Innovative organic breeding concepts: challenges and examples

Deliverable number	3.5
Dissemination level	Public
Delivery Date	September 2020
Status	Final
Lead beneficiary	DBH
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LIVESEED is funded by the European Union's Horizon 2020 under grant agreement No 727230 and by the Swiss State Secretariat for Education, Research and Innovation (SERI) under contract number 17.00090. The information provided reflects the views of the authors. The Research Executive Agency or the SERI are not responsible for any use that may be made of the information provided.

Document Version

Version	Date	Contributor	Summary of Changes
1.0	13-3 /13-5 2020	Edwin Nuijten	compilation of all available texts from: Véronique Chable, Paolo Annicchiarico, Adrian Rodriguez Burruezo, Stephanie Klaedtke, Ambrogio Costanzo, Salvatore Ceccarelli
2.0	13-8 2020	Edwin Nuijten	editing of available texts, writing of summary and discussion (to be reworked)
3.0	4-9 2020	Antje Kölling, Mariateresa Lazzaro, Ambrogio Costanzo, Pierre Riviere, Edwin Nuijten	adding several sections and reworking existing texts
4.1	15-9 2020	Edwin Nuijten	Final draft version
4.2	19-9 2020	Edith Lammerts van Bueren	Revision
4.3	24-9 2020	Edwin Nuijten	Revision
4.4	6-10 2020	Monika Messmer	Final revision

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Summary

In this review we have described what organic breeding is about and how it can contribute to organic agriculture and sustainable agriculture in general using new innovative perspectives from a systems-based approach. The partners involved in the writing of this review provided input on important issues to be included. Specific challenges and examples which are important for organic agriculture and organic breeding in particular were organised according to 14 topics. For each of these topics, experiences from EU-projects and national projects were complemented with information from literature review and key experts on particular knowledge fields. This review does not aim to be conclusive. Instead it aims to provide an overview of the key-issues for organic breeding and organic agriculture at large.

As perspective the systems-based approach was used, which is a holistic approach and helps to understand how various technical and socio-cultural aspects are interrelated. For example, socio-cultural and ethical aspects interrelated with technology development is described in Topic 1. For organic agriculture there is also a strong need to understand how intellectual property rights can be organised in different ways (see Topic 2). Awareness that there can be different approaches means also a broadening in concepts, such as collaborative approaches in breeding (e.g multi-actor and participatory approaches, see Topic 3) and to foster breeding for diversity based on agro-ecological principles (e.g. fostering the benefits of GxExS interaction instead of minimising them) and that resilience does not only include the agro-ecological sphere but also the socio-economic sphere (see Topic 4). This also implies developing novel financing approaches that fit to breeding approaches based on agro-ecological principles, e.g. that plant breeding is an integral part of society and as such the whole food system can be involved in different ways in financing organic breeding (Topic 5).

In order to foster organic breeding approaches, we need to further develop new effective organic breeding methodologies and a better understanding of the various facets, such as trade-offs between resilience, yield and quality in the breeding process (Topic 6). Organic breeding aims to emphasise quality as much as yield and as much as resilience. It also means developing guidelines for breeding for complex systems such as mixed cropping and agroforestry (see Topic 7). More crop diversity is considered as one of the ways to improve yield and yield stability of organic farming systems. Lessons learned about breeding for mixed cropping may be a starting point for breeding for agroforestry. Moreover, organic breeding emphasises a better understanding of the relationships between plants and microbes (see Topic 8). Evidence is gradually growing that well balanced plant – microbe interactions can contribute significantly to crop resilience and to managing pests and diseases. In combination with breeding for complex systems, this could prove to make organic food systems more robust.

The development of novel breeding methodologies also means developing further existing tools and/ or developing new tools. Topic 9 provides examples on how direct and indirect selection can be used for complex traits like Nutrient Use Efficiency (NUE) and Water Use Efficiency (WUE) which are not only important for organic agriculture, but for agriculture in general in order to mitigate the effects of climate change. For particular issues, molecular marker tools may be used, as in the case of pyramiding resistance genes to develop more durable resistance against phytophthora in potato (Topic 10). However, organic breeding seeks to design holistic, systems-based selection methods to develop durable resistance to pests and diseases. Resistance in organic systems should therewith be based on multiple genes, crop diversity and beneficial plant-microbe interactions - either targeting specific pathogens or by improving the robustness of the farming system as a whole (Topic 11). When it comes to breeding for weed suppressiveness, also a holistic perspective is used by understanding how both breeding and improved farming practices can reduce weed problems, e.g. so-called breeding for integrated weed management. Here we have to differentiate between weed tolerance and weed suppression ability (Topic 12). Not only new effective breeding methodologies and breeding methods need to be developed for annual crops in organics. Topic 13 deals with the effects of root stocks and scions on plant vigour in the case of perennial crops for organic agriculture. And finally, Topic 14

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describes decentralized-participatory breeding approaches based on dynamic management of evolutionary populations and how diversity-based breeding methods can be operationalised by utilising GxE interactions and benefiting from them instead of minimising them. This approach underlines the possibility of developing heterogeneous material and uniform varieties in the same process.

Together these fourteen topics describe and showcase how organic breeding can use and contribute to a holistic perspective in successful ways, e.g. that innovations in organic breeding are well connected with innovations in other knowledge fields such as agro-ecology, micro-biology, weed and disease management, sociology and economy (e.g. re-arrangements of the market) and law and governance (such as developments on intellectual property rights). Opportunities, challenges, and recommendations for various stakeholders are described in the final discussion section.

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Introduction

Organic plant breeding is one of the fields where gain and exchange of knowledge is important to improve organic agriculture. In order to contribute to organic farming, organic breeding needs new innovative perspectives from a systems-based approach. This means that organic breeding should not be considered as a technical exercise only, but that we also need to consider the social, ecological and ethical aspects, such as access to technology and financial resources, and the distribution of knowledge. Lammerts van Bueren et al (2018) emphasised the importance of connecting different perspectives. Another important aspect is to view organic breeding from a holistic perspective, meaning that innovations in organic breeding are well connected with innovations in other knowledge fields such as agro-ecology, micro-biology, weed and disease management, sociology and economy (e.g. re-arrangements of the market) and law and governance (such as development on Intellectual Property Rights). Another important element of organic breeding is to understand and respect processes of life, and to respect plants as living organisms (see ECO-PB paper 2012), based on the four IFOAM principles of care, ecology, fairness, and health (Nuijten et al. 2017). In order to translate these principles into the practice of organic breeding, the first step is to describe, understand and reflect upon various types of relationships, between technologies and working methods, between microbes and plants, between people, and between people and plants and technologies. In Science and Technology Studies (STS) various perspectives and theories have been developed on how to describe and understand these relationships. What they share in common is that technology is not neutral, but has relationships with people and plants, and that they influence each other; sometimes in very obvious ways and sometimes in tacit ways. In regard to relationships between people, various aspects need to be taken into account: economic, cultural and power aspects. The first step to understand these relationships better is to visualise, count and categorise them. In order to understand what type of relationships we want to stimulate, and which not, open discussions with all actors in the food system are needed (Chable et al. 2020). To foster processes of change, policymaking is very important, but often forgotten.

Definition of organic plant breeding

With the discussions on the compatibility of genetic modification with organic agriculture in the 1990's also definitions were developed of what organic plant breeding is (Lammerts van Bueren et al 1999, Wolfe et al. 2008). This has led to a distinction between **organic plant breeding** and **breeding for organic** (See Annex 1). The project LIVESEED refers to the **definition of organic plant breeding** provided in the International IFOAM Norms on Organic Production and Processing (Version 2014). Most important characteristics of organic breeding programs is that **all breeding steps from crossing till final selections take place under organic conditions** and that the applied breeding techniques are in accordance with the techniques listed in the Annex of the position paper on Compatibility of Breeding Techniques in Organic Systems of IFOAM Organics International from November 2017.

Besides value and process oriented organic plant breeding, **product-oriented breeding for organic** was defined by Wolfe et al. (2008). This differentiation was further developed in the position paper on organic plant breeding by the European Consortium for Organic Plant Breeding (ECO-PB) in 2012. Breeding programs for organic have a special focus on the breeding goals which are specific for organic agriculture (e.g. tolerance against seed borne diseases, weed tolerance, nutrient use efficiency), they do not apply critical breeding techniques, and selection occurred at least partially under organic conditions.

While organic breeding has not been integrated in legislation till the most recent revision of the EU organic regulation, several private labels already defined a certification standard for organic varieties. That fact that the new organic regulation (EU) 2018/848 defines "organic varieties suitable for organic agriculture" reflects the situation that organic food and farming is growing out of a niche market. With the growing importance and value of the organic market, it becomes more interesting to supply organic quality from the very beginning of the food chain - the breeding process. And with the growing

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interest in organic varieties, a quality control is needed to prevent misleading of consumers and fraud. However, the definition of "organic varieties suitable for organic agriculture" within the EU organic regulation is a first step and it is most likely that details in implementing this new part of the regulation will still be subject of evaluation and improvement under real life conditions.
Another reason for the growing importance of organic plant breeding can be found - at least for some species - in the progressively growing gap between breeding goals for conventional and organic farming. While varieties developed under conventional conditions e.g. 40 years ago were still suited also for organic conditions, this may not be the case for many varieties today. This issue may even gain importance if conventional plant breeders invest more and more in breeding technologies that are contested by the organic movement - so that many varieties newly developed in conventional breeding would not be eligible for organic farming anyway.

Organisation of the deliverable

Hence, this report deals with a range of aspects, such as social, ethical, economic, technical, plant related, disease related, and soil related) that are all important for the further development of organic breeding. It deals with aspects such as the efficiency of various tools, criteria, and methods for organic breeding (versus conventionally managed selection fields). It uses the systems-based breeding concept as a starting point. In this review we describe examples and challenges of fourteen topics important to the further development of organic breeding. These examples were drawn from various EU-projects and national projects. These 14 topics were identified by the partners involved in the writing of this review (see Annex 2). These topics are subdivided into three main sections, which are interconnected (see the flow chart below). The first section describes the broadening of perspectives on organic breeding, subdivided in 5 interrelated topics. Incorporating cultural and ethical aspects in breeding (Topic 1) means a broadening in perspectives on the use of Intellectual Property Rights (IPR) in organic breeding (Topic 2). A broadening in perspectives also means a broadening of concepts (Topics 3 and 4). Departing from the perspective that plant breeding is an integral part of society in order to be able to develop sustainable food system means also developing innovative ways of financing organic breeding (Topic 5). Broader perspectives on breeding (Section 1) results in broadening breeding strategies / methodologies for organic breeding, described in Section 2 (Topics 6, 7 and 8). The further development of breeding strategies / methodologies means further developing existing tools and / or developing new methods and tools, described in Section 3 (Topics 9 to 14).

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Flow chart of the organisation of the topics described in this report

Section 1: Broader perspectives on organic breeding
Topic 1) Incorporating cultural and ethical aspects into breeding
Topic 2) How organic breeding deals with IPR, patents, and geographical indication schemes (PDO and PGI)
Topic3) Collaborative approaches in breeding (such as community-based breeding, chain based breeding and participatory approaches)
Topic 4) Breeding for diversity based on agro-ecological principles: Concepts of genetic diversity, resilience, local adaptation, Examples on Genotype x Environment x Management interaction; and Genotype x Environment x Society interaction
Topic 5) Financing organic breeding initiatives

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Section 2: Breeding strategies for organic breeding
Topic 6) Trade-offs between resilience, yield and quality in the breeding process
Topic 7) Breeding for complex systems (such as mixed cropping, agroforestry)
Topic 8) Balanced plant – microbe interaction (such as plant communication and defence strategies)

↓

Section 3: Specific breeding tools and methods for organic
Topic 9) Direct versus indirect selection, and indirect parameters for complex traits like NUE and WUE
Topic 10) Use and efficiency of molecular tools, such as MAS, genomics, (epigenetic effects)
Topic 11) Inheritance of resistance to seed and soil borne diseases: From a gene-based to a cropping-systems perspective
Topic 12) Breeding for integrated weed management
Topic 13) Challenges and perspectives of root stock and scion in organic fruit tree breeding
Topic 14) Efficient breeding methods: Decentralized-Participatory Breeding for Organic Agriculture based on Dynamic Management of Evolutionary Populations

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Section 1: Broader perspectives on organic breeding

Topic 1) Incorporating cultural and ethical aspects into breeding

Introduction

To explain and underline that breeding is not only a technical exercise but also has cultural ethical aspects, in this section a number of questions relating to the cultural and ethical aspects of breeding are addressed.

Question 1 - What is the heritage of plant breeding activity? How did plant breeding methods evolve during plant domestication and the beginning of agriculture? In which way is modern plant breeding linked with the industrial vision of food systems?

Often, plant breeding is thought of as a technical activity. Today the development of molecular technologies dominates new developments in mainstream plant breeding. However, when taking a better look, one realises that culture and ethical aspects also play a role in mainstream breeding. *Our history can help us to understand.* First, let's come back to the beginning:

- From an anthropologist's perspective, it is difficult to understand why human beings have changed their way of life as hunter-gatherers and developed agriculture (Whitehouse and Kirleis, 2014).
- One hypothesis is the change of mind and conception of their position on Earth. They initiated an exploitation behaviour, leaving their friendly coexistence behind (Mannion, 1999; Scott, 2019).
- From Neolithic revolution to the 19th century, seeds have co-evolved with practices and environment and have shaped the cultural universe and landscapes (Purugganan, 2019).

With the development of industrialisation, which in agriculture resulted in the availability of mineral fertilisers and chemical pesticides, a standardisation trend co-evolved and influenced the vision of crops emphasising quantity and efficiency. In the 20th century the concept of homogeneity and stability emerged and a focus on a few main crops developed. This trend was supported by new scientific knowledge, such as in the fields of genetics and reproduction biology, and it was then possible to develop uniform crops, with the creation of the concept of "variety" around 1950. Moreover, these choices were reinforced by politicians, e.g. F1 hybrid development for maize was not only a technological development, but also a political choice with its origin in the USA (Kloppenburg, 2005; Sutch, 2008).

Breeding for homogeneity and distinctness has had clear socio-cultural consequences. For 10'000 years, farming and plant breeding were closely linked. With the agricultural industrialisation, a new job appeared, the plant breeder and a new kind of enterprise, the seed company. Since plant breeding had been separated from farming activities, plant breeding goals had to consider profitability of the seed company, through activities such as accelerating the breeding process using laboratory techniques such as the development of dihaploids, and enhancing and securing hybridisation techniques with cytoplasmic male sterility. These methods are not necessarily beneficial to farmers. Today, the profit can be increased by coupling patented traits (such as herbicide resistance) and the sale of the corresponding product. Moreover, plant breeding efforts were conducted to counterbalance the deleterious effects of homogeneity of the crops (Stukenbrock and McDonald, 2008) and their narrow genetic backgrounds (Kenini et al. 2012, e.g. looking for all forms of diseases and pest resistances). If these traits are not naturally present in the species, breeders have had, since the middle of the last century, a variety of artificial means (including genetic modification) to introduce these traits into the crop species, which calls for much debate about their compatibility with the

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organic principles. Nuijten et al. (2017) have carefully analysed all controversies in the light of underlying OA principles of health, ecology, fairness, and care. They pointed out that debates about the technical methods needed to solve certain problems cannot be fruitful if one does not understand underlying values of organic agriculture. This leads to the second question.

Question *2 – How are ethical and biological aspects interrelated? What are the socio-cultural and biological impacts of biotechnologies? How can we create awareness about these interconnections?*

It is clear that due to the IFOAM's basic principles of organic agriculture ethical aspects are very important in organic breeding. It is already widely accepted that genetic modification (including cytoplast fusion) and other technical interventions into the genome of plants are not allowed (e.g. ionizing radiation; transfer of isolated DNA, RNA, or proteins) in organic breeding (IFOAM .2014, 2017). In addition, plants should also be able to reproduce themselves (Lammerts van Bueren et al. 1999). Certain types of F1-hybrids are not able to reproduce because of cytoplasmatic male sterility (CMS) without restorer genes, which is undesirable from the perspective of the organic principles. The organic principles have ethical dimensions but also follows ecological and biological considerations to optimise the functioning of an agroecosystems. In the frame of a conventional vision of agriculture, the inputs are conceived as a "corrector", in order to control and optimise living processes/mechanisms. In an organic vision, the presence of pests and diseases are considered as a result of an inadequate/suboptimal farming practice and/or non-adapted cultivars and urge to adapt the farming system and seeds. How can we imagine that plants with CMS without restorer genes can mobilise all its adaptive potential when we know that its nuclear genome is not quite compatible with genetic information from mitochondria that is the energy centre of the cell?

Besides naturally occurring CMS in wild populations of plants (such as carrot and onion), the trait is artificially obtained in the case of cabbage and witloof chicory and cannot comply to the IFOAM principle of care (Billmann 2008). Cytoplasmic male sterile plants can be produced either through interspecific sexual crossings (alloplasmic lines) or by cytoplast fusions followed by backcrosses, where the nuclear genome from one species is combined with the cytoplasm of another (Kaul, 1988). In the first case, the novel nuclear-cytoplasmic combinations often result in aberrant expression of the mitochondrial (mt) genes and in the second, the cybrid plants (products of cell fusion) are selected in order to keep specific mitochondria genetic structure that induces the male sterility trait in the donor plant (reviewed in Carlsson et al., 2007). Different CMS phenotypes have been associated with certain open reading frames (orfs) composed of novel sequences of unknown origin combined with sequences of known mt genes. The expression of the CMS-associated mt orfs can often be reverted and the male sterile trait restored to fertility by nuclear restorer gene(s) (reviewed by Hanson and Bentolila, 2004). Nevertheless, for vegetable production the fertility restoration is not useful since the product is often harvested before the reproduction stage (brassica species, witloof chicory…). Then the plant is obliged to function in a biological context where the nuclear genetic information is only partially compatible with the mitochondrial genetic information. To illustrate this issue, in the literature we can find investigations in which many nuclear genes, associated with auxin response, ATP synthesis, pollen development and stress response, had delayed expression in Ogura-CMS brassica plants, compared to the maintainer line. This is consistent with the delay in growth and development of Ogura-CMS plants compared to plants with original cytoplasm (Dong et al. 2013). These studies have shown all sorts of abnormal proteins and stress protein expressions in CMS plants (Chen and Liu 2014). Even if most F1-hybrids based on CMS systems seem not to be affected by these abnormal proteins, what could be the ability of this type of CMS plants to adapt to new stressful conditions?

Moreover, the metabolic interactions between photosynthetic and respiratory metabolism (Araujo et al. 2014) are considerably important and remain both difficult to study and challenging to understand.

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Surprisingly, it was only recently that plant scientists started to dissect this complex relationship. However, it is now apparent that there are considerably more linkages between chloroplastic activity, including photorespiration, nitrogen metabolism and carbon fixation and nucleus genes.

Another field of investigation has been opened recently considering the holobiont concept for plants. The structure of the seed microbiome is recognised as an important factor in the development of colonization resistance against pathogens. Some recent studies showed that this microbiome is dependent on the cultivar (Ada et al. 2016; Rybokova et al. 2017.) Another recent study has specified the composition of bacteria and fungi microbiota in wheat roots which differed between modern and ancient cultivars. These ancient cultivars encompassed less pathogenic microorganisms than recent ones for bacteria both in terms of species richness and abundance (Mauger et al. 2020).

*A*s described above, and further elaborated in Topics 6 to 8 in particular, the improvement of certain biological aspects in plants (such as genetic and morphological uniformity and cytoplasmatic male sterility) have also disadvantages. From a theoretical perspective it can be concluded that, depending on the paradigmatic position, these disadvantages will have smaller or larger weight. However, there is more and more scientific evidence that agriculture focused on growth and technology is reaching its limits (Martin et al. 2016). We have ample information from biology, physiology and genetics to be able to underline the importance of the four basic principles of organic agriculture (ecology, health, fairness and care). We have previously discussed the threat associated with the uniformity and stability of a crop, which reduce resilience of the agroecosystems (Döring et al. 2013).

Although sciences like ecology can highlight the need for limitations to growth and the consequences of ignoring them, social sciences are necessary to diagnose societal mechanisms at work, how to correct them, and identify potential drivers of social change (Martin et al. 2016). Social sciences can help to clarify hidden and/or unconscious ethical values related to technologies and practices. They can also help us identify our blind spots. For example, *have we considered the overall societal impact of biological phenomena of traits like Cytoplasmatic Male Sterility (CMS)? Another unanswered question is what impact does it have on the quality of the products and robustness of plants?*

Question 3 - How can organic practices highlight and enhance the local potential of each environment? How can plant breeding reinforce crop adaptation and co-evolution with the local environmental conditions and socio-economic practices?

