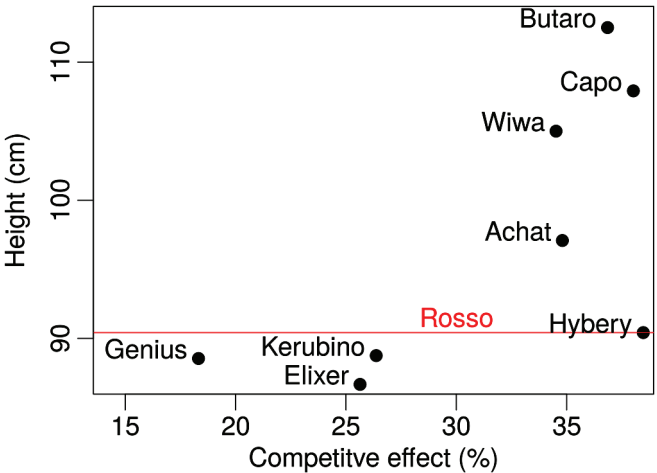


Fig. 1: Relation of competitive effect to canopy height. Red line indicates the canopy height of ‘Rosso’.

Table 1: Mean competitive effect (CE), variety effect based on ANOVA and heritability ( $h^2$ ). (# means with different letters are significantly different at the Tukey-test at  $p=0.05$ )

Year	Management	Mean CE <sup>#</sup>	Variety (F)	$h^2$
2016	Conventional	10.6% d	4.9 **	0.80
2016	Organic	21.0% c	7.5 ***	0.87
2017	Conventional	53.5% a	2.9 *	0.66
2017	Organic	41.4% b	3 *	0.67

### Keywords

Colored wheat · organic agriculture · *Triticum aestivum* · variety mixture · weed suppression

### Acknowledgments

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## Seed as common property - Breeding as a source for real economy, law and culture

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The worldwide loss of agricultural biodiversity is the result of a production process that generates maximum yields with the massive input of fertilizers and pesticides at the expense of environment and human health. On the one hand, there are enough kilocalories generated today to feed a world with a population of 14 billion people if not more than half of the primary production were not being destroyed. On the other hand, FAO has shown that 70-80% of the world's food is still produced by family farmers of which >80% cultivate a maximum of two hectares. Stabilizing and improving this production must therefore be a top priority.

FAO's extensive efforts with the International Seed Treaty (ITPGRFA) and the UN Convention on the Conservation of Biodiversity (CBD and Nagoya Protocol) have not been able to stop the loss of global (agro)biodiversity. The issue of food security is directly linked to the availability of seed. As in developing countries, seed was a common property in industrialized countries up to 100 years ago. Since then, seed has undergone a dramatic commercialization and privatization process. This process is also ongoing in the developing world, considerably affecting food supply and food sovereignty. The seed purchase not only makes a lot of farmers dependent on seed companies, but at the same time leads to the loss of the traditional adapted varieties and thus of agrobiodiversity.

Elinor Ostrom, the first Nobel Prize laureate for economics, has demonstrated how successful common property user communities (commoners) have been organized in the past and today. As often as Ostrom is cited in the commons movement – from open source software, to community projects in cities and municipalities, to agriculture, water supply, fisheries and economic theories – the «design principles» which she has identified for sustainable use of common property are rarely discussed. They are still inspiring and provocative even after more than 25 years since their first presentation.

The work of Elinor Ostrom gave reason to address the subject of seed and breeding from the perspective of the commons. However, it soon became clear that the transfer of the usage architecture of public domain natural resources such as water, pastures or fishing grounds to the conservation, utilization and breeding of seed and varieties is far from trivial. While depletion of natural resources have always been a concern, seed is characterized by the fact that it is lost only when not used anymore! That was and

still is the main reason for the alarming worldwide decline in agrobiodiversity.

Seed and crop varieties are associated with three different societal-social spheres. First, they are an economic or exchangeable commodity that is sold or passed on in the form of grains, seeds, cuttings or tubers. The same seed also forms a legal interest as a variety, whose use is regulated and protected in most countries of the world. Furthermore, it is also a fundamental cultural product and cultural heritage – similar to literature or music – which is dependent on the creativity, the perseverance and experience of a breeder or a breeder community. While the first sphere is still anchored in social consciousness, the second, and even more, the third, are prone to disappear. Modern molecular genetic methods are mostly overestimated and are of little relevance for the development of complex properties such as salt tolerance or drought resistance (see e.g. Gilbert 2014). Biodiversity is a result of the common evolution of man and nature (Vavilov 1932).

Organic breeding in Europe originated in the biodynamic movement and aspires to contribute to sustainable agriculture, biodiversity and food sovereignty. The spectrum of initiatives is wide and differentiated according to the objectives by each initiative. Most of them are organized as non-profit associations and thus show their closeness to common property and common good. Some initiatives are breeding for professional cultivation on a very high standard and in close interaction with their users. Others are concerned with the development of traditional cultivars and the conservation of traditional crops for agriculture and horticulture. Some projects involve participatory breeding with cooperation between scientists and farmers.

As in the objectives, the initiatives also differ in their social, political, legal and economic embeddedness. One of the major challenges for future development is to ensure the funding of future breeding activities. The requirements are particularly high when cultivating varieties for commercial organic farming, since quality expectations for organic raw materials and market products are higher and the cultivation conditions more demanding than in conventional agriculture.

Regarding the fact that nowadays only 1-5% of the varieties for organic farming are derived from organic farming, we face an enormous challenge. In contrast to the practice of organic propa-

gation of conventional varieties, the vision of using 100% of seed from organic breeding, as is being discussed in the ongoing revision of the EU organic regulation, is desirable but can hardly be implemented at present.

The importance of non-profit breeding initiatives can be justified historically and in principle. Historically, they are the continuation of the work of user communities through which the whole variety of crops has developed. In principle, breeding includes the following three elements: the regular reproduction and the selection of varieties, their distribution during periods of migration and the free exchange of seed among the different user communities as has been the case over the last 10 000 years across all continents.

With the help of examples, non-profit organic breeding initiatives are presented together with their rights and obligations:

- They breed many crop species and varieties for professional cultivation and hobby gardening. To meet the expectations of their customers, the intensity of the breeding, the methods used and the handling of the registration and the protection of their varieties can be very different. It appears that all forms of use, registration and ownership of varieties, as long as they are not patents, are compatible with the idea of common property and their user communities.
- The size of the user community is an important factor. On the one hand, it should be manageable because personal contact and familiarity create trust. On the other hand, it is a working hypothesis that, in the case of varieties for professional cultivation, all parties involved from the farmers to the entire downstream value chain, including the retail trade, can be considered to be members of the user community.
- Organic breeding contributes to a significant extent to the optimization of the achievements of organic production and thus to other subsistence commons. Organically bred varieties can cope with the nitrogen available in the soil, so that air and water are not polluted with artificial fertilizers. The varieties are open pollinated, *i.e.* self-propagating, and thus contribute to the increase of agrobiodiversity. Because production is not dependent on herbicides and pesticides, they support the ecosystem services of the biological and biodynamic producers for the benefit of the environment and health.
- The political request to use in the future only organically bred varieties in organic farming is an ambitious goal which can be achieved only with the material and ideal support of national and international government agencies. The financing of organic breeding is not possible solely through the sale of seed or through licensing fees for locally adapted regional varieties.
- Funding concepts and financial systems of non-profit plant breeding must relate to the users and their responsibility for both executive action and costs.

Future scenarios in industrialized countries:

- Of central importance is the geographical expansion of breeding activities beyond German-speaking regions.
- For this, the training of future responsible breeders will play a prominent role.
- Key factors for success are the differentiation, rationalization, co-ordination and interlinking of the activities as well as cooperation with new partners.

- Public relations activities aimed at the authorities and for promoting research and training centers must be expanded.

- Organic breeding improves the quality of products, as well as the raw materials for the value-added chain. Therefore, models for financing should involve all partners in the chain and the farming associations. For the former, a  $\frac{1}{10}$  of a percent fee on all fresh products is proposed, the latter could contribute with a steering and incentive tax.

- Organic breeding makes contributions to other commons. Since agrobiodiversity and ecosystem services are highly subsidized by governmental agencies the promotion of non-profit ecological breeding initiatives with money from these institutions is justified.

- The contribution of foundations is large and will remain so. Donors must recognize that breeding projects are always designed for cycles of 10-15 years, and therefore dependent on long-term commitments of funds.

In developing countries, breeding, seed propagation and cultivation are largely provided by the producers and production communities. In this way, agrobiodiversity is maintained to a large extent regionally and in some cases also newly created. At the same time, the challenge is to develop the traditional varieties quickly and effectively in the course of climate change, dwindling soil fertility and the partly low yields. This challenge has to be met in a difficult political environment and under the most difficult economic conditions. The rural communities are fragile, suffering from too few financial resources and often marginal recognition by governments and the international community.

Seed as a commons is dependent on user communities with structures, as has been detailed by Elinor Ostrom.

As a vision, we are proposing to actively create new seed and breeding communities as a third pillar alongside the international contracts (CBD and Nagoya Protocol, as well as ITPGRFA) to preserve agrobiodiversity.

Available instruments and recommendations for action:

- Food security and preservation of agrobiodiversity depend on a true assessment of the global costs and benefits of food production. As Sukhdev *et al.* (2016) emphasize, neither the maximization of the yields or profits per unit area nor the orientation towards the gross domestic product is suitable for this purpose. The one-sided reference to these two parameters obscures problems that are caused by high-yield agriculture. The authors estimate that high input agriculture accounts for 60% of biodiversity losses, 24% of greenhouse gas emissions and 33% deterioration in soil fertility. Last but not least, in many developing countries as well as in industrialized countries, there is not only undernourishment but also malnutrition, both resulting in rising healthcare costs.
- Nutritional sovereignty and agrobiodiversity depend on due consideration for farming communities. Their representatives must therefore be included in all negotiations where land sales to foreign investors, free trade zones and changes to seed laws are discussed and implemented.
- The recognition of traditional agriculture is essential for food sovereignty, agrobiodiversity and ecosystem services.
- The establishment of user communities that set their strategic and operational objectives and rules themselves, monitor their compliance, and punish non-compliance is dependent on recogni-

tion by the international community and national governments. Both support the formal establishment of such communities.

- On all continents, prototypes of user communities are developed for this purpose.
- In addition to their other activities, non-governmental organizations also contribute to the formation of user communities and the concrete formulation of the design principles identified by Elinor Ostrom.
- Together with farmers, they work out a monitoring system with which successes, problems and challenges can be recognized at an early stage. They support the actors in the further development and adaptation of these principles.
- Governments and authorities recognize, in addition to international treaties, this third form of protection for agrobiodiversity and provide the necessary legal and political freedom.
- In turn, the user communities undertake, with the support of many partner organizations, to intensify their cultivation methods permanently, to develop their crops continuously, to ensure seed storage and documentation, and to make their experience and knowledge available to other communities in a suitable format.
- By intensifying production, the existing crop species and varieties must not be destroyed. The support of traditional sustainable practices with locally adapted varieties is recognized by governments and international organizations as the key to achieving this.
- The economic existence of farmer communities must not be threatened by imports of food from industrialized countries. On the contrary, such communities should be supported in exporting any surpluses to other countries.

## Keywords

Biodiversity · common property · developing countries · organic plant breeding · seed system

## Note

The full version of the study of Wirz *et al.* (2017) is available in English, German or Italian upon request from the corresponding author.

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## Farm saved seed - Sensitive quality criteria and their impact to plant production

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Farm saved cereal seed is widely used in Austria. The percentage differs among species, on average it is about 50%. By specific testing services for farmers, AGES provides tests on seed quality for farm saved seed to prevent that farmers use seeds of poor quality. Thereby, the phytosanitary critical spread of seed related pathogens should be avoided. In an three years' evaluation period (2015-2017) relevant seed quality criteria of farm saved seed have been analysed and compared to the seed standards established for certified seed.

The results revealed that the seed quality of farm saved seed is very diverse, reaching from a very poor quality level up to fully compliance to common and legal seed standards. Among the tested criteria the main quality deficits are the content of other cereal seeds, a lower germination rate under stress conditions and, most relevant, an increased infection with *Tilletia* spp.

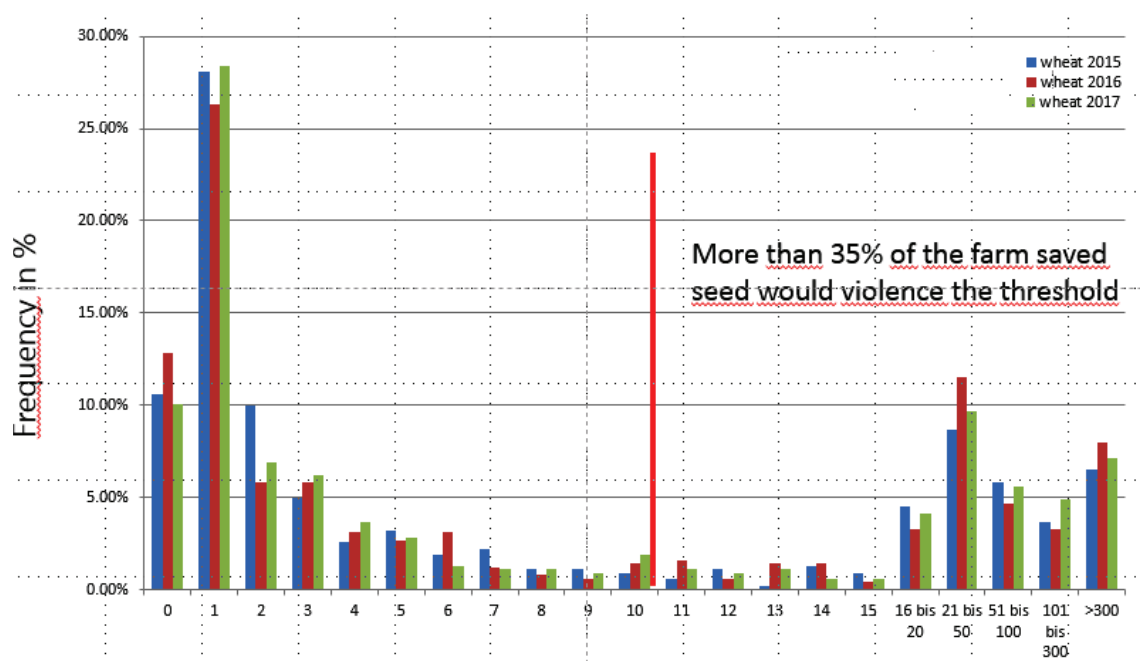
Other cereals seeds often couldn't be removed sufficiently by common seed treatment, therefore, it should be considered as

highly respected quality criteria. For some production branches, like malting barley or wheat of high baking quality, species and varietal purity is of high importance. Another outcome of the evaluation was the sensitiveness of triticale and rye to poor germination, which means that farm saved seed often do not catch the germination threshold of 85%.

Most relevant is the wide and hugh infestation with *Tilletia* spp. During the years 2015 to 2017 more than 35% of the farm saved seed samples showed an infection level higher than 10 spores per kernel, which is the current threshold for untreated seed use (Figure 1). Therefore, farm saved seed is considered as a major vector for infecting field production and for phytosanitary spread of the bunt pathogen.

### Keywords

Farm saved seed · germination · seed certification · *Tilletia*



**Figure 1:** Frequency of farm saved seed samples infected with 0 to >300 *Tilletia* spores per grain of wheat samples from 2015 to 2017



## Spectral sensing traits of nitrogen use efficiency in hybrid and line wheat cultivars

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### Abstract

Increasing nitrogen use efficiency is becoming increasingly important to cope with economic and ecological challenges in plant cultivation. Identifying the contribution of different traits contributing to dry matter production and nitrogen use efficiency may speed up the selection of more nitrogen-efficient genotypes in wheat. In a 3-year experiment with 13 hybrid and line wheat cultivars tested at 3 nitrogen levels, substantial differences in the grain N-uptake of approximately 30 kg ha<sup>-1</sup> at harvest were found. Using high-throughput spectral sensing, the predictability of traits influencing grain yield and N-uptake was investigated. The comparison of different methods and measurement dates revealed that models for N-uptake mostly led to closer correlations than those for dry matter traits. For most traits, simple vegetation indices from the group of red edge and water band indices delivered satisfactory results compared to multivariate PLSR-models. However, the complex trait of grain yield, which is also influenced by senescence characteristics, was better estimated by using PLSR-models. Generally, best correlations were obtained for measurements during the early grain-filling period, whereas a rather poor performance was observed during anthesis. Especially for dense canopies, optimized indices obtained from the passive hyperspectral sensor outperformed three tested active sensors. This may be attributed to the broader spectral bands, fewer bands and the weaker light source of the active sensors as compared to the passive sensor. The results support the application of spectral methods for high-throughput screening early in the season for selecting superior genotypes and for estimating the genetic potential.

### Keywords

High-throughput phenotyping · hyperspectral sensing · nitrogen uptake · Phenotrac · wheat phenomics · *Triticum aestivum*

### Introduction

With the tightening of regulations for nitrogen fertilization in crop production and the aggravating influence of climate change, the increase of nitrogen efficiency (NE) in quality wheat production and thus the selection for nitrogen-efficient genotypes will become even more important (Garnett *et al.* 2015). In wheat, the simultaneous consideration of grain yield and baking quality is decisive. In practice, the crude protein content in the grain is still used for this purpose. It can be extended with the grain yield to nitrogen uptake (Nup) of the harvest product (grain yield), which is closely related to the nitrogen uptake efficiency (NupEff) of the whole plant. This results in the definition of nitrogen efficiency as a product of nitrogen absorption and utilization efficiency (NE): NE = NupEff × NutEff (Moll *et al.* 1982). The nitrogen efficiency can be described from the contribution of different dry matter (DM) and N components (Figure 1).

Spectral methods have been used for detecting biomass (DM) and nitrogen uptake (Nup) in precision farming (Ali *et al.* 2016; Mistele & Schmidhalter 2006). Similar methods could also contribute to more efficient phenotyping of genotypes in order to detect the nitrogen efficiency (Araus & Cairns 2014). The aim of this study was to quantify the characteristics of NE in line and hybrid winter wheat cultivars in terms of their relevance for formation of grain yield (GY) and contribution to Nup and to compare them regarding their non-destructive spectral assessment.

### Materials and Methods

In a three-year trial (2014-2016) with line and hybrid winter wheat cultivars at Freising-Dürnast, organ-specific DM and Nup parameters were recorded at anthesis and grain filling. Depending on the year, 30 to 60 fertile culms were sampled and separated into leaves, stems and spikes at 2 to 4 growth stages. The areal conversion was carried out using the plant density. The experiment included 13 varieties at three N-levels, *i.e.* 4 hybrid cultivars: N1 = 100 kg N ha<sup>-1</sup>, N2 = 160 kg N ha<sup>-1</sup> and N3 = 220 kg N ha<sup>-1</sup>. In



Figure 1: Dry matter and nitrogen components contributing to grain yield and nitrogen uptake in chronological order.

2015, N1 was not sampled. Previously to plant sampling, spectral measurements were carried out in the 2<sup>nd</sup> and 3<sup>rd</sup> year (2015 and 2016) with the mobile multi-sensor platform *Phenotrac 4*, which, in addition to sensors having their own light source (active sensors), is equipped with a passive hyperspectral spectrometer (Erdle *et al.* 2013). Among the active sensors, a Greenseeker, CropCircle (CC) and Active Light Sensor (ALS) were tested (Kipp *et al.* 2014). In 2015, spectral sensing was performed on eight dates between 12<sup>th</sup> June (milk stage) and 22<sup>nd</sup> July (late dough) and in 2016 on 13 dates between 5<sup>th</sup> April (tillering) and 10<sup>th</sup> July (late dough) in order to compare different measurement dates.

The possibility of spectral estimation of the traits using 70 classical vegetation indices was tested through establishing linear models for all combinations of indices, measurement dates and plant traits. Models were established with data aggregated to cultivar  $\times$  N-level interactions from all N-levels and within the three N-levels. Additionally, the multivariate method of partial least squares regression (PLSR) was used to test for the best measurement date in each case. For this purpose, the spectral information of the passive spectrometer from 400 to 1000 nm was used. The evaluation was carried out with R (R Foundation for Statistical Computing, Vienna, Austria), and for PLSR models with the software Unscrambler® (Camo Software AS, Oslo, Norway).