In organic agriculture, the farming context and growing conditions often vary more compared to conventional agriculture. In addition, regionality of production is valued more highly, and there is also a preference for developing other socio-economic approaches to farming with more attention being paid to social, cultural and ethical aspects. In doing so, organic agriculture contributes to biodiversity on various levels: with more diverse crops, farming systems and increased soil life and above ground diversity.

For example, the project Farm Seed Opportunities showed a process of adaptation to local conditions when seed was produced on-farm over several years (FSO 2010). The FSO on-farm experiments at le Rheu, based on four crop species and run over three years, allowed us to obtain an accurate characterization of variety evolution over time and space in response to drastic environmental changes and contrasting farmers' practices on-farm. Overall, after only two to three years of on-farm growing and selection, there were significant changes for many traits assessed both on-farm and on-station. The significance and degree of evolution depended on the trait studied, the varieties, the farmers' practices and farm environmental conditions. This trend of on-farm evolution was also found for modern varieties, although there were fewer traits showing significant changes.

The DIVERSIFOOD project showed that there is potential for developing new plant breeding strategies for diversity and in coherence with the organic vision that also pays attention to cultural and ethical

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aspects (Chable et al. 2020). The following processes/trends are important (1) multi-actor and/or participatory plant breeding interrelated with community seed banks and (2) small scale companies embedded in their region. Basic guidelines are: 1) to work with the adaption potentialities of plants to its variable environment, instead of working with inputs to homogenise the environment; 2) intrinsic diversity is a prerequisite for adaptation and evolution with the agroecosystem, instead of striving for homogeneity, and 3) novel farm management approaches dealing with diseases, weeds, and nutrients (See Topics 9, 11 and12). These guidelines should be an integral part of the breeding work.

Outcomes

A truly sustainable intensification of food production will require re-engineering existing agro-ecosystems to introduce dynamic diversity in order to suppress the rapid emergence of new and highly damaging plant pathogens (McDonald and Stukenbrock, 2016). This implies also a *renewing of culture for an authentic organic agriculture. It means that in terms of organic breeding, questions need to be addressed about respect for the integrity of life, about food, about the value of food and seed. It also requires addressing the questions what the definition of organic seed is, and who will support the research for the transition.*

Maintaining and enriching cultural diversity helps broaden the perspectives on breeding, and hence on the maintenance and further development of biodiversity. It results in so-called GxExS interactions (Genotype x Environment x Society interactions). The results and importance of these interactions will be further elaborated in Topic 4. The meaning of words like organic variety depends on context. In DIVERSIFOOD it became clear that the meaning of landraces, local varieties, etc., are influenced by culture (Topic 4).

Looking at cultural and ethical aspects means looking implicitly at the economic aspects of breeding. Broadening cultural perspectives on breeding implies a broadening in the financial organisation of breeding, and that breeding is not only a commercial activity, but also a cultural activity, or a joint activity by the actors or value chain or food system. This will be further elaborated in Topic 5.

Challenges

The main challenges for the coming years is to find ways to make the interconnectedness between biological and ethical aspects more clear and easier to understand by the broad public.

Another challenge is to have open discussions with all food system actors on the questions of what the meaning of organic breeding is and what organic cultivars can be.

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Topic 2) How organic breeding deals with IPR, patents, and geographical indication schemes (PDO and PGI)

Introduction

Apart from the fact that there is common agreement within the organic sector that patents are not compatible with the IFOAM principles, the actual practices of organic breeding vary among practitioners. Some aim to develop uniform cultivars that fit DUS requirements in order to be able to register varieties with or without plant variety protection, others choose to develop cultivars with some levels of heterogeneity or purposefully aim to develop heterogeneous material such as composite cross populations or dynamic populations. Among the latter group there is common agreement to consider seeds and cultivars as a common good and to maintain open access to anybody who wants to further work with these seeds, either for seed multiplication, maintenance or further crop development. However, this approach is very different from the common opinion within the conventional breeding industry where it is considered normal to claim intellectual property rights over seeds, either through legislative ways like plant variety protection and patents or through technological approaches (through the development of F1-hybrids and the use of cytoplasmatic male sterility, CMS).

Challenges

As a result, it is a challenge how to deal with maintaining such open access in organic breeding. This challenge is becoming more real as organic agriculture is becoming part of mainstream agriculture and therefore an interesting market with good financial prospects. In Europe and North America several approaches have been developed on how to deal with it. In North America the so-called OSSI pledge (Open Seed Source Initiative pledge) has been developed (see osseeds.org). It pledges that breeders who use OSSI seeds do not claim ownership over these seeds. It has no legal binding, however, and is based on a moral appeal of the users. Kotschi and Horneburg (2018) suggested an Open Seed Source Licence model that does provide legal binding. The downside of this approach is that it requires a lot of legislative knowledge to work with it and narrows flexibility (Louwaars 2019). Nevertheless, there may still be the possibility of developing the Open Seed Source Licence further into a workable model. It may also still have the potential of being useful to support the financing of organic breeding (Topic 5). This represents a challenge of connecting the idea of open source with collecting financial support for organic breeding initiatives and ensuring that the output of the work is not being claimed by others but remains in the open access domain. At the moment, the concept of commons fits most logically with local networks based on personal relationships. However, for organising substantial financing larger initiatives or schemes are needed.

A potential alternative is the development of labels to show costumers that the product they buy results from organic breeding. At this stage the label Bioverita is the best-known example for that. It has the potential to be used across Europe. The label Bioverita has particular criteria and working methods the breeders should comply with. In the project DIVERSIFOOD various labelling strategies have been described and compared (Oehen, 2019), such as: Concepts for flagship label, trademark and other communication tools in support of the marketing of underutilized crops (food diversity). A more regional approach is the use of geographical indication schemes such as PDO and PGI (PDO: protected designation of origin, PGI protected geographical indication). These labelling strategies have in common that the emphasis is on the product and not on the varieties used to produce these products. In that way, in principal a wide range of products can be labelled. An advantage of such strategies is that it can attract and bind many stakeholders (Oehen et al. 2019). Organising such labels also goes with consider costs and a certain level of professionalization. That a labelling approach can have significant social impact on the people directly involved in local and regional networks is

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described in the example below on The French Peasant Seed Network (Réseau Semences Paysannes, RSP).

Example

Experiences from France on labelling

As a result of the development of peasant seeds in the field and their recognition, the question of the economic valorisation of the products resulting from the cultivated biodiversity arises more strongly within the networks of producers. Can proprietary tools such as brands and labels promote this valuation? The French Peasant Seed Network (Réseau Semences Paysannes, RSP) has been experimenting for 2 years (2016-2017) with the implementation of a collective private brand to identify products derived from peasant seeds (DIVERSIFOOD Innovation Factsheet #23, 2018).

As an industrial property right, its use is indeed not neutral: the shift from a use value of peasant seeds (linked to user rights) to a market value (linked to property rights and unbalanced power: added values are not well distributed) has consequences for peasant seed systems that are fragile from economic, organizational and legal points of view. The consequences that the RSP experienced during the two years of this pilot project led to the halt of the development of a collective private brand to label products derived from peasant seeds. The two main consequences are described below.

i) The micro-chains of peasant varieties (short chain or specialized) are currently under tension: the volumes are very limited, and we are witnessing speculative strategies generated by downstream players who aggressively invest in the organic sector. The stagnation or even the historical regression of mass distribution margins are now leading different major companies to invest in civil society to capture the value existing off-market to transform it into market value. The brand, initially thought by the producers to highlight practices and take into account actual production costs (including farm seed conservation and/or selection) has proved to be in practice a purely commercial tool in a logic of market segmentation. The RSP brand project was halted by the highly publicised repossession of the peasant seeds concept by the Carrefour Supermarket group, which set up a very effective marketing campaign (the Forbidden Market), at a time when the first products stamped with the RSP brand were going to be distributed in a specialised circuit (Biocoop).

ii) The majority of the producer's organization members of the RSP network market their product directly or in a short local chain. They are not seeking an umpteenth quality label knowing that most are already involved in other labels such as Organic Agriculture, Nature & Progrès or Demeter. Therefore, the demands for a brand are very minor in the RSP network and stem only from two "atypical" organizations of rather big organic vegetable producers marketing their products in long food chains. In these systems, the brand is also a guarantee outsourcing tool for the downstream players of the food chain. Putting the burden of proof on the producers can lead to negative effects: extra costs for the producers (certification to ensure traceability) and for the organizations (management of a brand), shift of working time towards the control of the guarantee and the marketing and not towards the structuring of peasant selection, risks of standardization of practices via national specifications that may lead in a loss of cultivated biodiversity at local level.

Based on the first experiences, RSP has come to the following conclusions:

- RSP is convinced that getting back diversity in the fields and on the plates will only be achieved by promoting and practising a peasant agro-ecological model with lots of small and diversified farms using diverse and locally adapted varieties and not by contributing to more and more industrialization of the organic sector.
- All actors of the organic sector should struggle together to firmly request full transparency on the breeding process to avoid having more and more undesired CMS hybrids or new GM technology within the varieties cultivated by organic farmers.
- RSP practitioners are considering seeds together with their know-how as a common which should be managed in a sustainable way by all the users from the field to the plate.

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LIVESEED is funded by the European Union's Horizon 2020 under grant agreement No 727230 and by the Swiss State Secretariat for Education, Research and Innovation (SERI) under contract number 17.00090.

Topic 3) Collaborative approaches in breeding (such as community based breeding, chain based breeding and participatory approaches)

Perspective

Just as a larger diversity in food systems is needed, also a larger diversity in breeding approaches is needed that is well connected to the different types of food systems (Lammerts van Bueren et al. 2018). This is particularly important for organic agriculture in order to better cope with biotic and abiotic stresses amongst others. Currently, most breeding is done for the anonymous value/food chains. In these value chains, the various steps of food production are broken down in specific steps, in a linear cooperation of specialists. The main interest of these value chain actors is producing and selling a product in a most efficient way. Within these current value chain organisations, to facilitate good cooperation and improve efficiency standardisation is very important.

However, for local and regional food systems where local cultural aspects also play a meaningful role, other breeding approaches may have benefits. In these approaches local actors take a larger role, or even a leading role. Such breeding approaches are described as collaborative and/or participatory approaches (Nuijten et al. 2017; Chable et al. 2020). In addition, instead of talking about a value chain, these local and regional initiatives can be better described as local and regional food systems or food networks, as they consist of networks of actors that often have several roles of food producers, processors and food sellers in various ways, sometimes simultaneously, where there is no sharp delineation of these tasks. In addition to producing products, these actors consider the nature of the process very important.

Collaborative approaches may also help reconnect agriculture with society. Involving consumers/ citizens in the selection process will help create awareness about agricultural practices and making citizens (feel) more involved. Consequently, dialogue between farmers and citizens can improve. In the scope of the DIVERSIFOOD project, these approaches have been further elaborated (Chable et al. 2020).

Since the 1980s, various initiatives have started to develop participatory breeding projects, mostly focused on involving farmers in order to select varieties that are well suited to the farmers' needs (Almekinders and Elings 2001, Ceccarelli and Grando 2020). Most participatory plant breeding can be best described as participatory variety selection. It means that farmers are involved in the selection of the best material towards the end of the breeding process. For breeders this is relatively simple to organise. It is also common practice for commercial breeders to test their elite material on farmers' fields to decide what to register. Involving farmers at earlier stages of the breeding process is more of a challenge. Although the benefits of involving farmers in on-farm breeding are clearly demonstrated, for example at early stages of barley breeding (Ceccarelli et al. 2001; Ceccarelli 2015), such approaches are not yet widely used, because of lack of incentives for breeders and because participatory approaches are not part of university curricula (Ceccarelli and Grando, 2020). Farmer selection in early breeding stages proved useful for pea breeding targeted to organic systems of Italy, where it provided higher yield gains than ordinary selection performed by breeders in a formal comparison of selected material (Annicchiarico et al. 2019). Implementation of participatory approaches implies a shift in thinking in regard to issues like seed and food sovereignty. Seed policies and the right to food were identified by De Schutter (2009), former UN special rapporteur on the right to food, as important for simultaneously enhancing agrobiodiversity and encouraging innovation.

Challenges

There is clearly a need to further develop effective approaches and to upscale successful approaches. This will require different styles of thinking. Because of cultural diversity, the breeding will need to be

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tailor made to local/regional conditions. In various initiatives different ideas may develop about issues like ownership of the results of plant breeding, and how to finance plant breeding. To upscale these approaches depends on various factors. Hekkert et al. (2007) identified the following factors to determine whether transitions come through: (1) entrepreneurial activities; (2) knowledge development; (3) knowledge exchange; (4) guidance of the search; (5) formation of markets; (6) mobilization of resources; and (7) counteracting resistance to change. These aspects are relevant in the context of chain-based breeding where various chain actors work together to develop a new product (Nuijten et al. 2017). These factors may not all be equally important when it comes to a transition towards more tailor-made technologies at local/regional level. In the case of community-based breeding, the development of a process at local level is more important. Serpolay et al. (2018) identified various aspects that are important to foster good collaboration. The following challenges and examples will be discussed here:

- *Factors important for sustainable collaboration*

As part of (re)developing local/regional food systems, a number of factors were identified in the DIVERSIFOOD project (Serpolay et al. 2018) to foster collaboration. These factors are a *common will* to undertake certain activities to foster change, to develop a *common vocabulary* in order to better understand each other and avoid confusion, which is also important for *trust building*, and contributes to *transparency*. *Facilitation* is important to support these processes and should not be underestimated. When setting up collaborative or participatory breeding, also *various types of resources* are indispensable, ranging from machinery and other facilities to sufficient financial resources. In addition, it is important that sufficient people are involved in order to have a well-*adapted distribution of the work.*

- *Sustaining long-lasting participatory and/or collaborative plant breeding*

In the Netherlands it is commonplace for farmers to be involved in potato breeding (Almekinders et al. 2014). Farmers do selection in the first three to four years of the breeding process, in this way sorting through a lot of progenies of crossings and maintaining only less than 1-5% of the original starting material. Today about 50% of the Dutch potato varieties have been developed in this way. Also, for organic potato breeding this model is being used. This approach was first supported by the Dutch government in the 1930s through subsidies (Van Loon 2019). Nowadays farmer-breeders can receive roughly 50% of the royalties once their selections make it to the market. This clearly shows that governments can have a decisive role in the development of new breeding approaches.

Long lasting collaboration between scientists and farmers can also be an option to develop participatory breeding approaches. An example is the development of organic dynamic populations of bread wheat in France. Scientists of INRA develop dynamic populations with high levels of diversity in which farmers in different regions with different pedo-climatic conditions make selections that are adapted to their conditions, using natural selection and/or simple mass selection methods. In the DIVERSIFOOD project a range of methods have been developed for scientists to support farmers in their selection work (Goldringer and Rivière, 2018). However, it is also fair to mention that such methods have been first described in the 1990s. Key questions are then what is needed to sustain such long lasting collaborations. One aspect is the collaboration itself, but an even more important aspect is the financing of such collaborations.

- *Financing initiatives*

The issue of financing will be further dealt with in detail in Topic 5. Here it suffices to mention that financing can be done in various ways according to different organisational models. In the case of chain-based breeding, the idea is that different value chain players support the development of new varieties. Often the chain players will need to see return to invest through special marketing, such as special products or special labels. In the case of community-based breeding, the underlying idea is that the focus is on the breeding process itself, and less on the end-product. In both cases, it is clear

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that breeding needs much investment and that breeding using collaborative and/or participatory approaches may reduce the costs, in combination with new cultivar concepts.

- *New cultivar concepts*

Nowadays the common cultivar concept is that cultivars should be Distinct, Uniform and Stable (DUS) in appearance, the reason being that they can be recognizable and distinguishable in order to be registered as precondition for commercialization of seed and to grant variety protection (breeders right). In addition, varieties of arable and forage crops need to pass the so called VCU test (Value for Cultivation and Use), proving its advantage over already registered varieties.

However, for some collaborative breeding initiatives, breeders' rights may not be an issue. Hence, the required uniformity (with often maximum 1% off-types allowed) is also of less importance and other forms of cultivar concepts can be used. Reaching sufficient uniformity is difficult and requires time and resources. For organic agriculture, cultivars with more genetic diversity have the advantage of better adaptability. Such cultivars are also easier to develop in local initiatives as they are aimed at local adaptation and marketing instead having to meet standard criteria of anonymous markets.

With the advent of the new EU organic regulation (2018/848) that will come into force in 2022 it will be possible to market also so-called Organic Heterogeneous Material (OHM) without prior DUS testing. Moreover, organic varieties suited for organic production (OV) have been defined in this regulation. Currently, the advantages of OHM are being explored, to achieve, amongst others, better yield stability, and better tolerance to diseases and abiotic stresses due to the higher level of genetic diversity of such material (Costanzo and Bickler 2020; Deliverable 2.8). Most work so far has been done with wheat, with the development of Composite Cross Populations (CCPs). For vegetables crops, the concept of OHM could also have advantages, such as in the case of mildew in lettuce and spinach. In tomato OHM is being developed in order to improve adaptability to local pedo-climatic conditions. In the scope of the of the new EU organic regulation a new 7-year temporary experiment will start to facilitate commercialization and characterisation of OV, e.g. through adjustments of existing DUS and VCU variety testing systems.

- *Testing for taste and nutritional quality*

So far, the most important traits for cultivar evaluation are yield, disease resistance and tolerance, and traits such as ease of harvesting, storability and uniformity. These are all traits to minimize risks on the farm and increase efficiency during transport and handling in the value chain. Quality can be separated into outer quality (uniformity, colour, shape) and inner quality (taste, nutritional value). Yield is easily quantifiable and easy to measure. Outer quality is also easy to observe. However, traits like taste and nutritional value are more difficult to evaluate, as they can be influenced by pedo-climatic conditions and farming styles. Also, taste may be very different depending on the time of evaluation such as in the case of pumpkin or cabbages. An evaluation shortly after harvest or after several months of storage can give very contrasting results. Another aspect of taste is that it can be very subjective. A solution would be to quantify taste by involving many people.

- *Involving consumers / citizens in collaborative plant breeding*

It is also suggested to involve consumers in taste evaluations. Consumers may often not be well trained to give exact descriptions of taste, but they can indicate what they like, and why. Involving many consumers can give more solid results. As taste can differ from one season to another, it is important to have taste evaluations over consecutive seasons to find out what breeding material has potential. Another advantage of involving consumers in taste tests is also that it helps to reconnect them with agriculture and to increase awareness about where their food comes from.

- *Awareness raising*

The interaction between farmers and citizens can help them develop more awareness about their own role in the food system and the roles of the others in the food system. Awareness may also lead to

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critical reflection and hence about how to further improve their own practices. This can also be a joint process, which can only be possible if there is sufficient trust and openness.

- Rethinking testing procedures for release and marketing
High costs for registration and variety maintenance are often barriers for the registration of new varieties. This is particularly the case for breeders with a small budget, or for crop cultivars developed for small markets. Collaborative approaches may facilitate testing and release of cultivars. In the case of the organically bred apple variety Collina, the breeder first involved colleagues in testing his variety before registration (Nuijten et al. 2018). Because his colleagues responded very positively, the breeder was sure that there was a market for his new apple variety and that he would be able to recover the registration costs.
In a collaborative breeding process of organic cultivars and/or OHM the development and testing phase may also partly overlap. When at a certain stage something interesting has developed with good field traits and good taste and nutritional value (a farmer selection or a dynamic population), a next step will be testing at a number of farmers' fields. To know whether the material is interesting for which farmers (with what sort of pedo-climatic conditions) it will be good to distribute the material to farmers with different pedo-climatic conditions. As not only yield and field traits but also taste and nutritional value will be evaluated, several extra years will be needed to know the actual performance of all these traits. Instead of a relatively short evaluation phase of two or three years, a longer evaluation may provide better results. In this way farmers may have the opportunity to find out what material really fits well under their conditions. Such approach implies distributing larger numbers of potential cultivars for testing to farmers and only register those cultivars that are really the best. With OHM there is also the possibility that certain farmers who conduct testing do some further selection themselves for further improvement of the OHM to fit their conditions best.

- Further development of collaborative approaches in breeding
As described by Ceccarelli and Grando (2020), the first participatory initiatives in breeding were aimed to develop cultivars better suited to farmers' needs. Collaborative approaches require a shift in thinking towards equal partnerships between breeders, farmers and other actors in the food system. Such shift in thinking requires time. For example, in the EU-project SOLIBAM (from 2010 to 2014) that aimed to develop integrated breeding approaches, the term resilience was defined at the level of the agro-ecosystem: "Resilience is the capacity of an ecosystem to respond to a perturbation by resisting damage and recovering quickly. A resilient system will reorganize while undergoing change so as to still retain essentially the same function, structure, identity, and feedbacks. Thus, resilience is linked to the adaptive capacity of a system in the face of change." In the following EU-project DIVERSIFOOD the "**resilience concept is extended to the whole food system**, including economic, social, political and cultural dimensions. Thus, resilience of the food system calls for adaptive capacities of the food chain at the agro-ecological and socio-economic level to provide sufficient high-quality food and to maintain its cohesion over time." (Collective publication, 2017).
Thus, the further development of collaborative approaches also requires a shift in thinking not only about agro-ecological issues, but also about socio-economic, political and institutional issues. In order to achieve this, a continuous process of reflection is needed as described by Rossi et al. (2019) as part of a continuous collective learning and innovation process in which all actors of the food system are involved (Chable et al. 2020).

Outcomes
In the past forty years, much knowledge has been developed about participatory and collaborative approaches in breeding. However, in the context of organic breeding, collaborative and multi-actor approaches need to be developed further and upscaled in order to become part of daily practice.

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LIVESEED is funded by the European Union's Horizon 2020 under grant agreement No 727230 and by the Swiss State Secretariat for Education, Research and Innovation (SERI) under contract number 17.00090.

Topic 4) Breeding for diversity based on agro-ecological principles: Concepts of genetic diversity, resilience, local adaptation, Examples on GxExM interaction; and GxExS interaction

Introduction

Following the principles of organic agriculture, i.e. ecology, health, fairness and care, an important challenge is how to benefit from genetic diversity in order to improve aspects of resilience, local adaptation and yield stability through Genotype x Environment interactions. Over time, awareness increased that the principles ecology, health, fairness and care are interrelated meaning that organic breeding should not only focus on the technical aspects of breeding methods, but instead should also take into account the socio-economic aspects (Chable et al. 2020, Lammerts van Bueren et al, 2018). Taking into account socio-economic aspects also means new perspectives on concepts like genetic diversity, resilience and local adaptation. Below, relevant outcomes of the EU projects SOLIBAM, DIVERSIFOOD and LIVESEED are described. The practical translation into new breeding methods utilising genetic diversity is described in Topic 14.

Concepts of genetic diversity

Here, the word 'cultivar' is used to encompass all types of cultivated varieties since the word 'variety' has been captured by regulation systems to designate uniform and stable varieties (see UPOV definition). In the LIVESEED project, the word 'cultivar' is meant to be more inclusive and is defined as follows: "general term for officially released varieties, landraces, less homogeneous populations, niche varieties, etc." For more details see Annex I.

Results of the EU-project DIVERSIFOOD showed that the high number of terms to qualify cultivated plants (e.g. landraces, local varieties, farmer/peasant varieties, heirloom varieties, populations, modern varieties, cultivars) represents many points of view to define them and the different visions behind them. These different viewpoints are related to different scientific, socio-economic and practice backgrounds, cultures and languages. Moreover, based on new insights, societal dynamics and scientific developments, interpretations of certain words may change over time. Hence, it is impossible to have clear, concise, and distinctive definitions for each of the categories.

In DIVERSIFOOD Deliverable 1.1 (2019) an attempt was made to differentiate cultivars using the type of market for which they have been bred as criterion:

1. Conventional agriculture: "Modern varieties are bred for geographical wide adaptation, which means that they should be marketed and then cultivated by farmers in different regions. That is possible thanks to external inputs in farming that homogenise most of the environmental factors. This criterion is also at the core of the breeding business model producing these varieties. In order to cover the cost of breeding (more and more in the hand of private companies) the same variety should be marketed and grown on as many hectares as possible. These modern varieties are registered and protected by plant breeders' rights that permit private breeders to recover the costs of breeding through the payment of royalties."
2. Organic agriculture: "Under the umbrella of varieties for organic agriculture one can find many different kinds of cultivars: from modern varieties only certified "organic seed" (and not bred organically) to local varieties or open pollinated varieties (in some cases also bred for organic). Hence, these cultivars originate from both conventional and organic breeding. The business model of organic breeding is still mainly based on donations by private foundation, because the royalties seem not to work well to compensate the costs of breeding: varieties are more locally adapted than the previous category and therefore each variety is cultivated on smaller surfaces reducing the income from royalties. They can be registered or protected with breeder's rights according to their degree of uniformity or novelty. The new organic regulation 848/2018 is opening a new space under this

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framework for two new categories: organic heterogeneous material (OHM) and organic variety suited for organic production (OV). Registration and certification processes for these new categories will be defined through delegated and implementing acts by the EU Commission in the coming year. This regulation will enter into force in 2022."

3. Local markets: "In these markets, locally adapted and collectively managed varieties play still an important role. They are usually heterogeneous cultivars that can be landraces, old varieties or new farmers' varieties bred in participatory plant breeding programmes. It is difficult to find a legal space for marketing them within the current framework due to their diversity. Therefore, they cannot be protected by IPRs and there is a growing interest in using open source or commons to manage them."

The concept of resilience

Similar to the concept of genetic diversity and concepts of varieties, many definitions exist of the word resilience. At the level of the agro-ecosystem, the EU-project SOLIBAM (2010-2014) has proposed the following definition of the word resilience: "Resilience is the capacity of an ecosystem to respond to a perturbation by resisting damage and recovering quickly. A resilient system will reorganize while undergoing change to still retain essentially the same function, structure, identity, and feedbacks. Thus, resilience is linked to the adaptive capacity of a system in the face of change".

Within the EU-project DIVERSIFOOD (2015-2019), the resilience concept was extended to the whole food system, including economic, social, political, and cultural dimensions. Thus, resilience of the food system calls for adaptive capacities of the food chain at the agro-ecological and socio-economic level to provide sufficient high-quality food and to maintain its cohesion over time.

The concept of local adaptation

It is often thought that farmer selections have local adaptation instead of broad adaptation because the farmer selections were often selected in particular localities. Research on various crops across the world shows that when we talk about local adaptation, we should define the actual agro-ecological conditions under which particular so-called 'local' varieties have been developed and that such varieties often do well under similar agro-ecological conditions in other regions as well (Mokuwa et al. 2013). However, adaptation to particular agro-ecological conditions will not automatically mean successful adoption by farmers in other regions. Adoption is also determined by adaptation to local economic and socio-cultural aspects, such as processing quality, taste and colour (Teeken et al. 2012, Chable et al. 2020).

GxE interaction, yield stability and yield reliability

The interaction of genotypes with cropping years can only be minimized by breeding varieties that are stable yielding across years. There are two concepts of yield stability, one relative to material that tends to maintain its yield constant across years (i.e., responding relatively better in unfavourable years), the other relative to material with low GxE interaction across years. The former has somewhat higher repeatability and heritability, allows statistically for a broader generalization and, most importantly, is more relevant for increasing the food security and the stability of agricultural income (Annicchiarico 2002). For selection or evaluation of plant material, mean performance and yield stability can be integrated into an index of reliability that assigns to stability a weight proportional to the expected degree of risk aversion by farmers (Annicchiarico 2009). The practical application of this knowledge is further described in Topic 14.

The evolvement of concepts such as GxExM and GxExS interactions

In the 1980's, some scientists started raising awareness about the importance of genotype x environment interaction (GxE interaction) and looking for the beneficial aspects (higher yields and better local adaptation) instead of trying to minimise GxE interactions (Ceccarelli 1989). In other words: where does which variety fit best. Topic 14 describes how to use population breeding strategies to benefit from GxE interactions for local adaptation. Gradually, awareness increased that

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also farm management and other socio-cultural aspects should be looked at. Hence the broadening of GxE interaction concept to **GxExM** (Kropff and Struik, 2002, Desclaux et al., 2008) **and GxExS interactions** (Ceccarelli and Grando, 2007), where the M stands for Management and the S stands for Society, respectively.

Examples on GxExM and GxExS interaction and breeding strategies

Selection for specific adaptation can exploit GxE interactions relative to environmental variation for many possible factors depending on climate (rainfall, low winter temperatures, etc.), soil, organic vs. conventional cropping, pure stand vs. mixed cropping and type of associated species/cultivars, etc. Breeding for specific adaptation can easily incorporate, and is reinforced by, farmer-participatory selection (Ceccarelli 2015), and is the ideal context for evolutionary selection schemes (e.g. Murphy et al. 2005). Breeding for specific adaptation can maximize the contribution of landrace and old cultivar genetic resources, as this material tends to have narrower adaptation than modern commercial varieties. While many landraces and old cultivars displayed specific adaptation to less favourable environments, the opposite response has also been observed for material that evolved in favourable environments (Annicchiarico and Piano 2005).

GxE interactions across organic vs. conventional cropping have special importance for organic breeding and organic agriculture. Several studies have provided direct or indirect evidence for greater performance under organic farming of material selected under organic management or selected for traits considered of special value for this management, e.g., for cereals (Murphy et al. 2007), grain legumes (Annicchiarico and Filippi, 2007) and vegetable crops (Serpolay et al. 2011). Also, relevant to breeding for organic systems are the reports of successful breeding for specific adaptation to intercropping (Annicchiarico and Proietti 2010).

Challenges and outcomes

One of the lessons learned of the DIVERSIFOOD project was that even though the majority of the partners involved in the project were very aware of the importance of integrating the technical and social aspects in breeding and the management of genetic diversity in general, partners realised they were part of a learning process (Rossi et al. 2019, Chable et al. 2020). The integration of different knowledge fields and perspectives is a process that requires continuous attention. Whereas technical aspects related to plant growth can be easily quantified and easily communicated, the socio-economic and cultural aspects that are also important in the process of improving resilience, utilising local adaptation and the maintenance of genetic diversity cannot be quantified and hence their communication is much more difficult. Hence, care needs to be taken to further foster the concepts outlined in this topic. Awareness of their importance is reflected in the following topics. Practical experiences with optimising and utilising GxExM interactions are described in Topic 14.

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Topic 5) Financing organic breeding initiatives

Problem description

Taking account of organic agriculture principles by IFOAM, the financing of organic breeding would differ substantially from that of conventional breeding. Indeed, as financial schemes need to fit to the respective organizational models and to the culture and ethical values of reference, the differences in terms of principles of organic *vs* conventional farming have relevant implications on the economic strategies applied in the breeding sector. In the organic sector breeding is not only a commercial activity, but also considered a cultural activity, or a joint activity by the actors or value chain or food system (see Topic 3). The organic sector values the increase of biodiversity, promotes free access to genetic resources and respects the integrity of the cell as smallest entity (ECO-PB 2012) and seeds as cultural heritage beyond the mere market value as production input (see Topic 1). Furthermore, organic agriculture differs from conventional agriculture concerning the use of diversity at different levels (see Topic 4, 7, 8 and 14). Organic farming requires the use of broad crop rotations. This, regarding breeding, determines the need to breed for a wide range of crop species, including minor and neglected crops, often with a relatively small total area per crop. Organic farming promotes the use of locally adapted cultivars and this for breeding means that the area under production of a single cultivar can be small, despite the share of organic farmland and the importance of the crop species the cultivar belongs to.

A recent report on ways to finance organic plant breeding by Kotschi and Doobe (2020) shows that the financing volume of organic plant breeding has been growing continuously by about 10% per year in the last years in Austria, Germany and Switzerland. At present, a total volume of approximately 5 million EUR per year is estimated. This is a success for the organic breeding sector but measured against the demand of organic seed and placed in a European perspective, this sum is still very low. It is about 1-2% of what yearly is invested in The Netherlands alone on conventional vegetable breeding (Kotschi and Doobe 2020).

The existing Organic Plant Breeding initiatives (many of which are non-profit organizations) are currently characterized by a high degree of efficiency in terms of cultivars produced with the available funding. However, an improvement in the financial basis is urgently needed in order to reach appropriate number of crops worked on, number of breeding sites, adequate technology used in the field and laboratory, infrastructure, etc. Additionally, a strong boost to the sector is needed to meet the target of phasing out derogations for non-treated conventional seed use planned by new European Organic Regulation 2018/848.

Despite the urgent need for organic breeding and organically bred cultivars seed production, financing of organic breeding remains a critical issue. The topic of adequate financing strategies, able to combine the respect of the organic sector values and the need for a strong increase in cultivar availability and organic seed production, is currently a challenge open to the whole organic sector. This is because the success in building an independent organic breeding and seed sector is strongly connected to the need to guarantee the integrity of organic products, and as such to maintain the integrity of the whole organic production cycle.

LIVESEED has contributed to the debate facilitating (with interviews, surveys and workshops) the dialogue among different actors of the organic sector (farmers, breeders, seed producers, processors, wholesalers, retailers, associations, consumers) to map the current financing strategies and design new business models for organic breeding. The work on this topic is included in a manuscript submitted by Winter et al. to the journal Agroecology and Sustainable Food Systems.

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Current financing strategies of breeding applied in the organic sector

Refinancing through royalties or seed sales (business model common in the conventional breeding sector) is a business model mostly used by conventional seed companies that also have Breeding for Organic programs. However, there is wide consensus in the Organic Plant Breeding (OPB) community that this business model cannot be easily applied to their context. The main reasons are that: (i) OPB aims to breed for many different crops and to produce high-diversity locally adapted cultivars and (ii) several OPB initiatives do not want to apply for variety protection (Wirz et al., 2017) in order to maximise the free access to genetic resources (see Topic 2). In cereal breeding (OPB), detailed economic figures were last recorded in 2013 (Kotschi & Wirz, 2015). These show that on average, license income, variety-development--contributions and seed sales cover only 9% of breeding costs, with a variation range of 3 to 15%. These data clearly show that alternative financing strategies must be identified to sustain organic plant breeding.

Public funding

Public breeding programmes in Europe have been strongly reduced in the last few decades. In most regions of Europe, the commercial enterprises are the major or even the only entity to place new cultivars on the market. Nevertheless, public contribution to plant breeding is still ongoing in some countries. For example, a positive case is potato breeding in the Netherlands: The current collaborative breeding approach is based on government financing that allowed 'Advising, coaching and encouraging breeders from 1938 onwards' (Van Loon 2019). Today, a culture exists where many farmer potato breeders breed for a hobby rather than for money.

Today, a major part of public funding used in organic breeding comes from EU research projects (e.g. H2020 DIVERSIFOOD, LIVESEED, ECOBREED and BRESOV) or national research programs. However, this type of public funding does not cover the cost for practical breeding work in the last years before cultivar release and comes with high administrative workloads for bureaucracy and management.

Private foundations

Private foundations (for example www.zukunftsstiftung-landwirtschaft.de) are important players in organic breeding financing via funds dedicated to organic breeders. The advantage of this financing strategy is that the annual donations are passed on to organic plant breeding with low administrative burden. However, also with this type of funding substantial resources may lack for the last 3 - 4 years of cultivar development prior to release. Other limitations are that foundations work on specific territories which limits the possibility to access to such financial resources in areas with limited presence of this type of donors. For example, at the moment the foundations specifically financing organic breeding are concentrated in Central Europe. As well, several foundations give priority for start-ups and because of this their support may not always be a suitable solution for long-term economic sustainability. How much financial resources foundations can invest depends also on the priority on social issues that they set internally and the economic framework (i.e., interest rates) and crisis. Private donor funds can be a substantial pillar of organic breeding financing but additional sources, especially resources coming from inside the sector are necessary for guaranteeing a strong and sustainable financial base.

Open Source Seed approach

The aim of the Open Source Seed approach (see Topic 2) is to create a seed sector based on seed as commons and to establish a counterweight to the patenting and monopoly of multinational seed companies. By securing seeds as common goods, the increasing shortage of freely available breeding material is to be stopped and the existence of small and medium-sized organic breeders, including farmer-breeders and community-based breeding initiatives, strengthened. Initial experience with the distribution of open-source licensed varieties has shown that consumers greatly appreciate this alternative to privatisation. It gives the individual the opportunity to take concrete action against

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monopoly in the seed sector, a motive that can significantly increase the demand for products from open-source varieties. Consumers can therefore generate a pull effect, not only for open-source seeds, but for organic breeding in general. Therefore, open source may become a successful narrative to raise consumer awareness and recognize the need for organic plant breeding. The concrete role of this strategy in terms of economic sustainability of organic breeding initiatives needs to be evaluated in the long-term and will depend on how the choice of the open-source licence will actually impact the direct involvement and economic support by the public.

Small scale value-chain collaborations

Refinancing strategy via direct involvement of the different actors of local food systems is a strategy often applied by decentralised farmer-breeder organisations and peasant seed networks. The direct sale or local and short value chains of the end products from the co-developed cultivars are an asset of this type of financing strategy. Additionally, using collaborative and/or participatory approaches and focusing on organic heterogeneous material and on solutions for local food systems may also help to limit the cost of cultivar development.

Looking more in general at value-chain collaborations, there are examples of small scale collaborations of value chain actors to produce cultivars suited to organic conditions (e.g. *Fair-Breeding®* and *Organic Seeds Sunflower*). More initiatives with collaborative financing strategy with the aim to select disease-resistant apple and potato cultivars, are active in the Netherlands, France, and Switzerland. These initiatives have developed various strategies to make the introduction of resistant cultivars into the organic market successful through different types of value chain partnerships (Nuijten et al. 2018). These examples underline that through smart novel approaches of market introduction and good networking new cultivars can enter the market successfully.

Outcomes and perspectives

In the organic sector, the diversity of breeding initiatives is considered a prerequisite for the restoration, maintenance and further development of the biological diversity of cultivated plants and for adaptation to local agroecosystem and socio-cultural specificities, which is indispensable for the major future challenge of agro-ecological transition of farming systems. Yet, it is clear that the economic implications for meeting the ecological, cultural and ethical principles of organic agriculture cannot be the sole responsibility of farmers and breeders but need to be shared along the value-chain for grounding the financing of organic breeding on a solid and sustainable basis.

The current funding options do not cover the resources needed to respond to farmers' and value chain needs of a broader assortment of cultivars adapted to organic conditions and for matching the phasing-out of non-organic seed use derogations (new EU Organic regulation 848/2018) by increasing the numbers of organic-bred cultivars. As a broader and more sustainable funding is needed for organic breeding, in the framework of LIVESEED activities in 2018 and 2019 (WP 3 and 4), different activities (including a farmers' survey, expert interviews and workshops) were conducted to collect the perspective of different actors of the organic sector (farmers, breeders, seed experts, food industry and retail sector professionals, association and civil society). This work was in collaboration with a parallel project conducted by FiBL in Germany and Switzerland, on demand of the federal association of organic food industry (BÖLW), and financially supported by the Mercator Foundation Switzerland and Software AG Foundation (Schäfer and Messmer, 2018). Given the success factors that could be deduced from the current experiences of financing breeding with collaborations along the value chain, this activity helped to summarise the opportunities for integrating organic breeding in value-chain partnerships. From this activity it emerged that the development of a pool funding strategy for organic breeding in Europe could serve as a central pillar for the financing of the different organic breeding organizational models. The pool funding concept is not new; for example, in the textile industry is ongoing since 2017 a participatory organic cotton breeding program (https://www.sgf-cotton.org/) that is co-financed by the Organic Cotton Accelerator

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(https://www.organiccottonaccelerator.org/). It was also proposed in 2009 by Osman et al. (2016) for financing organic wheat breeding in the Netherlands.

The central concept of the pool funding strategy proposed is that all value chain partners of the organic sector should make a collective effort to invest in organic breeding. If a small part (e.g. 0.1-0.2%) of turnover at the point of sale of organic products would be collected into a pool fund, which is coordinated and then distributed to individual organic breeding initiatives, a high level sector collaboration could boost the growth of organic breeding. An alternative could be that various chain actors carry the responsibility for various parts of the breeding (Nuijten 2019). This however needs very careful coordination and communication to keep all actors involved over a longer period.

At the moment, a pilot project (started in 2020) is ongoing to practically implement the pool funding business model in Germany. The project is coordinated by the federal association of organic food industry (BÖLW).