## Results and discussion

### Components of yield formation and N-uptake

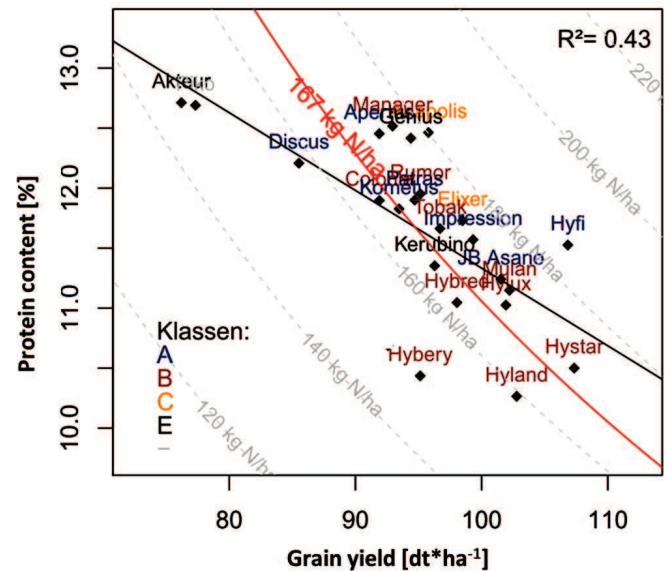
On average, about two thirds of the total biomass was formed until anthesis in the three experimental years. The yield differences between years were comparable to those between the N-levels, reaching from on average 7.9 t ha<sup>-1</sup> in 2015 to 9.1 t ha<sup>-1</sup> in 2016. On average, 28% of the biomass was translocated into the grain, which contributed 36-37% to the grain yield in all years.

For grain N-uptake, the proportion of nitrogen translocated from pre-anthesis uptake was always the most important component, which was found highest at low N fertilization (85% in N1 compared to 81% in N3). Only in N1, the average Nup exceeded the fertilized N (105 kg N ha<sup>-1</sup>), whereas only 139 kg N ha<sup>-1</sup> for N2 (160 kg ha<sup>-1</sup>) and 167 kg ha<sup>-1</sup> for N3 (220 kg N ha<sup>-1</sup>), respectively, were accounted for grain Nup. For some varieties, at the fertilization level of 220 kg N ha<sup>-1</sup>, no sufficient uptake was found to reach the limit of the average permissible N surplus of 50 kg N ha<sup>-1</sup> (Figure 2). Grouping according to quality groups could not be confirmed. Within the year  $\times$  N-stage combinations, the two components nitrogen translocation (N-TL) and post-anthesis Nup showed on average correlations with the grain Nup of  $r = 0.56$  and  $r = 0.52$ , respectively.

In 2015, additional plant sampling was conducted at cultivar-specific milk and dough ripeness. Total DM and Nup increased parallelly during grain filling (Figure 3). Little crossover between cultivars was observed, which represents an important prerequisite for making early spectral predictions of final grain yield and grain Nup.

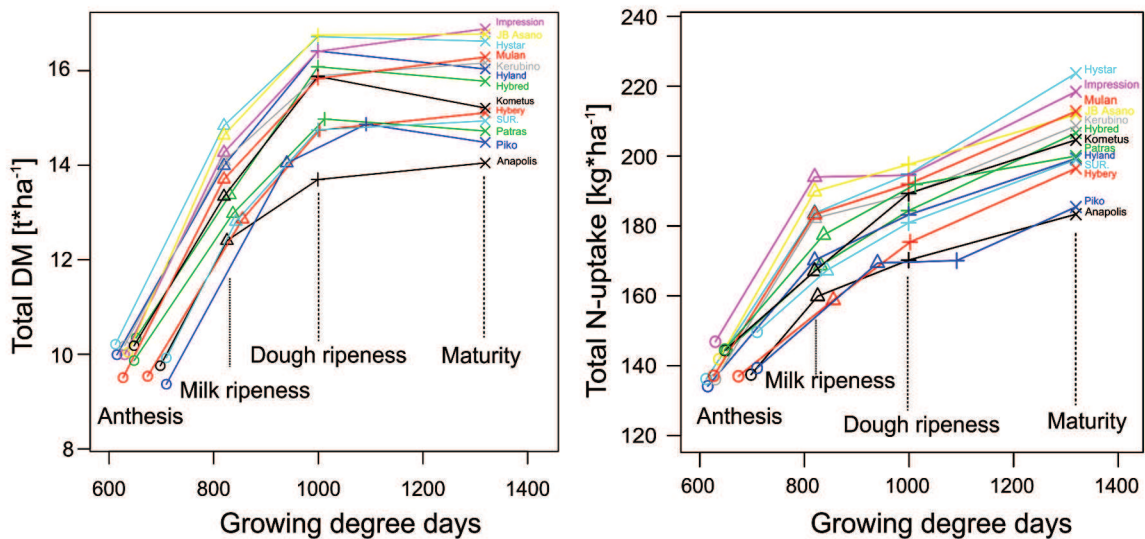
### Comparison of spectral predictability

Linear models and PLSR models were tested for both tested years. In both years, better models were found for the N-related traits than for the comparable DM-related traits (Table 1). With the best vegetation indices applied at the optimal measurement stage,



**Figure 2:** Grain protein content plotted against grain yield (14% moisture content) for the evaluated cultivars averaged over three years receiving 220 kg N ha<sup>-1</sup>, coloured by quality groups. Grey isolines indicate grain-N-uptake.

total Nup at anthesis was well estimated ( $R^2 = 0.83$  and  $0.86$  across N-levels, or  $0.60$  and  $0.65$  on average within the N-level in 2015 and 2016, respectively). An RMSE of 7 and 11 kg ha<sup>-1</sup> was achieved across the N-levels in both years. Slightly weaker associations were found for the individual organs, whereby in both years the best coefficients of determination were obtained for leaf-Nup ( $R^2 = 0.84$  and  $0.80$  across the N-levels). Similar values were obtained for leaf DM, whereas there were lower  $R^2$ -values reached for spike (not shown) and stem in both years. DM-translocation (max.  $R^2 = 0.43$ ) and post-anthesis assimilation (not shown, max.  $R^2 = 0.52$ ) showed poor to medium correlations, which may be due to the indirect character of the traits. For nitrogen, on the other hand, translocation was readily detectable ( $R^2 = 0.79$  and  $0.81$  in 2015 and 2016, respectively), since it mainly depends on the nitrogen uptake, whereas differences in the translocation efficiency were small. However, the post-anthesis Nup showed lower relationships (not shown, max.  $R^2 = 0.54$  within N-levels), however also contributed significantly less to the overall Nup (approx. 30% in 2015 and 13% in 2016). Both total DM and total Nup at maturity showed similar correlations like at anthesis. Similar to total Nup, it was possible to detect grain Nup ( $R^2 = 0.84$  and  $0.83$  in both years). Correlations with grain yield amounting to  $0.59$  and  $0.70$  in 2015 and 2016, respectively, with similar correlations within the N-levels, indicate the suitability of the models for distinguishing varietal properties. Despite the large number of indices tested, the group of 'Red-Edge' indices (Table 1: REIP, RVSI, R780/740, CC760/730, LCI) is excelling. These indices reflect the difference between the spectral regions of the red and NIR range, which becomes larger with higher biomass and N status. However, the widely used NDVI, which uses a wavelength in the NIR range (about 860 nm), was never among the best indices. Instead, some water-band indices showed good correlations (WBI, NDWI), which can be explained by the relationship between the foliar water content and the biomass as well as the weaker saturation of the bands in dense canopies (Peñuelas *et al.* 1993). The comparison of phenotyping methods at increased frequency in 2016 showed that measurements in the early to middle grain filling phase (milk



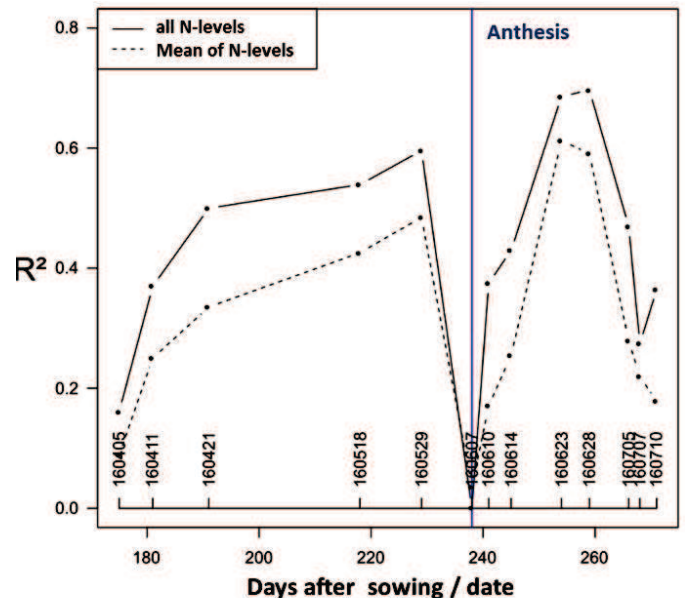
**Figure 3:** Temporal development from anthesis to maturity of total dry matter and total nitrogen uptake of 13 cultivars, averaged over N2 and N3 in 2015.

ripeness) were particularly suitable (ca. 7<sup>th</sup> to 28<sup>th</sup> June in 2016), which could be confirmed by a comparably good measurement date on 25<sup>th</sup> June, 2015 (Figure 4). On the other hand, hardly any correlations with grain yield could be found during ear emergence and anthesis, which could be caused by phenological differences due to the grain and anther color. However, already during booting, correlations were observed which, however, did not allow for exact predictions, but for a reduction in the breeding material to be recorded.

The PLSR-models mostly achieved similarly good correlations as the vegetation indices (Table 1). Particularly for the target traits grain-Nup and grain yield, improvements compared to the vegetation indices were found. This indicates an advantage of the combination of different wavelengths for sensing the phenological status. This effect seems to be important because anthesis biomass showed only medium relations to grain yield ( $r = 0.73$  in 2015) and is not sufficient for explaining the spectral prediction of the grain yield in the early grain filling phase. The three active sensors mostly performed less good compared to the best indices derived from the passive spectrometer. Exceptions were found in 2015 for some traits at anthesis, which could be due to the thinner canopy in this particular year. Among the active sensors, the ALS sensor showed the best correlations.