Challenges

A coordinated financing strategy implementing the pool funding concept in Europe would support to overcome the current limitation of segmented donations and still allow to keep the freedom for different organizational models of organic breeding to benefit from a common effort of the whole sector. Nevertheless, it is not desirable to have the financing of breeding completely dependent on a single strategy. It is also important to mention here that different breeding strategies (e.g. variety development, heterogeneous material, etc) go together with different organisational models and financing strategies (Nuijten 2016a, 2016b). It is preferable to have quantitatively relevant finance resources from pool funding based on value-chain collaboration backed up by likewise strong public contribution and private donations as three main pillars. Examples for distribution of finances across different initiatives can be found in Germany. The sector should show the interest and support to organic breeding by contributing to its financing as well as by advocating for higher public commitment in terms of resources but also of public institutions' direct involvement in organic breeding programs. Recently the Bavarian government invested into a public organic breeding platform. Furthermore, an increased awareness among civil society is necessary in order to increase the donations by private foundations. A diversified financing strategy (including public funding, resources from cross-sector pool funding strategy and private donations) is desirable for a sustainable growth of organic breeding and for allowing the diverse organizational models of OPB to be out- and up-scaled across Europe.

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Section 2: Breeding strategies for organic breeding

Topic 6) Trade-offs between resilience, yield and quality in the breeding process

Problem description

In organic agriculture the starting point to grow a good crop is a healthy soil. Measures to improve plant growth during the season are limited, but also undesired from the perspective of a holistic approach which is at the basis of organic. Hence, crop varieties need to have good yield stability and general resilience, rather than maximum yield potential. Improved resilience can also help buffering against climate variability and climate change. An important challenge in organic breeding is to maintain a favourable balance between resilience, yield and quality. First, it is important to further define crop resilience. Resilience can come in various ways. The common meaning is 'the capacity to recover quickly from difficulties'. It is also referred to as toughness, elasticity, or plasticity. However, the building of resilience in agro-ecosystems has not been well studied (Lin, 2011). In this section, the emphasis is on how plant breeding can contribute to building resilience in agro-ecosystems by improving particular plant traits.

There are many plant traits that can improve crop resilience, such as rooting depth and flexible root growth in order to catch water and nutrients, nutrient use efficiency (NUE) and water use efficiency (WUE) (both further described in Topic 9), plant microbe interaction (further described in Topic 8). Disease resistance can be part of resilience or, in other words, can contribute to resilience (see Topic 11). The above-mentioned traits apply to all crops. As crops have different patterns of growth, they can also have different coping strategies with unfavourable conditions. An example is tillering ability in rice: the higher the tillering ability, the better the plant can cope with adverse conditions such as abiotic stress (Mokuwa et al. 2013). Tillering ability in wheat and barley can also contribute to weed competitiveness (Mahajan and Chauhan, 2020, but perhaps this is not the case for all weeds (Worthington et al. 2013): Further details are described in Topic 12.

In addition to particular plant traits, crop resilience can be improved through increasing diversity in varieties, by developing heterogeneous material, such as dynamic populations and composite cross populations. In populations, improved resilience and adaptability can be achieved through processes of compensation and collaboration between plants, resulting in better yield stability (Döring et al. 2011, 2015). Diversity in crops can also be used to improve field tolerance against diseases, which will be discussed in sections 7 and 11. In this section the focus is on the improvement of plant traits relating to resilience, with emphasis on the trade-offs with yield and quality.

Yield is often measured in weights and volume, or in terms of economic or marketable yield: the total produce that can be marketed. The exact definition of marketable yield will differ per crop, and it also depends on the purposes for which it is used. For example, in the case of wheat, we often think in terms of grain yield. However, for animal husbandry, the straw yield can also be useful. Quality can be understood in different ways. In this section we understand quality as taste and nutritional quality, rather than merely outer quality (colour and shape). Storability, suitability for processing and cooking are other aspects of quality. Improving particular traits may affect other traits. Therefore, it is important to know which traits are interrelated and which traits are not? Generally, disease resistance is considered not to have a trade-off with yield. However, in practice, varieties with good disease resistance of field crops (such as wheat or potato) often have lower yield potential. It is often thought that relationships without trade-offs between resilience, yield and quality are difficult to find. Correlations can be easily understood as that improved resilience is related to reduced yield (Lazzaro

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et al. 2019). However, whether the correlation is related to underlying causal mechanisms are difficult questions to answer. One reason is that often different sets of varieties are compared such as old landraces and modern varieties of wheat which were developed for different purposes under different agro-ecological conditions. Below, examples of the complex relationships between resilience, yield and quality are given for several crops.

Examples

Reducing insect damage in cabbage

Thrips (*Thrips tabaci*) can be a real problem in organic cabbage cultivation. Resistance breeding against thrips is very difficult to impossible. There are no natural substances that can repel Thrips sufficiently. A good solution to reduce thrips damage is the amount of leaf surface wax. Earliness and Brix are other factors that can explain thrips damage (Voorrips et al. 2008). A clearly negative correlation between leaf surface wax and earliness was found in several studies (Trdan et al. 2004; Voorrips et al. 2008), meaning that late maturing varieties have a thicker waxy layer. Voorrips et al. (2010) concluded that a biological cause may be behind the correlation between thickness of the wax layer and earliness. A possible explanation could be that late maturing varieties have more time to convert energy in substances that can be then converted into waxy substances.

Žnidarčič et al. (2008) reported that a thick wax layer can also reduce damage caused by flea beetles (*Phyllotreta* spp.) and cabbage stink bugs (*Eurydema ventrale*). Whether a thick wax layer may or may not have a negative impact on quality is unclear. It is unlikely that a thick wax layer has a negative effect on nutritional quality, but it is suggested that there may be negative effects for processing like in the case of sauerkraut.

Trade-offs in cereals

A plant trait that are unlikely to have a trade-off with yield in wheat is for example the distance between the spikelets. Larger distances between spikelets allow a quicker drying of the spikelets and hence a reduction in spike diseases. However, the trade-off between yield and baking quality is quite well known for wheat and maize: higher yields are often related to lower baking quality (Osman et al. 2016). In the case of maize, a long-term selection experiment also showed a negative relationship between yield and content of oil and protein (Dudley et al. 1974). Other traits contributing to resilience may not have a trade off with yield and/or baking quality. Murphy et al. (2008) found no clear relationship between weed competitiveness and grain yield. As no causal relationship was found, it should be possible to improve both weed competitiveness and grain yield at the same time (Lammerts van Bueren et al. 2011). Allard (1988) reported that various loci can contribute simultaneously to different quantitative traits such as yield, disease resistance and plant morphology. Nutrient Use Efficiency can be improved through, amongst others, deeper rooting. According to Foulkes et al. (1998) older wheat varieties have better abilities to extract Nitrogen in low-N environments than modern ones. An explanation could be better cooperation with the soil microbiome (see Topic 8). Research on grass (*Lolium perenne*) showed that high root biomass can go together with high above ground biomass (Deru et al. 2014). In other words, there seemed not to be a trade-off between below and above ground biomass production. Anecdotal observations suggest this may also apply to cereal crops like wheat and maize.

Lupin: yield – alkaloid content – plant vigour

High alkaloid content makes lupin unsuited for food unless complex and water-consuming processing is applied. Novel registered cultivars ought to possess low alkaloid content, and a maximum threshold for total alkaloid content of 200 mg/kg has been fixed for lupin flour and foods by the Health Authorities of Great Britain, France, and Australia. However, high alkaloid content tends to confer

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greater fitness to white lupin via greater tolerance to biotic stresses exerted by pathogens and herbivores (Wink et al. 1998). This complicates the seed multiplication of sweet (low-alkaloid) cultivars, because occasional bitter-seed mutants do occur and tend to increase across generations of seed multiplication, sometimes jeopardizing the suitability of the cultivated material for human consumption. In the Netherlands, this negative relationship between sweetness and plant vigour has been observed. Another observation was that there were sweet-seeded lines with good yield potential (Nuijten and Prins 2014). Selection for low alkaloid content is a basic requirement for the food and feed market, and a great challenge for breeders. Moreover, also pedo-climatic conditions might increase seed alkaloid content above the threshold of 200 mg/kg (Annicchiarico et al. 2014).

Brassica: plant robustness – feed and food quality - CMS

Glucosinolates are known to improve protection against pests in Brassica, in that way improving plant robustness or resilience. In the case of rape seed, the question is what the effect of higher levels of glucosinolates is on animal feed quality. In the case of cabbage (including crops like broccoli and kohlrabi), it is reported that particular glucosinolates can contribute to human health, and that there is clear variation in glucosinolate levels between varieties (Rosa and Rodrigues 2001; Renaud et al 2014). If in field trials clear differences in yield are observed, the question is whether there is an actual relationship between yield and glucosinolate content, or whether the differences in yield and glucosinolate content are the result of different breeding material, processes and goals. Rosa and Rodrigues (2001) also found clear differences in glucosinolate content between early and late season cultivation. Renaud et al. (20014) found that particular glucosinolates were negatively related with carotenoids. Varieties highest in tocopherols and carotenoids were open pollinated and early maturing F1-hybrids (Renaud et al. 2014). In the case of cell fusion derived CMS F1-hyrids in cabbages (see Topic 1), much remains unclear about the actual relationships between CMS, stress proteins, robustness and vigour and measures taken to counterbalance possible negative relationships.

Challenges

The above examples provide an insight in the complexity of possible relationships between various traits. An important question is to better understand which traits are directly related, and which apparent relationships are caused by other factors. In the case of yield and protein content in cereals, it seems reasonable to argue that an increase in yield is related with a dilution effect in protein content. However, in the case of alkaloid content in lupin, it is difficult to understand the exact mechanism between alkaloid content and lower plant vigour. It could be that this relationship is based on the fact that the genes regulating low alkaloid content are neighbouring genes affecting plant vigour in a negative way and that there is actually now direct relationship.

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LIVESEED is funded by the European Union's Horizon 2020 under grant agreement No 727230 and by the Swiss State Secretariat for Education, Research and Innovation (SERI) under contract number 17.00090.

Topic 7) Breeding for complex systems (such as mixed cropping, agroforestry)

Introduction

Agricultural systems with higher levels of diversity can provide higher yields and yield stability with less inputs and often lower disease and pest pressure. A key limiting factor is that practitioners who want to use mixed cropping and agroforestry systems depend on cultivars bred for monoculture and are often not suited for use in complex systems. These practitioners are thus dependent on what is available on the market and need to find out by trial-and-error which cultivars can be used in complex systems. Another complicating factor is that such suitable cultivars may be taken from the market by the breeder when the cultivar has lost its value for cultivation in monoculture. Today, some experiences with breeding for mixed cropping is available. EU-projects like LIVESEED and ReMIX aim to develop tools for breeders to develop cultivars suited to mixed cropping. However, in the field of breeding for agroforestry systems, there is little information if any at all.

In this topic, first experiences in breeding for mixed cropping will be described briefly, followed by the challenges for breeding for agroforestry and how it can learn from lessons learned in breeding for mixed cropping.

Breeding for intercropping

Feed and forage crops

Intercropping usually implies the association of a legume and a non-legume species, owing to the several environmental and crop quality advantages provided by the presence of the legume. Breeding for intercropping has been given particular attention for forage crops, because of the long-standing cultivation of legume-grass mixtures. Legume companions in these mixtures or in annual legume-cereal mixtures often show a competitive disadvantage (Annicchiarico et al. 2019). Accordingly, compatible mixtures were defined as those that allow for a legume content large enough to optimise the benefits of its nitrogen fixation and superior feed quality value (Rhodes et al. 1994). Since the yield efficiency of a mixture depends mainly on the performance of its weaker partner (Harper 1977), selecting the less competitive species for greater competitive ability may be the main goal of breeding for intercropping, as proved by research on white clover (Annicchiarico and Proietti 2010).
On average, selection in pure stand exhibited about 40 % lower predicted yield gains than selection in target mixed stand conditions in a recent survey of case studies (Annicchiarico et al. 2019), encouraging the selection under intercropping or, possibly, in pure stand but on the basis of traits associated with greater general mixing ability and competitive ability. Breeding for compatibility with a wide range of plant companions is facilitated by the larger size of general-compatibility effects relative to specific-compatibility ones (Annicchiarico et al. 2019).

Food crops

Compared to feed crops, more breeding gaps exist in terms of breeding for mixed cropping. Whereas in the case of fodder crops, a field can be harvested at several crop development stages and still be used as feed, in the case of mixed cropping for food the main goal is often to harvest the grain of both crops (e.g. both the cereal and legume crop). Well-known examples are the combinations of wheat with faba bean or lupine, and barley with pea or lentil, depending on the pedo-climatic zone in Europe. The drier the climate and weather conditions, the higher the chances of a successful harvest. Given the potential advantages of mixed cropping, EU-projects like ReMIX and LIVESEED aim to gain a better understanding of important traits for successful mixed cropping and how to breed for such traits. Traits that are known to be important for successful mixed cropping are lodging tolerance and time of stem elongation and time of ripening. In the case of food crops, it is common practice to breed under

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monocrop conditions, but it is clear that cultivars developed for monocropping are not always the best partners in a mixed stand. For example, the combination of pea with barley can be prone to failure because both have rather poor lodging tolerance. Combinations of wheat and faba bean may fail due to differences in ripening time. A more successful approach is the combination of cereals (e.g. wheat or oat) with a companion crop like clover, either sown simultaneously with the cereal or sown about one or two months later. In both EU projects various experiments are ongoing to better understand what breeding methods can be used to develop cultivars suited to mixed cropping. In the case of mixed cropping for human consumption, involvement of the value chain (either local, regional, or anonymous) is important to be able to sell the produce. For example, after harvesting the produce from mixed cropping needs to be separated and still may contain grains of the other crop, which means that processors and further downstream chain actors need to be aware what the consequences could be in terms of allergies. Another aspect could be that cultivars suited for mixed cropping are not commonly used by processors and have other processing qualities than what the processors prefer. This means dialogue is needed with all value chain actors to discuss what solutions could be possible.

Breeding and Agroforestry

Introduction

A pressing challenge in modern agriculture is to sustain levels of productivity whilst ensuring environmental conservation. From this standpoint, agroforestry provides one solution as it delivers a range of ecosystem services whilst allowing continued productivity (Jose, 2009). Silvo-arable agroforestry systems involve the cultivation of crops and woody elements on the same piece of land. In temperate regions, the majority of silvo-arable systems that have been the object of research are alley cropping systems, i.e. arable fields delimited by tree rows, where the tree rows are monospecific and mostly dedicated to biomass production (Wolz and De Lucia, 2018).

The interaction between trees and crops can have both positive (complementary or facilitative) and negative (competitive) effects (Jose et al., 2004). The success of any agroforestry system depends on developing a better understanding of the biophysical mechanisms involved and using this knowledge to enhance complementary interactions and reduce competitive ones.

There are opportunities to minimise competitive effects through better design and management, but this can only go so far where there is the potential for breeding of crops to enhance traits better suited to tree-crop interactions in agroforestry systems. In this context it is important to better understand the various crop-tree interactions to ascertain which negative interactions can be alleviated through effective crop breeding for agroforestry systems. The role crop breeding can play in enhancing the productivity, stability and resilience of agroforestry systems needs to be better understood and the different constraints that (different) agroforestry systems pose need to be highlighted.

Breeding in the target environment is recognised as a priority to ensure local adaptation, and therefore crop resilience in organic and low-input systems (Ceccarelli, 1996; 1989). This concept has been widely explored and demonstrated in relation to marginal environments or organically-managed systems, in which cultivars selected in high-fertility, conventionally managed environments underperform and, conversely, cultivars that may be better suited, are discarded (Mikó et al 2014; Murphy et al., 2007). In fact, crop breeding is generally undertaken in, and for, homogeneous open fields, which might indeed result in crops that underperform in presence of the woody element. In theory, embedding the whole breeding process into agroforestry systems should result in agroforestry-adapted crops. However, when looking at how to implement this in practice, this idea risks being too simplistic. In fact, there are at least two aspects that make agroforestry systems different from any other potential ‘environment’ or ‘management system’ to be targeted in breeding: the explicit variation across space and across time.

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Table 1. Knowledge on tree-crop interaction

Type of interaction	Facilitative/complementary	Competitive/negative
Above-ground interaction	Buffering climatic extremes (windbreak)	Light interception by tree canopy
Below-ground interaction	Enrichment of nutrients from deeper layers	Competition for water
Complex and indirect interactions	Semi-natural environment as a source of functional biodiversity (IPM, IWM) Enhancement of soil microbiota	Semi-natural environment as a source of pests and weeds

Agroforestry systems are landscapes with explicit spatial variation

In a silvo-arable system, an arable crop rotation coexists with a perennial woody element which can assume different spatial arrangements. Two examples are the 'parkland', where trees are scattered in the arable field and is very common in subsistence tropical agriculture, and typical of specific sylvo-pastoral environments in Europe (like the Iberian 'dehesa'), and alley cropping, where arable fields are surrounded by tree rows, and is by far the most important configuration in temperate agriculture. This results in a diversified landscape composed of many micro-environments. Across a section of an ideal alley cropping system, in fact, there are at least three micro-environments: the mid-field, the tree row and the tree-crop interface.

The mid-field can be summarised as a section of the alley that is far enough from the tree rows so that the tree and the crop do not interact directly, but mostly through microclimate changes. Depending on the width of the alley, the 'mid-field environment' can either represent most of the field, when tree rows are very far apart, or be virtually non-existent, where the alley is very narrow. The indirect influence of tree rows on microclimate is especially due to a windbreak effect. In fact, even in the presence of short trees like in short-rotation systems, Böhm et al (2014) have found 50% wind speed reduction in the central point of 24 m-wide alleys in Germany. However, this latter work showed highly variable outcomes depending on where in the alley the wind speed is measured and on the tree rows orientation.

The tree row, which is in turn composed by (i) the trees and (ii) a non-cropped area beneath them, can be interpreted as a semi-natural environment. In fact, non-crop herbaceous vegetation develops under the tree rows in what Boinot et al. (2019) described as the understory vegetation strips (UVS). This latter work analysed the trade-off between the USV as a reservoir for weeds to the field and the USV as a hotspot for biodiversity conservation, finding that the positive function is prevalent whereas the negative function is limited to certain weed functional groups, like rhizomatous plants, whose dispersal is favoured by tillage. USV is a key component of the tree row to which, in all respects, all dynamics and management recommendations developed about field margins apply (Marshall and Moonen 2002), considering that the longitudinal development of the 'field margin' in an alley cropping system would be far greater than in a 'normal' landscape.

The tree-crop interface is the section of the alley where the crop and the tree row directly interact through competitive, niche complementarity or facilitative dynamics, both above- and below-ground. Complementary to the "mid-field", the tree-crop interface can represent the majority, or just a fraction of the alley. For example, Stamp et al (2009) found no yield difference in lucerne grown in 24m-wide alleys between black walnut rows in the US Midwest compared to open fields, whereas

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there was a significant yield reduction in 12m-wide alleys. Competition for light is the main above-ground interaction, generally reported to decrease crop yield (Artru et al. 2017, Dufour et al. 2013). In winter cereals, this yield reduction seems to be mostly ascribed to a reduced number of grains per ear and in part associated to a delayed phenological development (Inurreta-Aguirre et al 2018). Interestingly, the same dynamics does not seem to apply to nitrogen use efficiency: the grain protein in shady conditions was in fact increased under the trees shade in the experiment conducted by Dufour et al. (2013), which may suggest the existence of below-ground niche-complementarity mechanisms. Tree roots may compete with crop roots for water and nutrients. However, Cardinale et al. (2015) found an increased vertical development and depth of walnuts root systems when the trees were intercropped with durum wheat compared to tree pure stands. This phenomenon, possibly linked to the root-pruning effect of soil tillage, allows the trees to explore deeper soil layers and enhance nutrient recycling whilst diminishing below-ground competition with the adjacent crop. Further facilitation for nutrient cycling can be mediated by soil microbiota, as suggested by Querné et al. (2017) who found increased biological nitrogen fixation by Lucerne at the tree crop interface.