## Conclusions

The results show the suitability of spectral methods for estimating different traits related to DM and N which influence NE of winter wheat varieties. Multivariate methods seem to be particularly useful for the early assessment of complex characteristics, which are also influenced by phenological differences, whereas red edge and water-band indices appear to be suitable for the direct sensing of plant canopies. Active sensors hold potential and advantages with respect to costs and application, but there is a need for further improvement in measurement technology. Alternatively, applying multispectral cameras for high-throughput phenotyping with drones is promising and should be tested under practical conditions.



**Figure 4:** Correlations of the best water band index (WBI) with grain yield tested across and within three N-levels, plotted by measurement dates (days after sowing) in 2016.

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**Table 1:** Overview of correlation models for selected DM- and N-traits: MV, mean value; Nup, N-uptake; TL, translocation; VI, vegetation index; Anth., Anthesis. The values before (a) and after (b) the vertical line refer to the best models identified in 2015 and 2016 models, respectively. MV comprise N-levels included in the models (2015: N2-N3, 2016: N1-N3). The VI reaching best correlations for each trait tested over the season is reported. See The IDB Project (2017) for details on VI.

		Anth.-DM (total)	Anth.-DM (culm)	Anth.-DM (leaf)	DM-TL	Total DM	Grain Yield
MV (trait), all N-levels		9917 <sup>a</sup>  10919 <sup>b</sup>	6192 6935	1781 1932	3189 3177	15619 16570	8892 8525
R <sup>2</sup> , all N-levels	VI	0.69 0.57	0.58 0.38	0.84 0.87	0.20 0.27	0.70 0.57	0.59 0.70
	active VI	0.67 0.34	0.46 0.18	0.75 0.77	0.19 0.17	0.5 0.64	0.47 0.58
	PLSR	0.71 0.54	0.52 0.38	0.85 0.93	n.s. 0.24	0.75 0.54	0.71 0.81
R <sup>2</sup> , MV of N-levels (VI)		0.59 0.49	0.57 0.43	0.63 0.7	0.32 0.43	0.67 0.49	0.72 0.66
RMSE,	VI	318 1126	249 889	81 160	392 1040	516 1126	454 592
all N-levels	PLSR	348 1185	271 898	79 116	473 1076	712 1185	493 551
best VI		REIP REIP	REIP RVSI	LCI 780/740	PRI RVSI	RVSI REIP	WBI WBI
		Anth.-Nup (total)	Anth.-NA (culm)	Anth.-NA	N-TL	Total Nup	Grain Nup
MV (trait), all N-levels		126 141	48 58	48 51	104 113	183 171	162 143
R <sup>2</sup> ,	VI	0.83 0.86	0.75 0.63	0.84 0.80	0.79 0.81	0.84 0.86	0.84 0.83
	active VI	0.82 0.79	0.65 0.61	0.84 0.71	0.78 0.67	0.71 0.87	0.64 0.82
	PLSR	0.82 0.87	0.80 0.62	0.81 0.9	n.s. 0.24	0.91 0.89	0.88 0.96
R <sup>2</sup> , MV of N-levels (VI)		0.60 0.65	0.57 0.59	0.51 0.56	0.57 0.6	0.58 0.65	0.70 0.61
RMSE,	VI	7 11	3 7	3 8	6 10	10 11	9 10
all N-levels	PLSR	7 11	3 7	4 5	7 10	8 10	8 5
best VI		ALS RVSI	790/670 DSWI	ALS REIP	ALS RVSI	NDWI RVSI	WBI RVSI

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## LIVESEED - A project boosting varieties and seeds in organic farming funded by EU

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LIVESEED ([www.liveseed.eu](http://www.liveseed.eu)) is a project funded by EU H2020 research program and consists of 49 partner organizations coming from 17 Member States and Switzerland. LIVESEED aims to improve the sustainability, performance, and competitiveness of the organic sector by boosting organic seed production, developing novel breeding approaches to increase the choice of cultivars of various crop species adapted to organic and low-input agriculture for different pedo-climatic conditions in Europe and by harmonizing the implementation of the European regulations in relation to organic seed.

In order to achieve its goal, LIVESEED will specifically provide a level playing field regarding the use of organic seed across Europe. Some activities are analyzing the determinants of the current production and use of organic seed, the identification of breeding gaps of crops, increasing transparency of the EU organic seed market or improving the implementation of legislative requirements in close collaboration with national authorities in EU Member States.

A further work package is aimed to increase the volume and quality of organic seeds derived from cultivars suited for organic farming by developing and improving the efficiency of cultivar testing under organic farming. Suitable and adjusted protocols for 'Distinctiveness, Uniformity, Stability' (DUS) and 'Value for Cultivation and Use' (VCU) testing will be identified to foster the registration of open pollinated varieties and develop new descriptors for

the marketing of heterogeneous population. Furthermore, knowledge will be shared and training courses on smart practices for organic seed multiplication be organized across countries, as well as novel seed health strategies and technologies focusing on the vitality of organic seed will be investigated.

In the two above mentioned work packages AGES is involved in providing experience and adapting testing protocols and certification processes corresponding to the needs and characteristics of organic farming which have been established in Austria. AGES will contribute to an increased harmonization of the existing systems across Europe.

Further work packages within the project are, in brief, activities on accelerated breeding processes and adoption of new cultivars, improving the competitiveness of the organic seed sector by identifying gaps and bottlenecks in the market development of organic seeds and breeding and knowledge sharing and dissemination of LIVESEED results by building the capacity of breeders, seed producers, farmers, retailers, researchers and other actors of the food value chain.

The project has a duration for four years and will end in 2021.

### Keywords

Organic breeding · organic seed · seed certification · seed health





## Efficient and quantifiable scarification of *Sida hermaphrodita* seeds to overcome dormancy and increase germination

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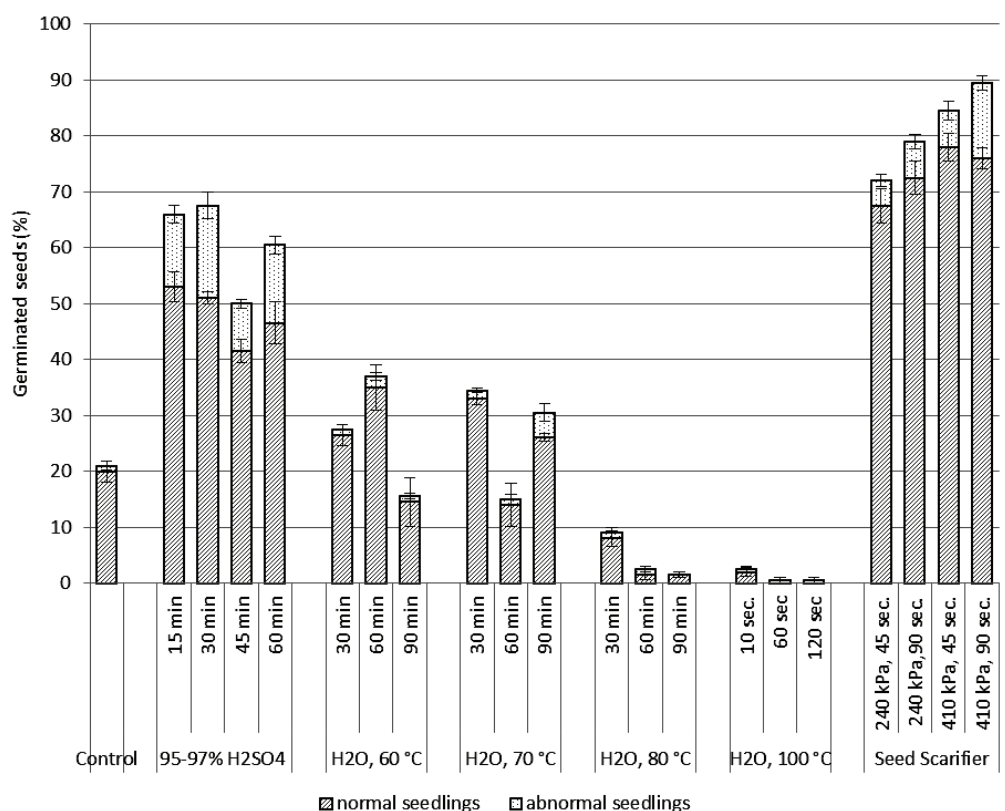
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To increase the biodiversity in the agricultural landscape, the perennial forb *Sida hermaphrodita*, which biomass is used for thermal utilization, can be a promising addition to the spectrum of bioenergy crops used in Austria. The plant shows multiple ecological benefits due to its perennial nature (reduced erosion), its thick and weed suppressing growth (no herbicides needed during the years of agricultural use), and its long flowering period (food source for pollinators). *S. hermaphrodita* belongs to the family *Malvaceae* and can be cultivated and harvested at the same site for over ten years, with low intensity care once established. However, stand establishment to date mostly occurs by cost- and work-intensive planting of pre-grown seedlings or root cuttings, since the seeds of *S. hermaphrodita* display a distinctive physical dormancy. An impermeable testa prevents the imbibition of water by the embryo, resulting in the failure of the otherwise intact and viable seed to start the process of germination. Different described methods were analysed in the research project SIDeCA (*Sida: Intelligent Densified Energy Carriers for Austria*) to efficiently and quantifiably break the dormancy of *S. hermaphrodita*.

Freshly harvested seeds from the field trial site Grabenegg were used in the laboratory experiments. To break physical dormancy, seeds were pretreated with (i) 95-97% H<sub>2</sub>SO<sub>4</sub> for 15, 30, 45 and 60 min; with (ii) 60°C, 70°C and 80°C hot water for 30, 60 and 90 min respectively; with (iii) 100°C hot water for 10, 60 and 120 s; and (iv) by using a pneumatic seed scarifier (PSS 2000) with four different factor levels (240 kPa for 45 s, 240 kPa for 90 s, 410 kPa for 45 s and 410 kPa for 90 s). The pretreated seeds and a control were then incubated at 20°C in the dark for 21 days, after which

the percentage of germinated seeds were determined. All germinated seeds were additionally differentiated into abnormal and normal seedlings, according to the development of their radicle and their cotyledons.