Agroforestry systems are perennial polycrops with explicit variation over time

There are two patterns of time variation in agroforestry systems: i) the growth of the trees, which generates different conditions whether the trees are young, middle-aged or long-standing, and ii) the management of the trees, which can cyclically alter tree-crop interactions. Pardon et al. (2018) analysed the different patterns of crop yield variation across the alleys for five crops in these three stages of development of the system. Yield depression close to the tree rows was clearly higher in mature systems than in young systems, and the authors attributed this not to nutrient competition (which were instead increased close to the trees) but to competition for water and for light. Other studies also reported yield decrease over time in mature agroforestry systems (Udawatta et al. 2014). Besides tree growth, human management can also alter tree-crop interactions cyclically, especially through various intensity of tree pruning, pollarding, and coppicing, as it is common in biomass-oriented management such as short-rotation forestry. Dufour et al. (2020) found a positive effect of pollarding on crop yields, but this effect was transient. In fact, three years after pollarding, the regrowth generated higher light competition than the non-pollarded trees.

How can adaptation to agroforestry be considered in a breeding program

From the above follows that not much information is available to what extent breeding for agroforestry systems can be possible, or to what extent agroforestry systems can be considered in breeding programs. Some information and inspiration can, however, be inferred from the literature on crop-trees interactions as a first step to develop guidelines for considering agroforestry in breeding programs:

- *crop selection (which crops are adapted to agroforestry)*

Results from Pardon et al (2018) suggest that winter cereals (wheat and barley) were less affected than spring crops (grain or silage maize, potato) by water and light competition at the tree-row interface in mature agroforestry systems. Within winter cereals, there is a suggestion that barley might be less sensitive to yield reductions than wheat (Dufour et al. 2020). Synchronisation of crop sequences with developmental stages or management of the trees can be a powerful strategy to minimise competitive effects. In fact, yield losses in young agroforestry systems are generally reported to be minimal. Crop life-cycles can be a key functional macro-trait to consider in this respect: spring sown crops can be more advisable to be grown in young or shortly coppiced/pollarded systems, whereas winter crops might be preferable under mature trees (Pardon et al 2018) as they would suffer relatively lower competition for light under mature trees, since crop vegetative development would correspond to leafless tree canopies.

- *cultivar testing and support to cultivar choice*

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The most critical aspect for the short-term optimisation of agroforestry performance of a given crop species is varietal evaluation. Varietal evaluation is time- and resource-intensive and, as such, may seem to be hardly applicable to the extreme variability of environments of agroforestry systems. However, methodological innovations in terms of data synthesis and integration of multiple sources (Brown et al. 2020) can be important paths to build the required evidence base. Evaluation of varietal performance into agroforestry systems can be as easy as obtaining profiles of crop performance across an alley cropping field width, as Pardon et al (2018) presented in terms of species comparison. An example of this is found in Smith et al. (2017), where such evaluation was conducted on wheat in 12m-wide alleys with standing and/or coppiced tree rows.

· *cultivar selection*

There are contrasting reports of adaptation of existing crop cultivars to agroforestry systems. Udawatta et al (2014) attributed significant yield reduction in a maize-soybean rotation in agroforestry to low shade and drought tolerance in the crops. On the contrary, Arenas-Corraiza et al. (2019) documented morphological and physiological acclimation to shade conditions, concluding that "current commercialised wheat and barley cultivars had sufficient plasticity for adaptation to shade". Comparing these two examples seems to confirm the importance of crop life cycle as a functional trait, and might suggest that breeding efforts for shade and drought tolerance should be given to summer crops. Evidence of varietal response to mycorrhizal symbiosis (Thirkell et al. 2019) and of enhanced microbial communities in AF systems suggest that breeding for enhanced symbiosis performance could result in better performance in AF systems (Topic 8).

· *evolutionary breeding*

The potential of genetically diversified populations of progressively adapting to environmental pressure through natural selection is the basis of evolutionary breeding (Phillips and Wolfe 2005) and can in theory be harnessed to derive crop populations adapted to a local agroforestry system. This could be especially relevant for Organic Heterogeneous Material (sensu the Revised Organic Regulation EU 848/2028): starting with seeds of a diverse population, farmers can develop a population adapted to a specific system, therefore circumventing the extreme difficulty of predicting adaptation to a potentially extreme diversity of environments. However, evolutionary breeding seems to bear a further potential. In an early-stage experience conducted in a mature alley-cropping system in the UK (Smith et al. 2017), a spring wheat Composite Cross Population seemed to respond differentially to its being multiplied in the centre of the alley vs. the west- or east-side of the tree rows and also to its being reproduced on the west- vs. the east-side of the tree rows. This suggests that fine microclimatic changes can be reflected in evolutionary breeding, potentially offering opportunities to use agroforestry systems as a breeding ground for populations adapted to multiple environments.

Conclusion

Crops are generally bred with a homogeneous open field in mind as a target environment, which can generate suboptimal crop performance in agroforestry systems. Crop breeding will need radical innovation and rethinking to be effective in this challenge. An agroforestry system is inherently different from an open field, as it constitutes, at the same time, a peculiar environment and a mosaic of micro-environments. Deep integration of crop breeding with management and system design is therefore essential. Choice of appropriate crop species for given configuration or development stages of the agroforestry system is a first critical step in optimising crop performance, followed by the quest of better adaptation within each crop species, which is the focus of plant breeding.

Thanks to the inclusion in the revised Organic Regulation of Organic Heterogeneous Material, evolutionary breeding can be a shortcut to obtain crops adapted to each individual system starting, ideally, from a highly diversified, base population. However, we suggest that the most essential process to optimise is that of variety evaluation and exploration of phenotypic diversity in response to the co-presence with trees, as a driver of future developments in breeding. This would need in turn

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methodological innovation, especially in terms of integration and synthesis of data from different sources. In fact, one can rightly question whether a variety testing programme could cover a wide number of varieties with such more complicated design, and the response is probably not. An essential concept in this respect is the adoption of a functional traits approach (Martin et al. 2015), by which a more pragmatic focus on key plant characteristics directly related to specific agro-ecosystem processes can simplify and optimise the generation of sound, and usable, evidence.

Challenges

More complex farming approaches like mixed cropping and agroforestry have proven to provide advantages in terms of increased yield and yield stability by using more sustainable farming practices. To optimise mixed cropping and agroforestry, plant breeding is considered very important in order to have better adaptation between crop types in complex systems as plant breeding has, perhaps for good reasons, focused on monocropping till today. The new organic regulation can provide new opportunities to organise plant breeding for complex systems in practice. However, it is very important to involve the whole value chain for farmers to be able to sell their produce. A practical first step would be to discuss with the value chain the advantages of mixed cropping and agroforestry approaches for their market and society in general.

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Topic 8) Balanced plant – microbe interaction (such as plant communication and defence strategies)

Introduction

In recent years, plant-associated microbes are receiving increasing attention in research in general and particular attention by researchers in the organic sector for their potential in contributing to agroecosystems resilience. Thanks to their abilities to decrease biotic and abiotic stresses and to support nutrient acquisition, microorganisms can be positively exploited to support agricultural sustainability (Hohmann et al. 2020). Current research on plant – microbe interaction broadens the agronomic approach of looking at microbes as potential source of diseases to be controlled to a more holistic vision of the necessity to promote a balanced plant – microbe interaction for optimizing agroecosystems functioning. Vandenkoornhuyse et al. (2015) have promoted the term holobiont, a concept that includes the plant and its associated microbes. Considering the holobiont concept from an agronomic perspective, plant functions (e.g. nutrient uptake, response to abiotic and biotic stresses, yield and yield stability) are determined by the interaction of the plant with the associated microbiome. Considering how important the impact of the microbiome on plant phenotypes can be, Oyserman et al. (2019) proposed to expand the model explaining a plant phenotype (Y) as the interaction genotype (G) x environment (E) with the inclusion of the microbiome (Mi) as additional explanatory factor. The extended model Y ~ G x E x Mi (not to be confused with the G x E x M concept described in Topic 4) describes a plant phenotype (e.g. yield) as determined by the genotype, environment and microbiome interactions. Knowledge on plant genetic determinants for beneficial interactions with individual microbes and entire microbial communities is growing rapidly (Hohmann et al. 2020). Several reports indicate that not only the host species but also the host genotype play a significant role in driving microbial community composition and activity, selecting for and against particular members of the microbial community. With the advent of modern sequencing tools and bioinformatics methodologies it becomes more and more feasible to explore not only the plant genome but also the interaction of the plant with the associated microbiome community in the roots, rhizosphere, and seeds. Breeding for the holobiont is based on the concept that the performance of a plant is not only determined by plant genes but also by the genes of the whole microbial community. The hypothesis is that plants that can attract a balanced microbial community in the soil will have a higher resilience against various stresses. However, microbe-assisted cultivation approaches face challenges that need to be addressed before a breakthrough of such technologies can be expected. Comparing different genetic backgrounds, the plant genotype was shown to play a small (~5% of variation) but significant role on microbiota composition (Hacquard *et al.* 2015), but to what extent genetic factors are responsible for shaping beneficial plant microbiomes is still poorly understood (Hohmann et al. 2020).

LIVESEED aims at developing holistic breeding strategies for resilience integrating the contributions from the host plant and its microbiome. LIVESEED consortium is engaged in bringing this research topic forward and closer to application by (i) promoting the dialogue within the research community and by addressing experimentally specific research questions related to plant – microbe interactions.

Examples

Gathering of experiences in the research community

On 6th December 2020, FiBL-CH organized a LIVESEED – EUCARPIA – ECO-PB workshop "Implementing Plant-Microbe Interactions in Plant Breeding" linked to the International miCROPe conference in Austria to exchange information with other researchers and disseminate first results of LIVESEED. The results of this activity have been summarised in the perspective paper "miCROPe 2019 – Emerging

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research priorities towards microbe-assisted crop production" with the intent to increase the visibility of the results and ongoing research on plant – microbe interaction (Hohmann et al. 2020).

From the examples presented during the conference and the workshop emerges that knowledge on plant genetic determinants for beneficial interactions with native soil microbiomes is growing rapidly. The perspective paper by Hohmann et al. (2020) mentions that it has become clear that the host genotype plays a significant role in driving microbial community composition and activity, selecting for and against particular members of the microbial community (Aira et al. 2010; Bulgarelli et al. 2015; Walters et al. 2018; Wille et al. 2019). There are a several reports on the host genotype influencing microbiome composition. However, the authors highlight how in order to exploit genotype effects in breeding it is necessary to identify single loci that explain microbiome structure and provide a list of studies that revealed genotypic variation of plant responsiveness to microbes. Schneider et al. (2019) demonstrate genotype-dependent colonisation success of mycorrhizal fungi. Sefloo et al. (2019) and Elhady et al. (2018) showed resistance mediated by individual strains or entire microbial communities, respectively. In the study by Faist et al. (2019) the topic of microbe recruitment under water and nutrient stress was investigated. The authors of the perspective paper report also the contributions by Hu et al. (2018) on responsiveness to soil microbial feedbacks. It is highlighted that the study by Shrestha et al. (2019) on bacterial quorum sensing molecules unravelled that the genetic variation in responsiveness to microbe-induced priming involved stronger activation of defence-related genes and cell wall structures.

Other studies led to the discovery of quantitative trait loci (QTL) as a first step towards marker-assisted selection of microbiome responsiveness: Bulgarelli (2019) identified a QTL associated with the recruitment of specific members of the microbiome and Wehner et al. (2019) a QTL for leaf rust resistance induced by microbes. Other studies focused on the relationship between root morphological traits and the soil microbiome for enhanced nutrient and water uptake (e.g. Galindo-Castañeda et al. 2019) and growth, drought and cold tolerance (e.g., Orozovic et al. 2019) with the objective to include plant-microbe interactions in breeding programmes. The topic of the seed-associated microbiota utility was presented in the workshop by Gabriele Berg (University of Technology Graz, Austria) and Matthieu Barret (INRAE, France; Adam et al. 2018; Torres-Cortes et al. 2018). In general, the structure of the seed microbiome is recognised as an important factor in the development of colonization resistance against pathogens. Some recent studies showed that the seed microbiome is dependent also on the cultivar (Ada et al. 2016, Rybakova et al. 2017). Another recent study has specified the composition of bacteria and fungi microbiota in wheat roots which differed between modern and ancient cultivars. These ancient cultivars encompassed less pathogenic microorganisms than recent ones for bacteria both in terms of species richness and abundance (Mauger et al. submitted/2020).

Even though this research field is very active and more and more results become available on the concept of plant - microbe interaction, plant genetics of and breeding for beneficial plant-microbiome interactions were highlighted during the conference and the workshop as underutilised but promising areas to improve crop resilience and yield stability. In order to facilitate the progression towards practical application of the breeding for the holobiont concept in the context of agroecological transition of farming systems, the organic sector should promote more research in this field and increase the involvement of breeders and farmers in designing potential future practical applications.

Experiences experimental work in LIVESEED

Review on insights to plant–microbe interactions

Wille et al. 2019 have published the review "Insights to plant-microbe interactions provide opportunities to improve resistance breeding against root diseases in grain legumes" in Plant, Cell &

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Environment journal. This review summarizes (a) the current knowledge of resistance against soil-borne pathogens in grain legumes, (b) evidence for genetic variation for rhizosphere-related traits, (c) the role of root exudation in microbe-mediated disease resistance and elaborates (d) how these traits can be incorporated in resistance breeding programmes.

Development of a high-throughput screening tool for pea

A high-throughput screening tool was developed by FiBL-CH that successfully differentiates susceptible and tolerant pea lines against a root pathogen complex in 3 weeks under controlled conditions using naturally infested soil. Preliminary results of the phenotypic root rot assessment in the field trials in Switzerland, France and Latvia could verify the resistance level of the selected pea lines selected with the screening tool. These results indicate the usefulness of the screening tool based on a naturally infested field involving the entire native soil microbiome as a key element for resistance breeding. Second year trials are ongoing in all three countries. A manuscript titled “Heritable variation in pea for resistance against a root rot complex and its characterisation by amplicon sequencing” is currently under review at the journal Frontiers in Plant Science (Wille et al. 2020 submitted).

Characterisation of the microbial root community of diseased pea

Next-generation sequencing (NGS) pipeline including complex biometric analysis of the fungal microbiome based on the ITS regions has been established and optimized by FiBL-CH. Highest differentiation of operational taxonomic units (OTUs) were found in the pea roots compared to the rhizosphere or bulk soil. The first analysis revealed quite a large number of potential pathogenic and beneficial taxa.

Pea lines showing contrasting tolerance levels to root rot were selected and tested in the screening tool using different soil from fields in Germany with a history of soil fatigue. After phenotypic data confirmed previous results, DNA was extracted from the root samples and sent for microbiome sequencing. Key microbial taxa with potential beneficial or pathogenic effects were quantified using qPCR methods and related to the different resistance parameters defined in the screening tool. Significant genotype effect as well as significant soil x genotype interaction on microbial community composition was detected. The set of contrasting pea genotypes were multiplied in several steps to conduct ongoing multi-year and multi-location field trials. First data on microbiome data of pea roots from field trials revealed that disease pressure and sampling time are main drivers of microbiome community diversity. OTU richness was higher in sick soil than in healthy soil and generally higher at early sampling time. Within the sites, sampling time had a big effect on both alpha and beta diversity. Different pea genotypes had only a minor effect on alpha and beta diversity in healthy soil.

Plant-associated microbial composition via seeds (seed microbiome)

In addition to the root microbiome, the influence of the environment on the submission of the plant-associated microbial composition via seeds (seed microbiome) is under test by LIVESEED. Seeds of the same set of pea cultivars propagated in different countries is under test for seed vitality by WR. Seed of the same genotypes has already been tested for Alternative oxidase (AOX) activity, which was proposed as a functional marker for resilience. Preliminary tests with inhibition of AOX enzymes by UEV and INRAe showed significant differences in relative germination rates in 2019 between the most tolerant and the most susceptible genotypes.

Farming system and population effect on microbial diversity in the maize rhizosphere

Analyses of the rhizosphere microbiota in different crop systems using different maize populations were conducted by IPC with support of FITOLAB in Portugal. The aim of this study was to understand how different production systems (conventional, organic and syntropic) and different open-pollinated maize populations (‘SinPre’ and ‘Pigarro’) can influence the microbiota of the rhizosphere. The data collected from the maize trial comprehends phenological data plus the structural diversity of the

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bacterial and fungal communities from the maize rhizosphere. The bacterial microbiota was determined from DNA extracted from maize rhizosphere samples based on the V3-V4 region of the bacterial 16S rRNA and ITS2 region for fungi using Illumina's MiSeq sequencing. This study revealed that organic farming systems result in higher bacterial and fungal diversity in the rhizosphere compared to conventional farming systems. The maize population 'Pigarro' had a significant higher genetic microbial diversity in the rhizosphere than the composite cross population 'SinPre' for both bacteria and fungi. However, 'SinPre' had a much higher percentage (61%) of fungal taxa that were specific to organic farming compared to 'Pigarro' (40%) indicating a specific adaptation towards organic farming. In general, the differentiation between farming system and between genotype was much more pronounced for fungal microbiome taxa than for bacterial taxa.

Outcomes and perspectives

The combination of emerging third generation sequencing technologies and new causal research approaches to better understand plant-microbe interactions can be used as helpful tools for more holistic breeding practices. Better understanding of plant-microbe interactions may not only be useful to develop more durable disease resistance (Topic 11) but may also help improve traits like nutrient use efficiency and potentially also tolerance to abiotic stresses like salinity (Topic 9). As such, better understanding of plant microbe interactions can be very important to develop more reliable and sustainable agronomic practices. Opportunities are particularly seen in the area of yield stability (increased resilience for challenging conditions) and productivity (maintaining yield while reducing inputs). In order to speed up applicability of this knowledge there is a strong need to work closely with farmers and to link controlled experiments with field conditions. In such a way, these tools can contribute significantly to systems-based breeding approaches.

Most importantly, these research findings provide a scientific basis for tailor-made approaches in regards to novel approaches to further broaden approaches and methods in resistance breeding (Topic 11) and breeding methods aiming for optimised GxExM interactions (Genotype x Environment x Management interactions (Topic 4 and 14), in that way contributing to increasing and optimising biodiversity at various scales.

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Section 3: Specific breeding tools for organic

Topic 9) direct versus indirect selection, and indirect parameters for complex traits like NUE and WUE

Introduction

Due to climate change many agro-climatic factors are evolving towards more stressful conditions. For example, particularly in Mediterranean areas, the climate change is causing lower amounts of rainfall and consequently a lower availability of water for crop irrigation. In fact, on the whole, the main water reserves for irrigation in these areas, i.e. reservoirs and dams, as well as aquifers, are gradually getting emptier. As a result, more efficient uses of the available water is necessary. Also, in Northern Europe rainfall is becoming less and more irregular due to climate change, which means that in the near future Northern Europe is facing similar challenges as the Mediterranean region.
Moreover, a lower availability of water for irrigation for the above-mentioned reasons is usually correlated with a lower quality, particularly in terms of saline concentration and levels of heavy metals and other plant-toxic elements. Therefore, both the amount of water available and/or its quality for irrigation in agriculture is expected to get worse due to the climatic change.
Finally, if we are aiming for a more sustainable agriculture, we need to search for a model of agriculture with a lower dependency of mineral inputs, particularly those synthetic or based on non-renewable mining resources (e.g. nitrate or phosphate mining). Another reason for a lower application of fertilizers is to prevent contamination in the environment when used in excess in agriculture (e.g. nitrates in aquifers, phosphate causing eutrophication of superficial water). Because of a lower dependency on mineral inputs, in organic agriculture farming practices are being developed to maintain a healthy soil (based on higher levels of organic content, a more active soil life and mineralization and better soil structure) to minimise the use of inputs.

In this regard, plant breeding can contribute to face all these challenges. Firstly, the use of plant diversity present in ancient landraces offers a great opportunity to succeed as the genetic pool they encompass comes from a low-input agriculture before the period of the green revolution. Therefore, ancient germplasm is expected to offer a better adaptation to stressful and/or low-input conditions than those genotypes bred for intensive high-input agriculture. Furthermore, this germplasm represents a broader genetic pool than that from modern varieties, which is very narrow (FAO, 2020). Even more, promoting the use of a more diverse gene pool will also contribute to mitigate genetic erosion and a more resilient agriculture. Secondly, there is a range of indirect parameters that can be used for selection against stress conditions, complementary to other (more direct) parameters. In the following section various examples are provided on how breeding can contribute to a more sustainable agriculture through direct and indirect selection for complex traits like Nutrient Use Efficiency (NUE) and Water Use Efficiency (WUE). Below detailed examples on pepper and tomato are described, complemented with experiences on other crops.