Non-pretreated seeds of *S. hermaphrodita* displayed a very low rate of germination (around 20% germinated seeds), which could be significantly increased with described pretreatments to break physical dormancy (Figure 1). Sulfuric acid treatment in general had a dormancy breaking effect, however the amount of abnormal seedlings also increased significantly. Pretreating seeds with hot water produced mixed results. While lower temperature levels for shorter amount of time increased germination slightly, an increase in temperature caused germination to decrease to a point where no more germination occurred. The best results were noticed



**Figure 1:** The different tested pretreatment methods and their effect on germination of *S. hermaphrodita* seeds

when the pneumatic seed scarifier was used, which had a highly significant, continuously increasing effect on germination. However, with an increase in pressure and duration of the pretreatment, a simultaneous increase in abnormal seedlings was noticed. When using a second degree polynomial as a trend function, a pretreatment optimum was determined at the factor level 410 kPa for 45 seconds.

To increase the germination rate of the seeds of the promising energy crop *S. hermaphrodita*, an efficient, quantifiable, and reproducible method to break the existent physical dormancy must be found. The effect of several different described pretreatment methods were tested in the laboratory, with a pneumatic seed scarifier being the most effective one. We determined the optimal pretreatment intensity with our seed scarifier to be at 410 kPa for 45 s. These results can be applied to produce *S. hermaphrodita* seeds with a high germination capacity, which could help to establish this energy crop by sowing, thereby reducing the costs and making the plant more attractive for interested farmers.

## Keywords

Energy crop · physical dormancy · pneumatic seed scarifier

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## Distribution of *Cannabis* chemotypes in European agricultural hemp cultivars

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In Europe around 50 officially approved agricultural hemp cultivars of *Cannabis sativa* L. are grown and should contain <0.2% tetrahydrocannabinol (THC) (EC 73/2009), a psychoactive cannabinoid. Next to this, numerous drug strains with a THC content up to >20% are grown illegally for drug production. So far, a differentiation of these two groups relies on a time-consuming quantitative biochemical assessment of cannabinoids in flowering plant material (EC 1122/2009).

The genetic determination of the so-called chemotype, a category superior to a cultivar, could be used for a more efficient differentiation and can also be applied to non-flowering material and to material devoid of cannabinoids (seeds and roots). Depending on the relative ratio of the cannabinoids THC and non-psychoactive cannabidiol (CBD) in mature inflorescences, three discrete chemical phenotypes (chemotypes) can be distinguished: a "THC-predominant" type, a "CBD-predominant" type and an "intermediate" chemotype. Chemotypes are qualitative traits, their use for certain cultivars or strains is man-made and depends on the breeder. They are not necessarily linked to the absolute THC yield. The distribution of chemotypes in 62 European agricultural hemp cultivars has systematically been investigated in this survey. The resulting database is available at AGES for forensic experts and breeders of agricultural hemp (<http://www.kiras.at/en/financed-proposals/detail/d/canndat/>).

### Keywords

Cannabidiol · *Cannabis sativa* · psychotropic substance · tetrahydrocannabinol



## ‘Sorting the wheat from the chaff’ - Comments on the spelt [:wheat] discussion

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### Abstract

The interest in spelt wheat and other so-called ‘ancient grains’ increased significantly in recent years. Compared to einkorn and emmer, however, spelt is not an ‘ancient’ wheat in terms of a cereal founder crop. Moreover, breeding programs in the second half of the 20<sup>th</sup> century changed the phenotype of spelt wheat from a tall, lodging prone and low input and low yielding crop to a semi-dwarf crop with high yield potential under higher inputs by crosses with common wheat. Since then, there’s a discussion on the ‘purity’ and/or authenticity of spelt among consumers, processors, growers and breeders. In the following, the history of European spelt wheat is outlined, demonstrating that the crop experienced repeatedly gene introgressions from common wheat. Methods claiming to differentiate between pure/traditional and modern varieties based on storage protein patterns are in practice not working for authenticity control. Honoring consumers’ trust in spelt products, irrespective of their motives, it is suggested that breeders stick to typical spelt characteristics during their selection and that processors keep the specific quality traits in spelt products. In turn, marketers are invited not to make false claims related to health issues.

### Keywords

Ancient grain · breeding · consumer trust · domestication · hulled wheat · *Triticum aestivum* · *Triticum spelta* · wheat evolution

### Introduction

The acreage of spelt wheat (*Triticum spelta* L.) increased significantly in recent years, especially in Central European countries (Grausgruber 2017). The reasons for this increase are manifold. First of all, the consumption of modern wheat varieties was blamed - without scientific evidence - to be responsible for all kinds of adverse health effects by some bestseller books (Brouns *et al.* 2013, Kelly 2015). As a consequence, many consumers turned to a gluten-free diet or to so-called ‘ancient grains’ such as amaranth, quinoa, millets, einkorn, emmer or spelt (WGC 2018), species which have been used to feed the world for thousands of years but were replaced by high yielding cash crops and varieties during the 20<sup>th</sup> century and were, therefore, never exposed to intensive breeding programmes (Longin & Würschum 2016). Moreover, the increased interest in traditional folk wisdom and medi-

cine rediscovered the medicinal and scientific writings of Hildegard of Bingen (1078-1179), the German Benedictine abbess, who considered spelt wheat to be superior to any other grain in the diet, an optimal food in all forms (bread, porridge, coffee) for all gastrointestinal problems (Strehlow & Hertzka 1988). Today, a wide range of spelt products, from cookies to bread, are available in Central Europe with Hildegard as eponym.

### Discussion on ‘pure spelt’

With the increased interest and acreage very soon a discussion on the ‘purity’ of spelt wheat started. Growers and processors of old varieties and/or landraces claimed the legacy of the traditional and pure spelt wheat cultivation for them, blaming modern spelt wheat varieties to be less nutritious and less healthy, and having spelt atypical baking quality due to gene introgressions from common wheat (*T. aestivum*). Brands and production chains were established excluding modern varieties from production, e.g. Urdinkel of IG Dinkel (Bärau, Switzerland; [www.urdinkel.ch](http://www.urdinkel.ch)). Most initiatives which grow ‘Urdinkel’ today rely on old varieties such as ‘Oberkulmer Rotkorn’, ‘Ostro’, ‘Bauländer Spelz’ or ‘Steiners Roter Tiroler’ or on varieties which were selected from (crosses between) old varieties, e.g. ‘Ebners Rotkorn’ or ‘Schwabenkorn’ (Münzing *et al.* 2009) although these varieties are less productive and taller with a tendency to lodging (Longin & Würschum 2014, Galbusera 2015). Moreover, there is no evidence that modern varieties are more “allergic” or less nutritious (Reents & Mück 1999). Various studies were carried out to classify spelt varieties, most of them based on polymorphism in the omega and gamma gliadin pattern (Schober & Kuhn 2003, Wieser 2006, Mayer *et al.* 2012, Koenig *et al.* 2015). However, all these methods failed to identify spelt wheat cultivars which have common wheat in their pedigrees perfectly (Becker *et al.* 2008, Breuer *et al.* 2014, Koenig *et al.* 2015).

### History of European spelt wheat cultivation

Spelt remnants were found in many archaeological excavations across Europe from the Late Neolithic to the Iron Age period. In most cases individual specimen were found among other cereals (Körber-Grohne 1989). From archaeological evidence it is assumed that the cultivation of *T. spelta* in Europe started around 2300 B.C. in parts of Switzerland (Akeret 2005). After the Iron Age, the retreat of spelt wheat to marginal areas in Europe started (Körber-Grohne 1989). From the Roman period until the High Medieval period, spelt wheat was the main crop in most parts of Alamannia

(syn. Alemannia, Suebia), the later Duchy of Swabia. Later on, rye (*Secale cereale*) became the dominant grain here, however, spelt wheat remained important, in northern Switzerland still as main crop (Gradmann 1909, Rösch *et al.* 1992, Schilperoord 2013). Spelt remained for long times also a main crop in areas of Asturias (Buxó i Capdevila 1989), southeast Belgium (Billen 1989, Deman 1989, de Moreau de Gerbey 1989) and the Carpathian mountains (Markus 1989).

### The origin of spelt wheat

For decades, the origin of spelt wheat was supposed to be Alemannia (Gradmann 1909), where the crop evolved between Stone and Bronze Age (Schiemann 1932). Kihara (1944) published the first sound theory regarding the origin of spelt wheat which was confirmed by the artificial synthesis of *T. spelta* by crossing wild emmer (*T. dicoccoides*) with Tausch's goatgrass (*T. tauschii*; syn. *Aegilops squarrosa*) by McFadden & Sears (1945, 1946a,b). Thereafter, Schiemann (1947, 1951) withdrew her first theory and supported the theory that spelt wheat evolved in the regions where *T. dicoccoides*, *T. dicoccum* and *T. tauschii* were naturally present, whereas Bertsch (1949, 1950) continued to claim southwestern Germany as centre of origin of spelt wheat. The findings of *T. spelta* in Iran (Kuckuck & Schiemann 1957) strongly supported southwestern Asia as the area of origination.

It was argued that spelt wheat found his way from Transcaucasia to Eastern Europe and Pannonia, and during the Migration Period with the Suebi to southwestern Germany where the crop was excellently adapted to the prevailing growing conditions (Andrews 1964). Decades later, many research studies on *e.g.* differences in spike disarticulation, allozyme polymorphism (Jaaska 1978), RFLP data (Dvorak & Luo 2001), and variation in the glutenin and gamma gliadin genes (von Buren 2001, Blatter *et al.* 2002, Yan *et al.* 2003, An *et al.* 2005) suggested that European and Asian spelt may be polyphyletic. The separate origins of European and Asian spelt were confirmed by Dvorak *et al.* (2012) studying the allele diversity in the *Tg* (tenacious glume), *Sog* (soft glume), *q* (speltoid spike) and *C* (compact spike) loci. The findings were consistent with suggestions that European spelt derived from a hybridization of hulled emmer with free-threshing hexaploid wheat (Schiemann 1932). The presence of the *C* allele in some European spelt accessions substantiated the hypothesis that at least for some European spelt genotypes the free-threshing hexaploid wheat was club wheat (*T. compactum*). Recently, Novoselskaya-Dragovich *et al.* (2018) confirmed the independent origin of European spelt investigating the polymorphism of LTR retrotransposons in hexploid wheats.

### Spelt wheat breeding in Europe

When the acreage of spelt wheat in Central Europe ceased at the beginning of the 20<sup>th</sup> century, breeding activities were initiated in Germany and Switzerland to improve the crop. In Baden, Stoll in Meckesheim and Lang in Gaiberg, in Württemberg, the Saatuchtanstalt Hohenheim, Zeiner at the Kgl. Staatsdomäne Neuhaus near Mergentheim and Aldinger at the Steiner'sche Schlossgut Laupheim, and in Bavaria, Fischer in Illertissen and the Fugger'sche Gutsverwaltung in Babenhausen, were involved in spelt wheat improvement (Hillmann 1910, Baur 1920). Almost all breeding stations focused on the selection of elite plants in existing landraces followed by pedigree selection. Thereby, *e.g.* the variety 'Steiner's roter Tiroler Dinkel' was selected from the landrace 'Roter Tiroler' (provenance Höchst, Vorarlberg) which was the most popular

In der beschriebenen Weise habe ich bis jetzt folgende Kreuzungen zwischen Weizen und Spelz mit Erfolg ausgeführt:

Main's standup-Winterweizen	♀ ×	brauner Winterkolbenspelz	♂
Square-head-	♀ ×	"	♂
"	♂ ×	"	♀
Bordier.	♀ ×	"	♂
Rivett's bearded (Rauhweizen)	♀ ×	"	♂

4\*\*

**Figure 1:** Excerpt of Stoll's work on spelt wheat describing the spelt × wheat crosses he carried out to improve grain yield, straw stiffness, rust resistance and ear morphology (Stoll 1902)

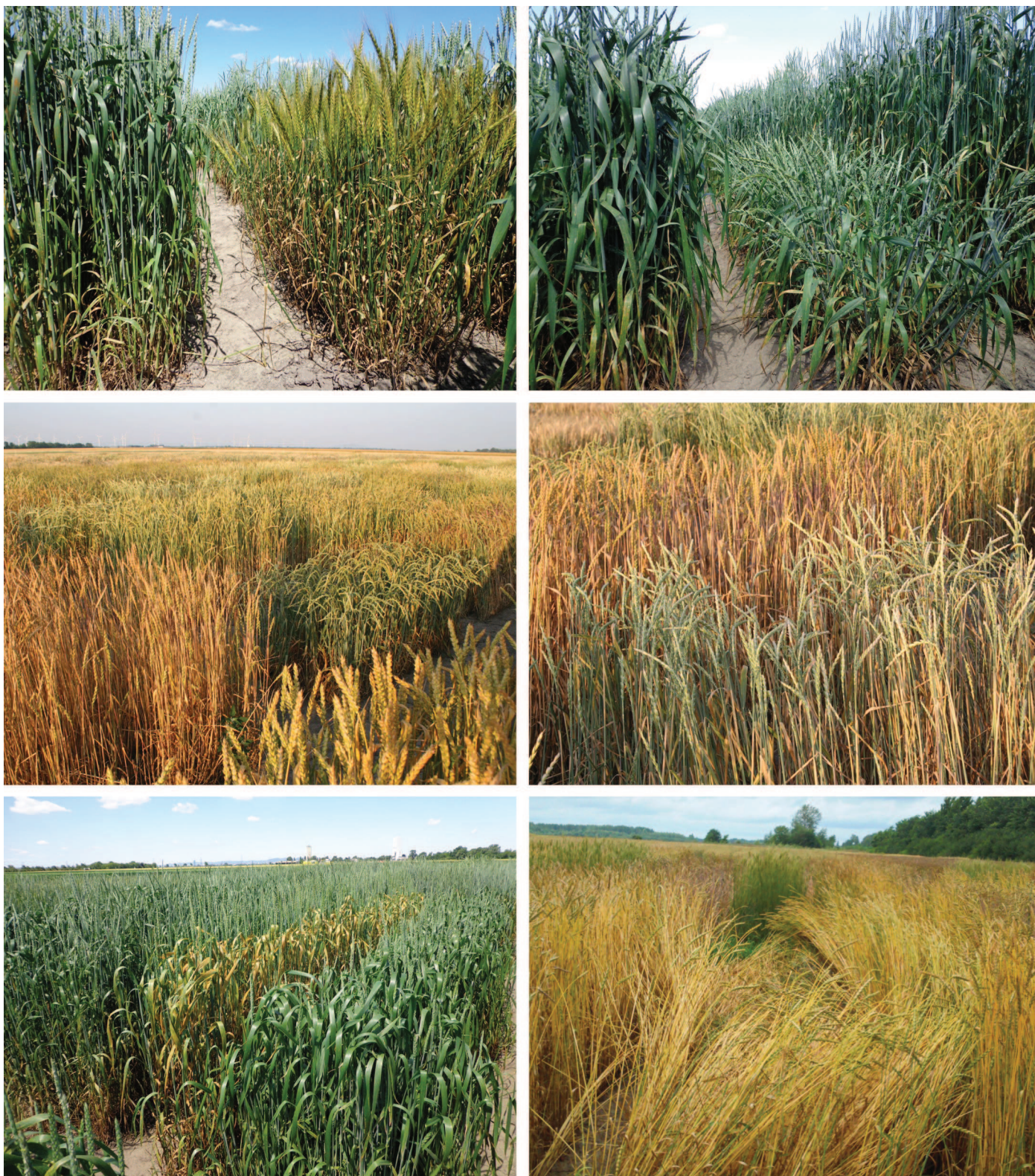
variety at that time in Swabia. Seeds of this landrace was regularly imported from Lustenau, Höchst and Bregenz in Vorarlberg across Lake Constance (Baur 1920).

Heinrich Stoll started already in 1894 with spelt wheat breeding and after no success in finding favorable spontaneous mutants, crosses with common wheat were established: 'Heine's Square-head', 'Bordier', 'Main's Standup' and 'Rivett's bearded' were crossed with 'Roter Tiroler' and 'Roter Winterspelz' (Figure 1) (Stoll 1902, Hillmann 1910, Baur 1920). From two crosses, three new varieties were developed (*i.e.* 'Stolls brauner Winterkolbenspelz', 'Stolls weißer Winterkolbenspelz', 'Stolls früher Riesenpelz') (Hillmann 1910). In Hohenheim, two lines (*i.e.* Roter Kolbendinkel Nr. 1, Schlegeldinkel 9a), which were obviously natural hybrids with common wheat, were selected and distributed to other breeding stations for further development and seed multiplication. Moreover, crosses between 'Schlegeldinkel' and 'Squarehead' wheat were carried out in 1904 (Hillmann 1910). Already Körnicke (1885) described in his book on cereal varieties three different spelt varieties (*i.e.* 'Weisser sammetiger Kolbenspelz', 'Weisser sammetiger Grannenspelz' and 'Roter sammetiger Grannenspelz') which were most likely spontaneous hybrids with common wheat. It seems that natural hybridizations between spelt and common wheat appeared not rarely in mixed or nearby crop stands.

In Switzerland, the identification of individual elite plants followed by pedigree selection in Swiss Rotkorn and Weißkorn landraces started in 1908. The work was carried out by farmers under the supervision of the federal seed testing and experimental stations in Oerlikon (Zurich) and Lausanne (Baur 1920, Schilperoord 2013). Selection criteria were *e.g.* plant height, awnedness, thousand grain weight and spike density. After official tests, seeds of the best lines (*e.g.* Oberkulm Nr. 3' as Rotkorn and 'Zuzgen Nr. 5' as Weißkorn type) were multiplied and distributed. A second selection cycle and official testing followed end of the 1920s which resulted in the release of further landrace varieties. Due to costs, however, the number of varieties was regionally reduced in the 1930s to two to three varieties each of the Rotkorn and Weißkorn type (Schilperoord 2013).

Despite these first breeding activities, spelt cultivation in Europe nearly disappeared in the second half of the 20<sup>th</sup> century. In the 1970s, the growing interest in unconventional food and low-input agriculture led to a regional revival of spelt wheat cultivation, especially in marginal areas. To counter the main problem of low productivity and susceptibility to lodging, modern breeding programs established in Belgium, Germany and Switzerland introgressed genes from common wheat to reduce plant height (*i.e.* *Rht* genes) as well as to improve grain yield and technological quality (Kling 1989, 1991, 2009; Winzeler 1989, 1991; Bertin *et al.*





**Figure 2:** Diversity in spelt wheat genetic resources, varieties and breeding lines with respect to ear morphology and habit, maturity, plant height, yellow rust and lodging resistance, and culm and ear discoloration during ripening (HealthyMinorCereals spelt diversity panel grown at Raasdorf, Austria, 2015 & 2017 - all photos by H. Grausgruber, except bottom right: spelt trial at Jõgeva, Estonia, 2015, by R. Koppel)

2001, Schilperoord 2013). Meanwhile small-scale breeding activities were established in many other European countries which resulted in the release of modern spelt cultivars, some of them originating from crosses with common wheat (e.g. in Poland and Italy) and showing more common wheat specific traits (i.e. high percentage of free-threshing grains, plump and medium long seeds). In Slovakia, 'PS Lubica' was selected from a cross between

common and spelt wheat and registered as common wheat as it is free-threshing but showing technological quality similar to spelt (Hanková et al. 2014). Contrary, 'Emiliano' was registered as free-threshing spelt conservation variety in Germany (Müller 2016).

By April 2018, 61 registered spelt wheat varieties are included in the plant variety database of the European Commission. Among these, seven varieties are registered as conservation varieties.



Most of the varieties originate from Germany, Italy and Switzerland. Further countries with current registrations are Hungary, Austria, Belgium, Poland, Slovenia, Spain, Croatia, Czech Republic and The Netherlands. This data illustrates that spelt wheat is today cultivated across Europe, far beyond its 'traditional' growing area in southwestern Germany, Switzerland, western Austria and southeastern Belgium.