Examples

Capsicum peppers and tomatoes: selection for WUE and response to salinity in Spain
In the frame of climate change, more efficient varieties are needed to produce under limited availability of water for irrigation and adapted to saline water. Tomatoes and peppers, two solanaceous crops, are among the most economically important vegetable species in the world and represent two cases of study for breeding for WUE and adaptation to salinity. Both species are highly water consuming crops and improving their WUE is a paramount objective for facing climate change. By contrast, in terms of salinity, tomatoes are moderately tolerant while peppers are very sensitive

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(Bojórquez-Quintal et al., 2014), so the challenge is more difficult in the latter. In fact, research aimed at screenings, genetics and physiology of tomatoes under saline conditions are quite abundant, while studies in peppers are new and quite scarce. In comparison, the studies about drought tolerance, i.e. WUE, have caught the attention of scientists more recently in both species, particularly in peppers (Penella et al., 2013; Grandillo and Cammareri, 2016).

Research findings in tomato

The studies on WUE in tomatoes have revealed that the stage of development is a key factor in the impact of the deficit in irrigation on fruit production, particularly at flowering and fruit set and maturity (Cahn et al., 2001; Harmanto et al., 2005; Chen et al., 2015; Nangare et al., 2016. But also i) sufficient availability of potassium may mitigate this impact (Liu et al., 2019) and ii) the genotype is very important, so that differences among cultivars are very obvious in terms of WUE and offers an opportunity for improving this trait through breeding (Wahb-Allah et al., 2011; Ezin et al., 2010; Albert, 2017).

Many agronomic and physiological traits have been evaluated in tomato screenings to assess WUE and responses to deficit irrigation to optimise the amount of water used: from general traits like height, width and biomass to others related to flowering and fruit set and yield, including physiological stress measurements like leaf fluorescence (Tardieu et al., 2017). Recently, high throughput phenotyping tools, based on RGB quality images, have been reported to discriminate genotypes in terms of more efficient soil water use (Danzi et al., 2019). These image parameters are aimed to integrate morphometric parameters of plants during their development: height, width, and biomass (Petrozza et al., 2014).

Regarding salt tolerance in tomato, there is a range of studies on the effect of this stress factor for decades: physiological, agronomic, breeding, genetics and metabolomics. Actually, it is one of the most tolerant crops and, as an example to underline this, it is the only crop which can be grown in the margins of the Albufera lake in Valencia, together with rice, using waste water high in salinity, coming from upstream fields. The response against salinity of this crop has been well characterized and many sources of variation with tolerance to this stress have been identified and even QTLs have been reported (Grandillo and Cammareri, 2016). Similarly to drought stress, many agronomic and physiological traits have been evaluated to assess the response of tomato to salt conditions in screenings and gene expression studies: plant height, leaf number, leaf length, width and surface, chlorophyll fluorescence, yield and yield components, such as days to flowering and to fruiting, days to maturity, number of flowers and fruit set in the intermediate clusters, fruit weight and fruit size, among others (Dasgan et al., 2002; Kumar et al., 2017). Shoot damage and leaf chlorosis and surface reduction are the first affected traits. However, other screening techniques have been developed to assess salinity tolerance in tomatoes at early development stages (Dasgan et al., 2002). Thus, genotypes differ significantly in shoot and root biomass and shoot damage, and higher shoot K^+/Na^+ and Ca^{2+}/Na^+ are related with lower shoot damage. Thus, these ion ratios can be considered for indirect selection. By contrast, no correlations are reported between the shoot-root dry weight and these ratios and Na^+ concentration classes. Other studies have reported that the key for genotypic differences in tolerance could be based in different abilities for Na+ exclusion by abscising older leaves and/or higher root development to survive in saline conditions (Raza et al., 2017).

Research findings in peppers

Considering peppers, the studies and screenings for WUE and salt conditions are more recent and scarcer, and they have been mainly performed in the Region of Valencia, based on ancient landraces provided by the UPV seedbank. The main reasons for this lack of information in comparison to tomato are: i) the economic importance and predominance of tomato and ii) that peppers are very susceptible to salinity, so very few efforts have been conducted previously. In these pioneer studies, also differences among genotypes were found in terms of plant development and biomass, fruit set, fruit

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size and quality. Indirect measurements based on leaf fluorescence were also performed to assess correlations with biomass and yield parameters (Penella et al. 2013 and 2016). The main conclusion of these studies is that photosynthesis rate measurements could be considered useful parameters to screen large collections of genotypes.

Use of rootstocks for improved salt tolerance
For both crops, it has been suggested to use tolerant genotypes as rootstocks (Colla et al., 2010; Penella et al., 2013). This strategy has also been used to mitigate the effects of soilborne pests and diseases. Nevertheless, these genotypes are usually very exotic and there is a lack of affinity between rootstock and scion, resulting in low yields. As an alternative, the use of hybridization between tolerant landraces and commercial varieties could be also considered to provide vigour to the (hybrid) rootstock itself and more affinity with the grafted commercial varieties. After this hybridization/grafting strategy for direct use, breeding programs could be performed using these hybrids as pre-breeding materials (see also Topic 13).

The effects of lower water use on product quality
Finally, regarding fruit quality, a limited water deficit may decrease fruit size in tomato, although its impact on water content and shape is lower or not significant, while it may have positive effect on other quality traits like firmness, colour, soluble solids and sugars, organic acids and ascorbic acid (Cui et al., 2019). However, the magnitude of the effect is highly dependent on the genotype, i.e. is the result of GxE interactions. In the case of peppers, drought may have a positive impact on ascorbic acid content and phenolics, but may decrease yield, and fruit weight and size and water content, particularly at the fully ripe stage (Lerma et al., 2012; Murcia-Asensi, 2020). However, salinity is clearly the worst stress and has dramatic effects on yield (3-4-fold decrease) and quality of peppers, i.e. ascorbic acid and phenolics, and only water content remains close to non-saline conditions, although the use of rootstocks may mitigate this impact (Murcia-Asensi et al., 2020). By contrast, although salinity usually decreases yield in tomato, the use of moderately saline water irrigation does not have much impact on yield, whilst it improves taste and flavor (aroma, sugars and organic acids).

Tolerance to low input conditions
Some of the main nutrients in plant growth are nitrogen and phosphorus and, as suggested in the introduction, organic farming can contribute to a more sustainable agriculture, needed to cope with the effects of climate change. Thus, breeding for more efficient cultivars in the use of N (NUE) and P (PUE) is of paramount importance. Then, these genotypes can be selected for both: i) conventional agriculture but with low input levels of N and P and ii) organic agriculture based on organic matter and its mineralization.
In this regard, NUE and PUE have been studied for years in many arable species, but the knowledge is comparatively scarce in vegetables, and very little is known about NUE and PUE in tomato and particularly in peppers. In the last years, two initiatives have been developed on this subject: SOLNUE project (started in 2019 and focused on NUE in solanaceous crops) and INIA RTA2013-00022-C02-02 (from 2014 to 2018, focused on PUE in peppers).
The SOLNUE project is focused on tomatoes and eggplant at the INRA and UPV. The Impact of low N on aerial biomass, yield, and root adaptation response in large collections of genotypes is being evaluated. Genotypes will be screened based on N intake in the aerial and root biomass. Also, gene expression is being evaluated and candidate genes will be assessed on the basis of very high NUE and very low NUE genotypes.
INIA RTA2013-00022-C02-02 project was aimed at PUE in peppers. This project was initiated based on previous experiences where very significant differences were found among *Capsicum* pepper varieties in terms of root biomass and architecture (Ribes-Moya et al., 2014). This project evaluated the impact of low P on aerial biomass and composition, yield and root adaptation response in large collections of genotypes. Also P mobilization from the root to the aerial parts was evaluated. Root biomass, root

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length and exploration capacity of secondary and tertiary roots was evaluated by high-throughput tools (scanned roots analysed with whinRhyzo software), including length and total percentages of primary, secondary and tertiary roots to the total length. Remarkable differences were found among genotypes in terms of PUE and root biomass and exploration capacity (Pereira-Dias et al., 2020).
Also, some experiments have been carried out on the use of rootstocks to improve PUE of the grafted variety under low-P conditions and some genotypes have shown a good performance to provide a high PUE in the scion (Pereira-Dias et al., 2020).
Finally, in the frame of a comparative study between organic agriculture (where only organic matter/ manure was provided as fertilizer) and conventional management (synthetic/mining fertilizers applied), significant differences were found between the two systems in terms of both root biomass and exploring capacity and alkaline-fosfatase activity at the rhizosphere, being considerably higher under organic conditions (Fita et al., 2014). Also, a significant GxE interaction was detected, suggesting that some genotypes may show better response to organic conditions than others in regards to these parameters.

Relationships between NUE, root development and arbuscular mycorrhizal fungi (AMF)

Lammerts van Bueren and Struik (2017) reviewed and compared breeding strategies to improve NUE by increasing N uptake, N utilization efficiency, and N harvest index, in arable seed forming crops versus leafy and non-leafy vegetable crops, each involving many different crop physiological mechanisms and agronomic traits, that express themselves differently under low- and high input conditions. Head forming crops (lettuce, cabbage) depend on the prolonged photosynthesis of outer leaves to provide the carbon sources for continued N supply and growth of the photosynthetically less active, younger inner leaves. Grain crops largely depend on prolonged N availability for uptake and on availability of N in stover for remobilization to the grains. Improving root performance is relevant for all crop types, but especially short cycle vegetable crops (such as lettuce and spinach) benefit from early below-ground vigour. Lammerts van Bueren and Struik (2017) found sufficient genetic variation available among modern cultivars to further improve nitrogen use efficiency, but it requires interdisciplinary research approach (integrating agronomy and crop physiology) and efficient selection strategies to make rapid progress in breeding.

In an experiment conducted with maize, where conventional and reduced tillage were evaluated under different fertilization regimes (unfertilized vs. different levels of slurry total N and mineral NPK fertilizer), Messmer et al. (2010) observed significant effects of the genotype and fertilization regimes on NitUE measured as dry matter yield. Despite the fact that mineral NPK fertilizer provided on the whole higher dry matter yield than slurry and N-unfertilized, higher fertilization levels were positively correlated with chlorophyll content, plant height and N content, but negatively correlated with N use efficiency, suggesting that maize plants evolve towards higher N use efficiency ratios under low N input. Furthermore, significant genotype x tillage x fertilization interactions may occur, which must be considered in order to optimize variety selection for each condition. Also, fertilization regime had effect on root colonization as arbuscular mycorrhizal fungi (AMF) were higher under unfertilized conditions than under mineral NPK treatments, which suggests that root-fungi symbiosis increases to promote higher nutrient acquisition when availability of nutrients is lower for maize plants. In fact, arbuscular mycorrhization improved general dry yield under unfertilized conditions, while mycorrhization abundance was negatively correlated to dry matter yield under high N conditions. Thus, this may indicate that maize-AMF is beneficial under low input conditions, but it can be counter-productive under high fertilizer conditions due to the parasitic nature of the fungi. Finally, genotypes differed significantly in the species detected by PCR in AMF.

Even brassica, which were supposed, until very recently, not to have the ability for mycorrhizal associations have been found to show low levels of colonization in some species (Regvar et al., 2003)

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differ in this trait. Also, genetic variation has been reported for PUE in B. oleracea and cauliflowers with higher proportion of fine roots have been shown more Nit-UE (White 2007; Kage et al., 2003).

Outcomes and challenges

Breeding for abiotic stresses is of paramount importance to face climatic change and to achieve gradually a more sustainable agriculture, particularly in those areas: i) where intensive agriculture is nowadays predominant and ii) affected dramatically by the climate change and their consequences. The use of a gene pool more diverse than the current modern varieties in breeding is essential as ancient landraces and ecotypes, particularly those developed before the green revolution, have evolved under less intensive and more stressful conditions. Thus, there are more opportunities to find the genetic factors in these genotypes that enable a better adaptation to organic and low-input agriculture. Also, we must not forget that the rootstock strategy (see also Topic 13 for perennial crops) may provide an additional strategy to face low-input conditions (drought, salinity or low nutrients conditions). So, breeding must also play a role to select good genotypes for rootstocks.

In terms of methodology we must work not only in the evaluation of the above ground parts of the plant in response to stress conditions, but the root and soil interaction is essential. Studies on the root response (length, flexibility in growth, vigour, and architecture) based on biomass and composition are necessary. Studies on root images is also a powerful tool to understand what is happening in the plant parts above the ground. The root-soil interaction is also a key factor in plants to face water stress conditions and low nutrient conditions (see also Topic 8). Thus, the most efficient genotypes usually show a high ability to get mycorrhizal associations in their rhizosphere, enabling a more efficient use of limited inputs. Thus, such ability for mycorrhizal associations and production of root exudates stimulating such beneficial associations must be explored for indirect selection. Another important lesson is that mycorrhizal associations can be beneficial under low input conditions (typical for organic agriculture) but may be counterproductive under high input conditions.

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LIVESEED is funded by the European Union's Horizon 2020 under grant agreement No 727230 and by the Swiss State Secretariat for Education, Research and Innovation (SERI) under contract number 17.00090.

Topic 10) Use and efficiency of molecular tools, such as MAS, and genomics

Introduction

Organic agriculture aims to use a broad perspective on agriculture, including its relationship with society, integrating agro-ecological, socio-economic, political and institutional perspectives. This broad perspective has been expressed in the IFOAM principles of ecology, care, fairness and health. These principles also help guide the development of organic breeding. For example, it clarifies what techniques are compatible with organic agriculture and which techniques are not compatible. It is clear that genetic modification is not compatible with the principles of organic agriculture. New techniques such as CRISPR-Cas are also incompatible with these principles. Instead of seeing molecular tools as the future for plant breeding, organic agriculture aims to maintain a broad perspective. It seeks to develop effective alternative approaches well suited to the IFOAM principles, ideally seeking collaboration with the whole value chain and/or society at large. Some of these approaches have been described as chain-based breeding, community-based breeding (Nuijten et al. 2017) or systems-based breeding (Lammerts van Bueren et al. 2018). These approaches do not exclude the use of molecular markers, but rather aim to understand which questions can be addressed best with which methods.
To further understand the compatibility of molecular markers with organic breeding, it is useful to take the three perspectives as described by Wolfe et al. (2008) as point of departure. They distinguished three approaches in breeding:

1) Breeding programmes for conventional agriculture (BFCA)
2) Breeding programmes for organic agriculture (BFOA)
3) Breeding programmes within organic agriculture (OPB).

For all three approaches, variety development must exclude the use of genetic modification. In addition, BFOA and OPB must apply breeding techniques that are in accordance with the techniques listed in the Annex of the position paper of IFOAM International for organic cultivation from November 2017. An important difference between BFOA and OPB is that in BFOA only the last stages of the breeding process should be under organic conditions and that in OPB the whole breeding process should be under organic conditions. Hence, the use of molecular tools in BFOA and OPB is also different. In BFOA, the use of molecular tools may be similar to BFCA. In OPB molecular markers are considered to be used as additional tool only when certain traits cannot be selected for at plant level in the field (ECO-PB 2012). Some organic breeders, however, argue that molecular tools should not be used at all as breeding techniques are not neutral objects but have a socio-technical script in them, unconsciously influencing the way of working when applying them (see also Topic 1). A more principal point of view is that in the case genetic modification has been used in the development of molecular markers organic breeding should abstain from using such tools. Presently, molecular markers are mainly applied as diagnostic tools for the selection of certain traits and determination of genetic diversity in OPB of arable crops and fruits, while the majority of OPB for vegetable crops refrain from this tool.

Developments in conventional breeding

In this section, the current developments in molecular marker development in the context of conventional breeding are described.

Marker development and plant genotyping: a changing scenario in conventional breeding
From the perspective of conventional agriculture, marker-assisted selection (MAS) and its development represented by genomic selection (GS) are techniques of which their use can primarily be foreseen for trait-based breeding, that considers traits like crop yield as a synthetic trait. Considering crop yield a synthetic trait with a focus on adaptation to specific environments or cropping conditions may make GS relevant also for ecosystem-based breeding as defined in Lammerts van Bueren et al. (2018).

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In the last three decades, crop improvement research (mainly aimed to non-organic farming) has increasingly invested in the development of molecular markers that could assist plant selection. For long, however, the adoption of molecular breeding has been quite limited , because the small number of available markers and the high cost of their utilization prevented the development and cost-efficient exploitation of marker-based selection for quantitative traits (such as most traits of practical agronomic interest) and strongly limited the possibility to identify markers linked with genes of traits under monogenic or oligogenic control (such as the resistance to most diseases). Other possible limitations were represented by the limited exploration of trait-marker associations due to the use of narrowly-based plant material such as a biparental population, the detection of linkages of modest practical interest if markers are linked to unfavourable alleles, and QTL-environment interactions. The shortcoming of narrow based plant material was overcome by genome wide association mapping (GWAS) based on a broad set of 200 to 500 genotypes representative for the breeding material.
Moreover, the progress in the development of marker types (from RFLP and RAPD to AFLP, SSR, DArT, SNP, etc.) and genotyping procedures (up to next-generation ones) has allowed for a huge increase in the available marker numbers and a drastic decrease of the marker cost per data point. Conventional breeders suggest a turning point in which genomic selection may become more economically convenient than phenotypic selection for improving crop yield in well-defined target environments and other key complex traits, in several contexts and breeding schemes. For example, genotyping-by-sequencing (GBS; Elshire et al. 2011), which skips sequence discovery and explore SNP polymorphism in DNA fragments cut by a restriction enzyme, can provide several thousands of polymorphic SNP markers at a cost of € 40-50 per genotyped entry (inbred line; candidate parent plant for synthetics; etc.), which is lower than the cost of SNP array-based techniques and in most instances is probably 4- to 6-fold lower than the cost per entry of the ordinary field-based evaluation for the yield trait. Obviously, not only the lower cost but also the ability of the molecular tool to predict the trait influences the actual efficiency of this tool relative to phenotypic selection.
Further savings from the use of molecular breeding approaches can derive from selection for multiple traits (as usually done in breeding programs), since genotyping costs do not increase with the number of selected traits, while phenotyping costs do. Future technological progress is likely to further increase the convenience of molecular breeding approaches.

Genomic selection (GS)

When working under predictable conditions, such is the case in conventional agriculture, a conventional point of view is that the best way of exploiting marker information is by genomic selection given the importance of complex, polygenic traits (such as crop yield in different environments, drought tolerance, protein content, etc.) in order to reduce/replace field testing. In contrast to marker studies linked with individual genes of monogenic traits or with quantitative trait loci (QTL), genomic selection is based on the concept that all traits can be predicted by the large number of genotypic data (>40’000) if the calibration is done properly. This approach implies the development and application of a statistical model for the prediction of breeding values based on the joint analysis of phenotyping and genotyping data of a training population that represents well the target genetic base and target environments (Meuwissen et al. 2001; Heffner et al. 2009). If the model is sufficiently predictive, it can be exploited by genotyping a large number of genotypes from the genetic base, to identify a small subset of inbred lines promoted to evaluation under field conditions or a set of parents for breeding a synthetic variety.
Of crucial importance for success of GS are the choice of a representative sample of at least 500 genotypes i.e., the training population, derived from the breeding gene pool and that the phenotyping conditions must represent well the target environments and their variation, which implies phenotyping the training population in many environments and across 2-3 years.
The usefulness of a genomic model defined for a given genetic base also for selection in another genetic base is one of the aspects in focus by research and is likely to differ depending on the trait, the species and the plant material. GS definition for a genetically-broad genetic base is preferable to

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ensure the applicability of the model to the majority of the relevant genetic resources, although this may imply lower genome-enabled predictive ability than genome-based predictions for a narrow genetic base (because of the greater genomic complexity of the broadly-based reference population). For example, the loss of predictive ability for alfalfa biomass yield using a model developed for non-dormant germplasm to predict dormant germplasm or vice versa approached 30% (Annicchiarico et al. 2015).

Marker-assisted selection

Unlike GS, MAS exploits just a few markers for plant selection. Several opportunities emerged for the possible adoption of MAS in breeding for organic farming. MAS can allow the pyramiding of multiple monogenic traits, such as pest and disease resistances and some quality traits (Xu and Crouch 2008; Das et al. 2017). One of the assets of MAS is its ability to facilitate and speed up the introgression of exotic and wild alleles and, thereby, increasing the crop genetic diversity and resilience, which are features of key interest in organic systems (Lammerts van Bueren et al. 2010). Examples in which MAS has been successfully applied to practical breeding were reported, among others, for improved resistance to different diseases in wheat and barley (Miedaner and Korzun 2012), to powdery mildew in pea (Ghafoor and McPhee 2012), and to PVY in potato (Ottoman et al. 2009).

Recently developed genotyping techniques such as Genotyping by Sequencing (GBS) can offer greater opportunities also for MAS, because of much greater exploration and exploitation of trait-marker linkages in the genome. Also here, the study of genetically-broad plant material, such as a diversified germplasm collection, may provide greater information on useful alleles and markers (via a genome-wide association study) than that of narrowly-based material, although at the cost of lower linkage detection ability. Genomic Selection may prove useful also for oligogenic traits, such as resistance to diseases controlled by various genes (e.g., resistance to common bunt in wheat) or possibly showing a horizontal resistance pattern, by incorporating many relevant linked markers and weighing them according to their impact on the breeding value and possible gene complementation. The increasing availability of sequenced crop genomes is bound to widen the opportunities for and increasing the efficiency of MAS and Genomic Selection, by favouring the identification of relevant genes and target genomic areas.

Challenges for organic

Applicability of molecular marker tools depends on the type of breeding approach. In this section we consider the three directions as described in the introductory part in relation to organic agriculture:

1) Breeding programmes for conventional agriculture (BFCA)
2) Breeding programmes for organic agriculture (BFOA)
3) Breeding programmes within organic agriculture (OPB).

Considerations are different for each of these breeding directions and include technical, social, ethical and political aspects. As genomic selection is replacing selection steps in the field, this application is rather debated in the organic sector, as the co-evolution and interaction of the plant in the living soil is of prime importance. Unlike GS, MAS exploits just a few markers for plant selection to supplement selection under organic conditions.

BFCA

Molecular breeding is particularly suited to large conventional breeding programs targeting geographically-wide regions, of which investment in equipment and personnel for laboratories and bioinformatics can be paid for by incomes arising from large markets of their varieties (which are usually bred primarily for conventional systems). Marker-based selection for tolerance to biotic stresses that are particularly difficult to control can have a major appeal, while other specific traits (e.g. nutrient use efficiency or tolerance to key abiotic stresses), or the global adaptation to well-

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defined growing condition as expressed by crop yield, may become increasingly interesting for GS in time.

BFOA

There is nearly no information on the potential usefulness of GS to breed for crop yield in organic systems. One study assessed GS for organically grown pea in Northern and Central Italy based on recombinant inbred populations of three parental lines (Annicchiarico et al. 2019b), while most studies are not conducted in the target environment. Genotype × environment (GE) interaction patterns showed much greater Genotype × Year than Genotype × Location interaction, supporting in this case the breeding for wide adaptation to the region. When assessing selection gains in independent selection environments, GS compared with phenotypic selection exhibited at least 80% predicted greater efficiency for selection of lines within the same cross, and 20% greater efficiency for lines from different crosses that shared one parent with the cross for which the GS model was developed.
With reference to legume crops, GS tended to display greater efficiency than phenotypic selection for pea grain yield selection under severe drought (Annicchiarico et al. 2017) and alfalfa selection for biomass yield (Annicchiarico et al. 2015), and was able to predict the grain yield responses in climatically-contrasting environments of white lupin landraces (Annicchiarico et al. 2019a). Its potential usefulness to select for yield in contrasting environments and quality traits of sweet lupin lines is under assessment in the project LIVESEED.

Especially for relatively small breeding programs (such as breeding for organic farming, targeting organic systems), the application of GS or MAS requires careful optimization with respect to lab and bioinformatics equipment and personnel and the level of outsourcing. A low-cost genotyping technique such as GBS could conveniently be outsourced, sending DNA (or possibly leaf samples) and receiving sequencing data (used for home-made SNP calling) or SNP data that underwent the SNP calling. Even the bioinformatician's work needed for development and application of genomic selection may be outsourced. However, an initial investment is required for meaningful and reliable phenotyping in target environments to develop GS or MAS procedures. It is important to mention here that organic farming systems are more diverse than conventional farming systems and, moreover, diversity in farming systems is considered an important goal in organic movements. Also, these GS and MAS procedures require the prior careful definition of the target genetic base in the context of the target environment, as their information on useful genomic areas and alleles partly depend on the germplasm sample used for the studies. This means that, for example, molecular markers may be useful in the case of breeding for Nutrient Use Efficiency. On the other hand, as organic farming systems differ much from one another, it is not yet clear to what extent molecular markers can be really useful in the case of breeding for Nutrient Use Efficiency when taking into account the diversity of farming systems and an increase in costs with a larger number of target environments.
Farmer-participatory breeding may display greater selection gains and efficiency than conventional breeding (Ceccarelli 2015). A recent study focusing on pea breeding for Italian organic environments showed that farmers' preferences as expressed by an appreciation score exhibited greater selection efficiency and farmer's acceptability of selected material than the breeder selection index. Breeding values based on the farmer selection index or the farmer acceptability score exhibited greater correlation with grain yields in independent environments than those from breeder selection criteria (Annicchiarico et al. 2019c). These results illustrate the importance of farmers' evaluation; however, they require validation from new selection experiments.

Another drawback of molecular breeding is the proprietary aspect of some important techniques. For example, GBS has been patented a few years ago, and this resulted in a paucity of providers thereafter (while a licence needs be obtained for in-house application).

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In theory, cooperation among breeding programs could be very important for application of molecular breeding targeted to organic systems, to share the challenges and costs implied by genotyping for developing molecular tools and then applying them for large-scale molecular selection on the one hand, and phenotyping aimed to development of the tools on the other. Consortia of private breeders and public research institutions could be highly relevant, too. In conclusion, molecular tools could be seen as possible additional tools for BFOA, whose adoption needs, anyway, to be embedded into an ecological perspective of key traits and environments targeted by a breeding program, via approaches still to be defined.

OPB

In the case of OPB, socio-economic and ethical aspects need to be considered carefully, together with the technical aspects. One important aspect in the case of OPB is that no IP-rights (e.g. patents) can be obtained on cultivars using molecular markers. In addition, molecular markers used should be publicly available.

Another important aspect is that organic breeding must be conducted under organic conditions, e.g. the plants should be selected under regular organic growing conditions (see the definition of organic plant breeding in Annex 1). So far, it seems molecular markers can be useful for marker assisted selection (MAS) as an additional diagnostic tool to improve durable disease resistance like in the case of resistance breeding against phytophthora in potato or common bunt resistance breeding in wheat (see Topic 11). Another use could be to describe the soil microbiome in order to select for microbiome mediated disease tolerance (Topic 8), or to describe genetic diversity in a base population, or to better understand genetic differences between cultivars.

In contrast to MAS, genomic selection (GS) aiming to replace selection steps in the field, is still debated among the organic sector, as the co-evolution and interaction of the plant in the living soil is of prime importance.

Outcomes

As described above, molecular breeding is particularly useful for the identification of genetic diversity in the breeding material and development of parental and/or inbred lines, common in conventional breeding. In the case of the breeding of open-pollinated varieties or organic heterogeneous material molecular markers may be useful to identify the shift of gene frequencies or integration of individual genes by MAS. To conclude, in the case of organic plant breeding, a case-by-case decision will need to be taken to decide whether MAS or GS are useful or whether other approaches (Topics 6 to 9 and 11 to 14) may be preferable from a holistic perspective, also taking into consideration socio-economic and ethical aspects (Topic 1 to 5).

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LIVESEED is funded by the European Union's Horizon 2020 under grant agreement No 727230 and by the Swiss State Secretariat for Education, Research and Innovation (SERI) under contract number 17.00090.

Topic 11) Inheritance of resistance to seed and soil borne diseases: From a gene-based to a cropping-systems perspective

Introduction

With the widespread development and availability of chemical pesticides for seed treatment and crop management from the 1950s onwards, plant breeding programs have not always considered resistance breeding against fungal diseases a priority for many crops. This is particularly true if good chemical control is available such as the case with late blight (*Phytophthora infestans*) in the case of potato or common bunt (*Tilletia tritici)* in the case of wheat. In organic agriculture many diseases can be managed with quite well with good crop rotation, soil and manuring practices (Finkh et al. 2015). However, specific organic breeding programs have increasingly engaged in resistance breeding (such as for late blight and common bunt), to strengthen genetic disease control and make organic systems less vulnerable to plant diseases.

Resistance can be defined as the "inherent capacity of a plant to prevent or restrict the entry or subsequent activities of a pathogenic agent when the plant is exposed, under suitable environmental conditions, to sufficient inoculum of a pathogen to cause disease" (Bhargava and Srivastava, 2019). Different types of resistance exist and are dependent both on the genetic variability in the host plant and in the potential pathogen. Resistance can be horizontal, or quantitative, when it controls a broad range of races of a given pathogen. Horizontal resistance is usually conferred by a combination of many genes, each with a minor effect (polygenic). On the other hand, vertical, or qualitative, resistance results in a total resistance against one or few races, but none against others. It is usually conferred by one or a few major genes, which are easily identified and transferred from one genotype to another. However, relying on vertical, monogenic resistance presents a risk, as widespread and continuous use of a particular cultivar or type of resistance may lead to the development of a new pathogenic race or to a shift in pest population. The continuous adaptation of pathogen populations in order to "break" or circumvent resistance genes they are confronted with has been described as an "arms race". Resistances obtained by stacking or pyramiding several resistance genes are therefore preferable, as more difficult to break and therefore more durable. Disease resistance is different from tolerance, as the latter refers to the ability of a plant to limit the impact of a given disease on its development and health.

In the following sections, three cases will be presented to illustrate the state of the art of organic resistance breeding, as well as the limits and uncertainties linked to resistance breeding: Breeding for common bunt resistance in bread wheat, anthracnose resistance in lupine and resistance against several diseases – *Phytophthora, Rhizoctonia* and virus Y - in potato. The objective is to illustrate how resistance breeding interacts with broader visions of crop plants, of crop ecology and socio-economic aspects of organic systems, as well as the challenges that emerge from those interactions.

Examples

Breeding for resistance against Common bunt in bread wheat

Resistance breeding against common bunt is being carried out within several organic wheat breeding projects in Europe. Several partially resistant winter wheat varieties have been registered and marketed. This example illustrates how breeding for genetic resistance is one relevant strategy to be combined with others, and that other defence mechanisms, breeding objectives and management levers should not be left aside.

<u>Disease and issue</u>

Common bunt, caused by the fungi *Tilletia caries* and *T. foetida*, has been mentioned as a devastating disease in wheat crops as early as antiquity and was one of the earliest plant diseases to undergo systematic study (Agrios, 2005). The disease is mainly seed-borne but can also subsist and reinfect

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plants from soil. The disease can be well controlled in conventional wheat production since the widespread use of chemical fungicides as seed treatments but has reappeared as a priority disease organic cereal production (Matanguihan et al., 2011). With rising awareness of this issue, a comprehensive strategy for bunt management in organic systems has been developed in the last roughly 20 years, including cultivation practices, good seed multiplication practices, organic seed treatments and resistance breeding.

Resistance breeding mechanisms and projects

Currently, 36 pathogenic races of *T. caries* and 15 races of *T. foetida* have been identified based on their reaction to 14 wheat differential lines each putatively containing one of 14 recognized bunt resistance genes, although new resistance genes and combinations of resistance genes are being discovered (Goates and Bockelman, 2012). Several European organic breeding programs have bred and released winter wheat varieties with some bunt resistance, among them the bio-dynamic wheat breeding and research programs at Dottenfelderhof and Cultivari Darzau in Germany, Getreidezüchtung Peter Kunz in Switzerland, and Agrologica in Denmark.

According to the gene-by-gene model, single resistance genes or combinations of resistance genes usually correspond to specific bunt races, and do not confer absolute resistance to all pathogenic strains. Users of varieties qualified as "bunt resistant" need to understand that the efficiency of a given resistance will depend on the bunt races predominantly present in their region and that these can evolve over time. For instance, the resistance genes present in German wheat varieties were evaluated before integrating resistant varieties into French variety trials, to match with those bunt races that are prevalent in France (Cadot et al., 2018). To address this issue, Agrologica has initiated a study of the virulence of European bunt populations to prioritise most relevant resistance genes for organic wheat breeding in the framework of the LIVESEED project. To combine a larger number of resistance genes than can be stacked into a pure line, Agrologica has also developed a more diversified wheat population (e.g. a composite cross population). This constitutes a novel approach to integrate a larger number of resistance genes and control more bunt races at once. A diversified population does not meet DUS standards for variety registration and therefore needs to be marketed as a population.

Considerations and challenges

Organic wheat breeders engaged in resistance breeding against common bunt, as well as other actors of organic wheat cultivation, have experienced several challenges and considerations that need to be taken into account as regards the broader vision of organic systems.

- According to one Swiss breeder, the simple mechanistic view of just "stacking" resistance genes into cultivars is too reductionist, as the breeder's eye remains essential when selecting for harmonious cultivars. It allows taking into account the interactions of a plant's genetics with its environment and with maternal effects to select for well-balanced varieties that are well adapted to organic conditions, for example. As counter-example, the variety 'Renan', released in the 1980's but still widely cultivated on organic farms in France, is mentioned: it indeed combines resistances to several plant diseases, but is altogether rather unsuited for organic cultivation, if only because of its short straw. Resistance breeding must thus be attentive to an integrated approach as not to forget about the overall quality and potential of a given breeding line.
- Focusing on genetic bunt resistance, based on known resistance genes, also bears the risk of losing sight of other defence mechanisms, such as the phenomenon of field resistance observed by Gaudet and Puchalski (Gaudet and Puchalski, 1989; Matanguihan et al., 2011). These are yet unknown resistance mechanisms conferring wide disease resistance under field conditions, but which fail to be expressed under controlled environment conditions. Similarly, Anders Borgen at Agrologica has observed that winter wheat variety 'Stanka' can be infected by common bunt at early growth stages but may activate unknown defence mechanisms suppressing the fungus after tillering. Among French farmers working with heritage and farmers' wheat varieties, questions are

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also emerging regarding the role of complex, locally adapted microbial communities associated with plants and seeds, as well as the role of crop diversity, to counter-balance the common bunt fungi. Two workshops on Implementing Plant-Microbe Interactions in Plant Breeding organised by EUCARPIA (European Association for Research on Plant Breeding) in 2015 and 2019 show that similar questions are emerging in the arena of breeding and pre-breeding research, opening new perspectives for organic breeding. This also resonates with the vision of plant health as a dynamic process based on complex interactions between plants and their environment (Spieß, 1996).

- Whereas resistance breeding may offer perspectives for the breeding of new varieties from crosses, other solutions are necessary in the context of community seed networks growing and multiplying farmers' and heritage varieties. This involves seed testing and preventive seed treatments, but also organisational aspects such as seed growers individually and collectively taking responsibility for seed health and making sure to have sufficient back-up seed stock in case of infection during multiplication. The interest of farmers within the Réseau Semences Paysannes for the issue in France is one indicator for the need for strategies adapted to crop diversity, as well as the interest generated by conferences and demonstrations on bunt control at the European Diversity Cereal Festival held in Denmark in 2019.
- Resistance breeding should not be perceived as a waiver of sound growing systems. In France, as in several other countries (Matanguihan et al., 2011), common bunt reappeared rather prominently about 10 years ago on organic farms. As a corollary of the widespread use of chemical fungicides as seed treatments, farmers' knowledge and awareness concerning common bunt and its prevention had strongly declined, and no alternative seed treatments were available. Since then, control mechanisms have been identified and put to use. Nevertheless, several bunt outbreaks were again observed in 2019. In some cases, large acreages were hit after non-treated seed had been sown within a narrow crop rotation in French regions specialised in arable cropping, in particular wheat. In this context, the call for introducing bunt resistant wheat varieties in the French seed market became stronger. Although resistant varieties may indeed be a temporary solution and "safety net" in such a situation, it is important to keep insisting on sound cropping practices, including diversified rotations and close observation.

Anthracnose resistance in sweet lupine

Sweet lupine is an emerging crop in Europe, currently representing a small acreage and of little economic importance. Anthracnose is one important barrier to the development of sweet lupine cultivation, particularly in Central Europe, and breeding for anthracnose resistance in organic breeding projects is still in its beginnings. This example shows the difficulties and limits of resistance breeding, but also how organic breeding projects are an opportunity to develop comprehensive strategies for a crop, and in particular emerging crops.

<u>Disease and issue</u>

Due to its high protein content and well-balanced amino-acid content, interest for sweet lupine as alternative to soybean has arisen for human and animal consumption in Europe. Among the cultivated species of lupine, white (*Lupinus albus* L.) and blue lupine (*L. angustifolius* L.) have received most attention. White lupine has the advantage of higher general yield potential and of presenting winter and spring-types, thus allowing for autumn sowing at least in milder climates. However, white lupine is more susceptible to anthracnose. Indeed, anthracnose (caused by *Colletotrichum lupini*), a seed-borne fungal disease with strong destructive potential, constitutes a key barrier to the development of organic lupine production. In addition, seed monitoring and reliable detection of anthracnose in seed lots remains difficult to date. Altogether, these issues contribute to the fact that there is no commercial production of organic white lupine seed in Europe to date. Only non-treated conventional seed is available.

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Resistance breeding mechanisms and projects

Varieties of white lupine with good tolerance to anthracnose have been bred and marketed in Australia but are not available in Europe. Several breeding programs for white lupine exist in Europe, among them two focused on organic agriculture: a breeding program at FiBL (in collaboration with Getreidezüchtung Peter Kunz) in Switzerland and another one at the Louis Bolk Institute in the Netherlands. The former has a particular focus on breeding for anthracnose resistance in white lupine. Other white lupin breeding programmes are conducted in France by Jouffray-Drillaud, in Italy by CREA in Italy, and in Germany by Saatzucht Triesdorf in Bavaria, which allegedly bred the first variety with improved anthracnose resistance, Frieda, released by the Deutsche Saatveredelung (DSV) in spring 2019. A second variety, Celina, will be released in spring 2020. Although these breeders are not working explicitly for organic agriculture, their work is also recognized among stakeholders of the organic sector.

Screening for anthracnose resistance in Switzerland has revealed some promising germplasm as parental lines for future resistance breeding (Arncken et al., 2018). A composite cross population has been created from these promising lines as a basis for further breeding. However, in contrast to anthracnose resistance in other legume crops, resistance in white lupine is a quantitative trait of which the first QTL have been identified in Australian material (Książkiewicz et al., 2017). Although much of lupine genetics is yet to be explored, long-lasting, horizontal resistance thus seems difficult to obtain. An additional difficulty is that sweet lupine suitable for consumption by humans or animals due to low alkaloid levels, has a narrow genetic basis. Currently, no wild relatives are known from which resistance genes could be crossed into white lupine, but there are some landraces from different Mediterranean and African countries with increased tolerance. But, as they are bitter lupins containing high levels of quinolizidine alkaloids, selection within crosses is needed to get sweet-seed lupins with low alkaloid content.

The discovery of molecular markers closely linked to resistance genes has paved the way to more effective and cost-efficient selection for resistance to anthracnose (Książkiewicz et al., 2017). Following this finding, a PCR-based screening tool is under development at the Institute of Plant Genetics in Poznan (Poland), whereas FiBL-CH and CREA in the scope of LIVESEED are developing a genome wide association mapping approach to better account for the oligogenic nature of the trait.

There are also other aspects contributing to solve the anthracnose challenge in lupins. As the pathogen is possibly always present in the plant but the disease outbreak normally only occurs under the moist and warm conditions that dominate the weather in mid Europe from June to August, the search for genotypes with adapted development dynamics could largely contribute to meet the challenge. In years with high anthracnose incidence, early maturating genotypes were superior to late ones, “escaping” from the devastating late phase of the epidemic. In parallel, FiBL-CH is exploring the diversity and virulence of different strains of *Colletotrichum lupine* and exploring different physical and biological seed treatments in the scope of LIVESEED Task 2.3.3.

Considerations and challenges

In Europe, lupine is an emerging crop, which interests both organic and conventional sectors. As lupine cultivation currently has little economic importance, resources for lupine breeding are scarce. However, lupine’s status as an emerging crop also presents opportunities, as developing both lupine breeding and cultivation allows devising a truly comprehensive strategy, which from the beginning takes the needs of the organic system into account. In such a comprehensive strategy, resistance breeding probably has its role to play along with other approaches, such as improved seed monitoring, developing and improved understanding of the fungus causing anthracnose and how it interacts with microbiota associated to lupine, as well as possible physical, biological or disinfective seed treatments. As for the case of wheat above, integrating the creation of a CCP in a resistance breeding strategy is illustrative of how organic breeding finds original ways of combining genetic resistance with novel breeding tools and concepts. Another approach that has also been tried by the FiBL-CH project could be the implementation of mixed cropping systems. This could establish beneficial plant-plant interaction and help as a barrier against secondary anthracnose infection during the growing season. However, to date no cropping partner could be identified that was able to improve the system compared to pure stand cultivation of white lupins. An additional problem is that a mixed stand may be beneficial for the development of other diseases in lupine, although in general mixed cropping has a diminishing effect on crop diseases compared to pure stands.