The currently available varieties can be grouped roughly in three classes: (i) old landraces and traditional varieties, and (conservation) varieties selected from them with low to medium yield, tall plant height and susceptibility to lodging, but individually high resistance to diseases; (ii) modern varieties with reduced plant height, improved lodging tolerance, medium to very high yield (especially under high-input conditions), but partly spelt atypical characteristics, e.g. higher percentage of free threshing grains, shorter and more plumb seeds; (iii) modern varieties from organic breeding programs with improved lodging tolerance despite relatively tall plant height, medium to high yield (especially under low-input/organic production), and spelt typical characteristics, e.g. long, narrow and sharp-edged grains, culm coloring during ripening (Figure 2).

## Conclusions

During its history, European spelt wheat experienced gene introgressions from common wheat several times. Already its origin is based on a natural hybridization between emmer and common wheat. It is also documented that breeding at the end of the 19<sup>th</sup> century often relied on the selection of offsprings from natural outcrossings between spelt and common wheat, appearing in mixed and/or neighboring crop stands. Hence, it can be assumed that natural hybridizations between wheat (sub)species appeared many a time, especially in mixed crop stands. At the same time, first spelt breeders in Germany started with spelt × common wheat crosses with the aim to improve yield, threshability and lodging resistance. With the introgression of the genes of the Green Revolution in the second half of the 20<sup>th</sup> century, spelt wheat changed its phenotype significantly from a tall crop, excellently adapted to low-input and adverse growing conditions, to a semi-dwarf crop with high yield potential at mineral fertilization and application of growth regulators.

Consequently, the terminology of 'pure spelt' varieties is not defined but just randomly used for marketing. The use of the gliadin pattern for the classification of spelt wheat varieties does not reflect the percentage of common wheat introgression, but is only the (random) result of selection. Thereby it can be explained why is such analyses old and tall varieties (e.g. 'Altgold', 'Steiners roter Tiroler', 'Oberkulmer', 'Ostro') group together with modern, semi-dwarf varieties (e.g. 'Badenkrone', 'Cosmos', 'Spy' 'Zollernspelz') (Koenig *et al.* 2015).

Without doubt, spelt wheat needs, as well as other crops, genetic improvement to cope with changes in growing conditions due to global warming, e.g. new (races of) pathogens, reduced vegetation period, heat stress during grain filling, etc. Therefore, using genes from other wheat (sub)species may be necessary. However, it must be considered that despite the still high interest in 'ancient wheats', the market is highly volatile and sensible. Therefore, breeders have to consider in their selection characteristic spelt traits such as the hulledness, size and shape of the grain, ear morphology, process of ripening with the typical discoloration of the culm, specific taste, chemical composition and product quality.

Many consumers prefer spelt products for whatever the reason. Both processors and breeders have to appreciate the consumers' trust in spelt wheat by providing an authentic product.

Besides common wheat, the diversity present in other spelt gene pools such as the Spanish or Asian, or Georgian spelt (*T. macha*) (Cao *et al.* 1998, Elía *et al.* 2004, Novoselskaya-Dragovich *et al.* 2018) can be used to broaden the the genetic basis of European spelt.

## Keywords

Ancient grain · breeding history · centre of diversity · domestication · *Triticum spelta* · wheat evolution

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## Grain yield of Austrian wheat varieties and the prospect of their distribution in Georgia

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### Abstract

Georgia is a country of ancient wheat cultivation with a great diversity caused by the cultivation in different soil climatic conditions. Each region of Georgia is cultivated with regionally adapted wheat landraces. In the 1950s wheat varieties from France, Italy, Hungary, Germany and Russia were introduced for evaluation and inclusion in breeding programs. In 2014, eight modern Austrian wheat varieties were introduced within a cooperation between Austria and Georgia and tested at the facilities of the Scientific Research Center of Agriculture.

### Keywords

Adaptation · baking quality · grain yield · *Triticum aestivum*

### Introduction

Wheat is an ancient crop in Georgian agriculture and within its evolution many distinct types were domesticated in this center of diversity, e.g. Georgian spelt wheat (*Triticum macha*), Colchic emmer (*T. palaeocolchicum*), Persian wheat (*T. carthlicum*) or Zanduri wheat (*T. timopheevi*) (Mosulishvili *et al.* 2017). For centuries Georgia was self-sufficient in wheat production, however, during the last century Georgia was transformed into the 'fruit basket' of the Soviet Union and wheat production ceased. Therefore, between 1992 and 2016 Georgia had to import between 343 000 and 919 000 tons of wheat from Europe, Russia and Ukraine to meet domestic consumption (www.indexmundi.com). In recent years Georgian wheat production dropped below 50 000 ha with a maximum yield of 25 dt ha<sup>-1</sup> (www.fao.org/faostat/). To increase wheat production, modern varieties from abroad are tested for their adaptation and performance under Georgian conditions by the Scientific Research Center of Agriculture (SRCA).

### Materials und Methods

Eight Austrian common wheat (*T. aestivum*) varieties (Table 1) were grown from 2014 to 2016 in the main wheat production regions of Georgia, i.e. Dedoplistskaro (Shavchrelebi), located at 602 m a.s.l. altitude and characterized by an average annual temperature of 10.1°C and 585 annual precipitation; Telavi (Gulgula): 396 m a.s.l., 11.8°C and 770 mm; Akhaltsikhe (Vale, Chorati): 1120 m a.s.l., 9.0°C and 698 mm; Khashuri: 680 m a.s.l., 9.7°C and 565

mm (Kevkhishvili 1998). The trials were laid out with a plot size of 100 m<sup>2</sup> and two replications.

During growth, the plots were scored for seedling emergence, winter damage, heading, flowering and maturity. Resistance was assessed against yellow and stem rust, Septoria, Fusarium head blight and powdery mildew. From each variety 25 plants were taken to record plant height, number of productive tillers, ear length, number of spikelets per ear, number of grains per ear, ear weight and the thousand-kernel weight. Grain yield was determined based on 50 ears and by harvesting a 1 m<sup>2</sup> subplot of each plot.

In 2015, resistance against leaf and stem rust was determined at Kobuleti (Scientific direction of Phytopathology and Biodiversity, BRSU Scientific Center) using artificial infections. The plants' disease reaction was scored according to the modified Cobb scale: 0, immune; R, resistant; MR, moderately resistant; M, intermediate; MS, moderately susceptible; S, susceptible (<http://wheatdoctor.org/scoring-leaf-rust>).

### Results and discussion

#### Disease resistance

The evaluation of fungal disease resistance is shown in Table 1. Except for 'Balitus', the Austrian wheat varieties showed excellent to acceptable leaf rust resistance, whereas resistance against stem rust was not observed. Against other leaf spots the resistance was good. 'Amandus' and 'Amicus' proved their excellent disease resistance also under natural conditions, especially in 2016 when an excess of precipitation in spring and high temperatures in summer resulted in an increased pressure of leaf rust.

#### Agronomic traits and yield components

The phenological observations revealed that 'Fidelius' and 'Lukullus' are too late in maturity for growing in low rainfall regions such as the Shiraki plains, as the grain filling period coincided in this region with high temperatures and low precipitation. Due to its earliness, 'Lupus' was identified as the most drought tolerant variety here. The results of agronomic traits and yield components are presented in Table 2.

**Plant height.** Values for plant height ranged from 75 to 98 cm. The tallest plants were observed in Dedoplistskaro, whereas the lowest plant heights were recorded in Akhaltsikhe. Lodging was

**Table 1:** Evaluation of disease resistance of Austrian winter wheat varieties

Variety	Leafrust	Stem rust	Unidentified yellow leaf spots (%)
Amandus	0	MS	10
Amicus	R(2+)	MS	10
Balitus	MS(3-)	MS	5
Gallus	MR(2+-3)	S	5
Fidelius	MR(2)	MS	5
Lukullus	MR (2)	MS	1-5
Lupus	M (3)	MS	Septoria:10%; brown spots:15%
Urbanus	MR (2-3)	MS	20

**Table 2:** Agronomic and yield components of the Austrian wheat varieties at four Georgian locations (mean values 2015-2016; PHT, plant height; FT, number of fertile tillers; EL, ear length; SPE, number of spikelets per ear; KPE, number of kernels per ear; EW, ear weight; TKW, thousand-kernel weight). Highest/best performance is printed in bold.

Variety/Location	PHT (cm)	FT (n)	EL (cm)	SPE (n)	KPE (n)	EW (g)	TKW (g)
Dedoplistskaro (Shavchrelebi)							
Amandus	<b>75.5</b>	8.2	10.6	20.9	52.2	2.8	<b>48.6</b>
Amicus	78.7	6.9	12.0	21.7	54.0	2.3	39.8
Balitus	92.4	6.0	11.6	20.9	39.8	1.9	46.0
Gallus	87.6	7.4	12.1	19.3	61.4	2.3	44.4
Fidelius	84.1	8.1	10.0	22.2	55.3	1.6	45.2
Lukullus	92.8	<b>9.1</b>	<b>13.9</b>	21.5	54.4	2.4	40.2
Lupus	97.3	8.7	12.2	<b>22.5</b>	<b>73.2</b>	<b>3.4</b>	37.2
Urbanus	90.6	8.2	13.5	21.6	46.1	1.8	42.6
Telavi (Gulgula)							
Amandus	81.3	2.2	8.5	16.2	38.7	2.0	<b>50.4</b>
Amicus	<b>79.0</b>	4.1	<b>11.3</b>	<b>20.9</b>	51.7	2.3	40.8
Balitus	90.7	4.3	10.7	21.4	49.8	2.3	44.0
Gallus	93.3	<b>4.8</b>	10.6	18.4	<b>52.5</b>	<b>2.9</b>	48.2
Fidelius	90.4	3.5	8.8	18.3	48.9	2.2	44.4
Lukullus	97.8	3.7	10.1	19.5	49.0	2.1	45.6
Lupus	90.0	3.8	10.2	17.9	42.7	2.0	43.2
Urbanus	89.8	4.1	10.0	19.6	47.4	2.0	45.4
Khashuri							
Amandus	39.6	4.0	8.5	16.4	41.1	1.9	<b>50.0</b>
Amicus	<b>81.3</b>	4.0	<b>10.6</b>	<b>20.9</b>	52.2	2.1	40.6
Balitus	80.8	3.3	10.3	19.6	50.8	2.4	42.8
Gallus	88.5	<b>4.1</b>	10.0	16.2	<b>46.4</b>	<b>2.0</b>	45.2
Fidelius	87.8	4.3	9.3	19.6	49.2	2.1	40.8
Lukullus	87.0	3.7	10.5	19.0	47.6	1.9	40.8
Lupus	92.3	4.1	10.3	20.8	58.1	2.4	37.0
Urbanus	77.4	3.1	9.3	16.6	26.7	1.2	42.4
Akhaltsikhe (Vale, Chorati)							
Amandus	<b>70.6</b>	3.1	8.6	15.3	39.5	1.9	<b>53.4</b>
Amicus	72.6	2.2	9.3	<b>18.3</b>	54.3	2.3	40.0
Balitus	76.9	2.4	<b>10.1</b>	17.9	52.1	2.2	45.2
Gallus	81.0	<b>3.8</b>	9.2	15.3	49.3	2.5	46.2
Fidelius	80.1	3.1	9.0	18.2	<b>61.2</b>	<b>2.6</b>	44.2
Lukullus	78.9	1.8	9.4	<b>18.3</b>	45.3	2.2	46.8
Lupus	86.2	2.5	9.4	18.2	47.1	2.1	45.0
Urbanus	75.3	1.7	9.1	17.7	38.9	1.9	52.8

recorded only partially for 'Lukullus' and 'Lupus' in 2016 at Dedoplistskaro due to frequent rainfall.

*Productive tillers.* The number of fertile tillers is a key indicator of productivity; 700-800 ears m<sup>-2</sup> are recommended for high yields. The Austrian wheat varieties were characterized by medium numbers of fertile tillers and ranged from 1.7 to 9.1. The highest values

**Table 3:** Mean grain yield (2015-2016) of Austrian winter wheat varieties at four Georgian locations (GYLD, grain yield; YLD50E, grain yield of 50 ears; DEVC, deviation from check variety 'Bezostaya 1').

Variety/Location	GYLD (g/m <sup>2</sup> )	YLD50E (g)	DEVC (dt ha <sup>-1</sup> )
<b>Dedoplistskaro (Shavchrelebi)</b>			
Amandus	<b>888.5</b>	124	+36
Amicus	811.5	91	+29
Balitus	725.0	94	+20
Gallus	535.0	113	+0.1
Fidelius	808.0	107	+29
Lukullus	714.5	88	+19
Lupus	739.5	117	+21
Urbanus	664.0	95	+13
Bezostaya 1	534.0	92	-
<b>Telavi (Gulgula)</b>			
Amandus	664.0	124	+15
Amicus	642.0	101	+11
Balitus	637.0	109	+11
Gallus	<b>724.0</b>	105	+21
Fidelius	655.5	107	+15
Lukullus	700.0	102	+19
Lupus	610.0	109	+10
Urbanus	630.0	113	+12
Bezostaya 1	513.5	93	-
<b>Khashuri</b>			
Amandus	<b>639.0</b>	93	+8
Amicus	593.5	98	+3
Balitus	561.0	105	-
Gallus	575.0	100	+2
Fidelius	617.0	101	+6
Lukullus	582.5	96	+2
Lupus	483.0	95	-8
Urbanus	475.5	79	-8
Bezostaya 1	561.5	95	-
<b>Akhaltzikhe (Vale, Chorati)</b>			
Amandus	696.5	111	+14
Amicus	779.5	114	+22
Balitus	531.5	114	-3
Gallus	582.0	110	+2
Fidelius	662.0	130	+10
Lukullus	678.0	126	+12
Lupus	550.5	116	-1
Urbanus	<b>783.5</b>	118	+22
Bezostaya 1	555.5	99	-

were recorded at Dedoplistskaro, the lowest in Akhaltsikhe. A genotype × location interaction was observed, *e.g.* in Dedoplistskaro 'Lukullus' and 'Balitus' had the highest and lowest number, respectively, whereas in Telavi 'Gallus' and 'Amandus', in Khashuri 'Fidelius' (4.3) and 'Urbanus' (3.1), and in Akhaltsikhe 'Gallus' and 'Urbanus' were the extremes. Summarizing, all varieties can be assumed to be suitable for production in the Kakheti and Kartli regions, while 'Gallus' and 'Fidelius' are suitable for the Akhaltsikhe region.

*Ear traits and thousand-kernel weight.* As for other traits the highest performance for ear length, number of spikelets per ear, number of grains per ear and ear weight were realized in Dedoplistskaro. The Austrian wheat varieties show a medium spike density. The highest TKW was observed for 'Amandus'. 'Lukullus' and 'Urbanus' showed high values in almost all locations except Dedoplistskaro.

*Yield.* The grain yield of the Austrian wheat varieties and check variety 'Bezostaya 1' is presented in Table 3. Except 'Balitus', 'Lupus' and 'Urbanus' at Khashuri, and 'Balitus' and 'Lupus' at



Akhaltsikhe, the Austrian wheat varieties outyielded the check variety 'Bezostaya 1' in all environments. Generally, the highest grain yields were observed in Dedoplistskaro and Akhaltsikhe regions. A significant genotype  $\times$  location interaction was observed for 'Gallus' which was similar in its performance to 'Bezostaya 1' in three locations, however, was the highest yielding variety in Telavi. Similarly, 'Urbanus' performed inferior compared to almost all other Austrian varieties in Dedoplistskaro, Telavi and Khashura, however, was the highest performing variety in Akhaltsikhe. Therefore, it is demonstrated that environmental conditions in Georgia are manifold and an intensive testing of foreign wheat varieties is necessary to select varieties with either an acceptable general adaptation or the best regional adaptation.

**Baking quality.** Baking quality is closely related to the quantity and quality of gluten. In the harvest of the 2015 crop the gluten content was determined as an indirect measurement of baking quality. The standard requirement of  $\geq 22\%$  were met by 'Urbanus' (25%), 'Lukullus' (23.9%), 'Lupus' (23.2%), and 'Amicus' (22.8%). 'Amandus' (20.8%), 'Balitus' (19.8%), 'Gallus' (18%) and 'Fidelius' (17.8%) clearly failed the minimum requirement. The highest gluten content was observed for check 'Bezostaya 1' (25.8%). It must be noted that the rather low gluten levels were influenced by continuous rainfall during the period of observation.

## Conclusions

The field trials of Austrian winter wheat varieties in Georgia from 2014 to 2016 revealed that Austrian varieties are best suited for growing in the Dedoplistskaro and Akhaltsikheregion where they can reach stable and high grain yields. A significant genotype by location interaction revealed that from the investigated germplasm varieties can be selected with regional adaptation to all test environments which can significantly outyield the old Russian check variety 'Bezostaya 1' by 8 to 36 dt ha<sup>-1</sup> in low and high productive areas, respectively. The Austrian varieties were also characterized by a high lodging tolerance and excellent to good resistance levels against fungal leaf diseases.

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Working and harvesting on weekends - determined by culture and does it affect yield?

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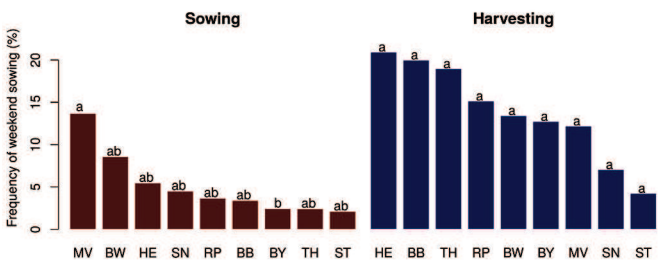
The necessity to sow or harvest on weekends is often a matter of debate. To support discussions and decisions with empirical evidence, we analysed the frequency of weekend sowings and harvests in official variety recommendation trials of winter wheat in Germany. Particularly, we investigate the following hypotheses: (i) Are there differences between the states (Bundesländer) and can these differences be explained by economic and religious covariates? (ii) Is there a change over years? (iii) Does sowing or harvesting on weekends have an effect on yield?

We extracted the dates of sowing and harvest and grain yield from publicly available reports of the German *Landessortenversuche* for winter wheat in the years 2001-2016. The dataset contained 1029 trials. Census data on gross domestic product (GDP), religious affiliation, and employment in agriculture were obtained from the Federal Statistical Office of Germany. For both sowing and harvesting, we used a binary dummy variable, indicating if a trial was sown or harvested on weekdays (0) or weekends (1). A mixed effect logistic regression model with binomial error distribution and logit link was applied with the terms state and year as fixed and trial location as random, similar to repeated measure analysis. Significance of differences between states was assessed by pairwise comparisons and adjusted means are reported after backtransformation. In order to determine the effect on grain yield, one-way ANOVA was performed with the dummy variable as categorical factor.

Weekend sowing and harvesting differs between states (Figure 1), but only the difference between Mecklenburg Western Pomerania (MV) and Bavaria (BY) was significant for sowing. For both, sowing and harvesting, Saxony-Anhalt (ST) showed the lowest frequency of weekend working. Although no significant relations between any of the tested covariates could be detected, there seems to be a (possibly spurious) relation between Protestantism and weekend work (Table 1). A significant ( $P<0.05$ ) increase in weekend harvesting was found: the odd ratio increases by 6% per year. However, there was no significant change in weekend sowing. Trials sown on weekends had on average a yield increase of 2.2 dt ha<sup>-1</sup>, but effects were not significant.

**Table 1:** Correlations between the frequency of weekend sowing and harvesting and economic and religious covariates.

Spearman rank correlation	% Weekend work	
	Sowing	Harvesting
Total GDP	0.13 ns	0.30 ns
GDP per capita	0.12 ns	0.37 ns
% Catholics	-0.02 ns	0.13 ns
% Protestants	0.40 ns	0.57 ns
% Others	-0.20 ns	-0.32 ns
Agric. Employees	-0.27 ns	-0.32 ns



**Figure 1:** Frequency of weekend sowing and harvesting per state (MV, Mecklenburg Western Pomerania; BW, Baden-Württemberg; HE, Hesse; SN, Saxony; RP, Rhineland Palatinate; BB, Brandenburg; BY, Bayern; TH, Thuringia; ST, Saxony-Anhalt).

Key words

Germany · grain yield · harvest · sowing · variety trial

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