Managing several diseases in potato

Examples of how three important plant diseases are managed in potato breeding and cultivation illustrate how resistance breeding also interacts with organisational and societal aspects.

Diseases and issues

The fungal diseases caused by *Phytophthora infestans* (late potato blight) and *Rhizoctonia solani* (black scurf) are considered as highly devastating diseases in organic potato crops. As a vegetatively propagated crop, potato is also prone to a number of pathogens which can accumulate in vegetative tissues, such as virus Y (Tiemens et al. 2013). As a crop of economic importance in Europe, both in organic and in conventional agriculture, a large number of research and breeding projects are financed to improve potato health, with different strategies.

Resistance breeding and research

Since 2009, the Dutch organic potato breeding project Bio Impuls is based on the close collaboration between breeding companies, research institutions and farmer-breeders to breed Phytophthora resistant varieties for the organic sector. Organic farmer-breeders are strongly involved in the project, which allows both to profit from farmers' experience and knowledge and to reduce costs: based on their breeding objectives and experience, farmer-breeders request specific potato crosses, which are then conducted by the pre-breeding program of Wageningen University or by the breeding companies. Potato seeds are then provided to the farmer-breeders for selection and vegetative multiplication over several generations. The most promising candidates from farmers' selections are then evaluated for further traits such as broad adaptation and yield stability by the breeding companies and, eventually, if proven to be good released as varieties. Similar collaborative potato breeding schemes exist in other countries, such as France, but a specificity of the Dutch approach is that intellectual property rights and royalties are shared between breeding companies and the collaborating farmers, usually on a 50-50 basis (Lammerts van Bueren, 2010). Up to today, 10 varieties with multiple-gene resistance have been released from the project.

Although research on Rhizoctonia resistance has been ongoing for several decades in different countries (Dowley, 1972; Olanya et al., 2009; Zhang et al., 2014), an alternative approach to managing Rhizoctonia on potato has been explored in the Netherlands, based on farmers' experiences. Dutch organic farmers have observed a gradual reduction of Rhizoctonia in their potato crops when they use their own seed potatoes, as opposed to seed potatoes produced on other farms. They also observe a progressive reduction of the disease when they convert to organic farming. Dutch researchers have studied this phenomenon known as "Rhizoctonia decline" and concluded that it is due to soil life and disease-suppressing soil microorganisms. There seems to be a mutual adaptation between soil and potato-skin microbiota that account for Rhizoctonia decline when using farm-saved seed potatoes. In this example, the specific experience of organic farmers has pointed to an approach to Rhizoctonia management that may be a complement or alternative to resistance breeding.

As a final example, let's take the management of virus Y in potato. In most Western seed potato production schemes, this virus is controlled by systematically going through in-vitro cuttings under sterile conditions at regular intervals. Among widely used potato varieties, none have satisfactory resistance to virus Y. The German research project ECOPOT-RESI, in which an organic potato farmer and breeder participated, explored potential sources of genetic resistances to this virus. However, only few wild relatives presenting resistance genes can directly be crossed with cultivated potato. Even for those relatives that can be directly crossed, laborious backcrosses are necessary to obtain

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potato types acceptable for cultivation. Cell fusion has been proposed as genetic engineering technique that allows to circumvent and facilitate the integration of genetic resistances from wild potato relatives into cultivated potato (Julius-Kühn-Institut, 2016). However, as cell fusion has been defined as genetic modification exempted from the GMO regulation, its use in organic breeding has been refused by the International Federation of Organic Agriculture Movements (IFOAM Organics International, 2017) and banned by several private organic label associations. Back-crosses may take more time, but also have the advantage of developing new diversity. The management of virus Y in organic seed potatoes has also been studied at ITAB in France in the framework of a student's research project (Le Grumelec, 2019). On the one hand, tolerance mechanisms to virus Y were identified as promising alternatives to genetic resistance. But, some seed potato producers feared that tolerant potato varieties, which do not express disease symptoms despite the virus being present in the crop, may act as virus-reservoirs in the landscape, thus presenting a risk for other varieties.

Considerations and challenges

The example of potato health management points to several considerations and challenges as regards resistance breeding for organic systems.

The Bio Impuls project does not only show the added value of integrating farmers' resources, knowledge and experience to develop potato varieties adapted to organic growing conditions and consumer needs (such as taste), but also demonstrates how organic breeding projects can benefit from existing traditions in conventional breeding such as the sharing of intellectual property rights and royalties . Such cases can contribute to redesigning the socio-economic grounds of resistance breeding.

The phenomenon of "Rhizoctonia decline" illustrates how organic growing conditions foster complex plant-microbe interactions, which can in turn favour plant health. Accordingly, organic research and breeding projects can embrace approaches more orientated towards crop ecology as complement or alternative to genetic resistance.

Considerations on research on the management of virus Y in Germany and France have raised two sets of issues. Firstly, resistance genes present in crop wild relatives raise the issue of which breeding and genetic engineering techniques are acceptable according to organic principles. With new challenges ahead, it is important to have clearly defined pros and cons of techniques departing from the organic principles as defined by IFOAM Organics International. This issue is also relevant for many other crops where crop wild relatives contain promising resistance genes. Secondly, the French study on virus Y management in seed potatoes identifies a dilemma as disease tolerance is considered as a strategy that could be deployed in parallel of other approaches: if tolerant varieties are considered a reservoir of viral inoculum and as a risk for more susceptible varieties, actors of seed potato production and potato breeding may be tempted to opt for a simplistic vision and production scheme taking into account only genetically resistant or susceptible varieties, thus setting aside other more complex and durable plant defence mechanisms. This question seems relevant for organic systems as it aims to base agricultural production on complex local ecological interactions rather than one-size-fits-all solutions.

Outcomes

Beyond the general call for more resistance breeding to strengthen organic cropping systems and to generally reduce pesticide use, relevant questions concerning how to incorporate genetic resistance in wider plant health strategies and farming systems remain. Resistance breeding is a powerful tool to strengthen plant health in organic systems, but it cannot be considered as a stand-alone solution to ensure plant health. Organic breeding projects should consider resistance breeding in relation to several other aspects of cropping and wider agricultural systems, including its complementarity with other plant health approaches including preventive measures, the social and economic models in which it is embedded and the principles and values behind organic agriculture.

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Complementarity with other approaches to plant health

Users of resistant varieties may be tempted by a dichotomous and overly simplistic view which accounts only for genetically resistant or susceptible varieties. In such a perspective, disease resistant varieties may be perceived as a panacea, creating blind-spots as regards to other defence mechanisms and resilience factors in cropping systems. These factors include, amongst others, collaborative approaches as addressed under Topic 3, crop diversity and local adaptation, addressed under Topic 4, and balanced plant-microbe interactions, plant communication and defence strategies, discussed under Topic 8. Avoiding the creation of such blind spots seems key for developing comprehensive approaches to plant health management. For that the broader context of organic breeding will need to be clearly explained continuously.

Integration in fair social and economic models

As for any plant breeding project, resistance breeding requires human and financial resources, as well as time. On several occasions and concerning different crops, the author of this section has heard actors describe the economic quandary of commercial breeding and seed companies when it comes to releasing crop varieties resistant to seed-borne diseases, as these varieties present an advantage for farm-saved seed and may thus weaken incomes from royalties. Collaborative or participatory breeding projects involving farmers and other actors interested in resistant varieties therefore have a potential to advance resistance breeding, as shown by the Dutch potato breeding project Bio Impuls. In this perspective, finding fair solutions for intellectual property rights and benefit sharing are a necessary prerequisite as well as novel financing strategies for organic breeding (Topic 5).

Compliance with organic principles and values

For many actors of organic farming, challenges in plant health management arise not so much because of a ban on chemical treatments, but rather because they endeavour to engage with plant health through equilibria in living systems, rather than “going to war” against potential pathogens through disinfection or genetic resistance (the term “arms race” between resistant plants and pathogenic strains is quite illustrative of this). IFOAM Organics International has formulated this idea in its principles of “health” and of “ecology” (IFOAM Organics International, 2005). The former is based on the hypothesis that “healthy soils produce healthy crops that foster the health of animals and people”. The second bases production on ecological processes. Whereas resistance breeding focuses on the genetic composition of plants to ensure plant health, other approaches widen the scope to take into account soil health and the microbiology associated to soils and plants. This diversity of plant health approaches seems vital both for organic agriculture and for maintaining crop diversity (Döring et al., 2012; Klaedtke, 2017).

When sources of resistance genes lie beyond a cultivated species itself, i.e. in crop wild relatives, breeding projects are rapidly confronted with the question of which techniques can be used to introgress those genes into the crop. For instance, departing from the principles of organic agriculture as defined by IFOAM, the European Consortium for Organic Plant Breeding has formulated a position which excludes cell fusion from organic breeding as it is a technical interference below the cell level (ECO-PB, 2012). Organic breeding projects have the responsibility to employ breeding techniques that comply with such principles, including for resistance breeding. On the other hand, organic resistance breeding projects have also been the opportunity to develop innovative breeding techniques in accordance with organic principles, such as the creation of wheat and lupine CCPs as a way to combine several resistance genes, at the same time also creating new diversity.

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Topic 12) Breeding for integrated weed management

Introduction

Crop-weed interactions are extremely variable, depending on the weed species, the abundance and composition of the weed community, climatic and environmental factors. The complex nature of crop-weeds interaction is particularly challenging from a breeding point of view (Hoad et al. 2012). Weed management has generally been the object of crop management, that, ideally, can be adapted to the specificities of a given field in a given climatic pattern easier than breeding an adequate crop variety could. The absence of herbicides in organic farming, as well as environmental concerns and growing emergence of herbicide-resistant weed populations in conventional farming have generated growing interest in managing weeds through optimising crop interference (Van Der Meulen and Chauhan, 2015). This can be achieved through an integrated approach, known as Integrated Weed Management, which is based on a diversity of strategies and tactics for weed control, including the breeding and use of appropriate cultivars (Buhler 2002; Swanton and Weise 1991).

Mechanisms of crop interference

Crop interference is a complex matter that can be dissected in two main mechanisms: allelopathy and competition. Both are complex and quantitatively inherited traits with high levels of GxE interactions, and therefore require appropriate designs and protocols for genetic gains to be obtained, as described by Worthington and Reberg-Horton (2015). Allelopathy is the ability of the crop to directly hamper the growth of coexisting weeds through exudates. Competition is the indirect interference between weeds and crop mediated by the limited environmental resources, and can be in turn subdivided into:

1- Weed Suppressive Ability (WSA), i.e. the ability of a crop to outcompete weeds and minimise their growth, and
2- Weed Tolerance Ability (WTA), i.e. the ability of a crop to withstand the presence of weeds minimising the damage.

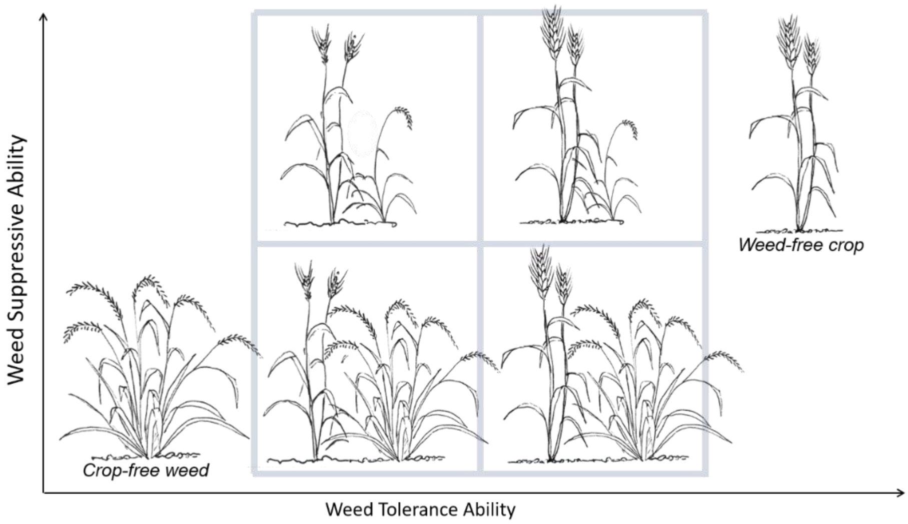

Figure 12.1. Competitive interactions between crop (wheat) and weed (ryegrass) in terms of weed tolerance and weed suppressive ability (Drawings by Ambrogio Costanzo, ORC UK).

Allelopathy

Allelopathy is an ecological mechanism through which a plant releases chemical compounds (allelochemicals) that can interfere with physiological functions (respiration, ion uptake, photosynthesis) of neighbouring plants (Kholi et al, 1997). Several crop species are known to express

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allelopathic properties, notably rye (*Secale cereale*), sorghum (*Sorghum bicolor*) and many Brassicacee, and their effect can be harnessed at various levels, including rotation planning, management of residues, cover crops and intercropping and cultivar selection (Jabran et al. 2015). Besides these key allelopathic species, genetic variation in allelopathic properties has been found in many important crops, including wheat (Bertholdsson 2010).

Weed tolerance ability

Weed tolerance ability (WTA) is the ability of a crop to minimise the difference in performance (e.g. yield) in the co-presence of weeds compared to its performance in weed-free conditions. Genetic variation within and between cultivars has been identified, which implies there is a possibility to breed for weed tolerance. However, a weed-tolerant crop does not necessarily control weeds, which can exacerbate the problem in the long term via e.g. excessive production of seeds. However, understanding this competitive mechanism can be useful to breed for crops able to withstand weed pressure. Horneburg et al. (2017) proposed that soybean, a species with notably low weed tolerance, can successfully be directly selected under the pressure of ten different crop species, used to simulate different mechanisms of weed interference.

Weed suppressive ability

Weed suppressive ability (WSA) is the ability of a crop to maximise the difference between weed growth in the presence of the crop and weed growth in the absence of the crop. The key aspect of a weed suppressive crop is a fast development of the canopy that can reduce the emergence, density, growth and seed production of weeds, and therefore lies in a fast occupation of space and resources. Enhanced WSA can be ensured by breeding and crop management. Management is especially important in arable crops such as small-grain cereals. Crop spatial arrangement (Evers and Bastiaans, 2016), particularly increase in seeding rates and reduced row spacing (Jha et al. 2017) are critical in enhancing crop WSA. The identification of genetic variation underlines the possibility to breed for weed suppressive ability. This is a very important tool to maintain weed seedbanks at a low level and/or slow down the build-up of aggressive weed seedbanks.

Critical periods

Weed presence does not generate yield damage regardless of when it occurs. On the contrary, the competitive damage is especially generated in specific time frames during the crop's growth cycle, known as 'critical period' for weed competition. This timeframe is experimentally determined comparing crop yields and/or weed biomass from plots that have been kept weedy or weed-free, and in presence or absence of the crop, in a range of intervals. The critical period for weed competition in wheat has been determined to be very early in the growth cycle, i.e. between two and four weeks after emergence, in relatively warm climates (Southern Brazil, Agostinetto et al. 2008), and between November and February in winter wheat in oceanic climates i.e. between one and three months after emergence(Great Britain, Welsh et al. 2008). Similarly, both cabbage and cucumber suffered significant yield losses in presence of weeds in the first three to five weeks after transplanting (Weaver, 1984). For soybean not only the early stages are of importance for weed tolerance ability but also the weed suppressive ability before harvest as this effects the weed seed return and the build-up of seed banks and thus weed pressure in the following crops. Experiments to determine the critical periods have generally been targeting optimal timings of mechanical weed control measures but can be of extreme relevance in selecting crop traits that are most likely to affect crop-weed interaction in a positive way.

Weeds life strategies

Like every spontaneous plant, weeds can be classified into different life-cycle strategies that determine their chances of survival and colonisation in different environments. Grime (2001) classified

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these strategies in three primary categories of Competitors, Stress-tolerant and Ruderal (C-S-R theory), summarised as follows:

- **Competitive** plants thrive in low-disturbance and low-stress environments and concentrate their resources on outcompeting other plants.
- **Stress-tolerant** plants thrive in condition of low disturbance and prolonged environmental stress, concentrating their resources on withstanding the stress through physiological variability.
- **Ruderal** plants that thrive in highly and recently disturbed environments, concentrating their resources in growth rates, fast growth cycle and maximised production of seeds.

Different cropping systems might favour a shift towards one of the three categories, for instance high-intensity soil labour can indeed favour outbreaks of ruderals, whereas high-fertility situations can favour the accumulation of competitors. Likewise, Gunton et al. (2011) showed that crop architecture and sowing date, more than crop species and type, were associated with the C-S-R classification of weed communities across 651 arable fields in France, and suggested associations between graminoid crops and ruderals, tall single stem crops and competitor-ruderals, and rosette crops and competitors. This might be very relevant for breeding crops in which canopy architecture has a strong genetic variability. For instance, Korres and Froud-William (2002) found that increased wheat seed rate was a more successful tool than cultivar choice to enhance weed suppression in Britain, but this was not valid for some of the tested cultivars, particularly the top-competitive, historic Maris Widgeon cultivar.

Breeding history and crop interference

Breeding progress in the second half of the 20th Century has undoubtedly increased the yield potential of key crops, by focusing on crop ideotypes such as the short-strawed, single-ear wheat plant described by Donald (1968). Such breeding progress has often been charged with having only focused on yield, generating plants which were highly dependent on external input and not adapted to low input conditions (Yapa, 1993). Wheat is perhaps the plant whose functional traits changed the most in the second half of the 20th Century, following the introduction of the dwarfing genes which clearly reduced straw length (Flintham et al. 1997). Several authors have hypothesized that the shortened straw, useful in preventing lodging and increasing the harvest index, could have had a detrimental effect on crop competitiveness, thereby generating a potential dependence on herbicides. In particular, Zhu and Zhang (2013) suggested that better competitiveness of old (not dwarf) cultivars is not limited to the different canopy architecture, but to different dynamics of resource allocation to the root systems. In this work, the tested old cultivar showed a marked root redundancy, interpreted as an indicator of competitiveness, compared to the modern one, whose lower investment in roots was instead interpreted as a ‘cooperative’ strategy, which can indeed maximise yields by minimising intra-specific competition in monocrop and weed-free conditions.
Similar trends were reported for many other crops. Notable examples are barley with a well-known allelopathic potential that might well have been diluted in over a century of breeding (Bertholdsson 2004), and soybean (Hammer et al. 2018).

Key relevant traits

To be able to conduct efficient selection, it is important to know which traits can be selected for directly, and which traits can be selected for indirectly. Given earlier research findings, it seems reasonable to suggest that these traits may differ per crop and cultivation system. Various traits have been suggested for direct selection. These include the production of allelopathic compounds, seedling vigour, early growth rates, straw length in cereals, and below-ground traits. Traits for indirect selection can include tolerance to mechanical weed management, growth cycles and avoidance strategies, and adaptation to better weed-suppressive mixed cropping systems. However, such indirect selection strategies cannot be selected for with single plant progenies and can only be used in larger plots.

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Outcomes and challenges

Crop weed competition can be a critical tool for integrated weed management, especially for organic farming where the use of herbicides is not allowed. Gaba et al. (2018) showed that crop competition in winter wheat is by far the most important effect in reducing weeds, when compared to management practices. Weed-proof crops are therefore increasingly sought for. Most of the scientific literature on cultivar effects on weeds, however, bears the problem of having mostly considered model weeds and/or artificial weed populations. Additionally, frequent confusion between WSA and WTA does not help making results replicable. For example, Ghaouti et al. (2016) concluded that competitive ability of faba bean genotypes against the model weed *Camelina sativa* was maximised in highly heterozygous genotypes. However, the conclusion might be misleading unless the reader appreciates that the key measured variable for 'competitive ability' is crop yield loss in weedy conditions, i.e. essentially WTA, not WSA.

The divergences and convergences between suppressive and tolerance mechanisms are clearly shown in a work on Australian barley by Mahajan and Chauhan (2020), who suggest that scoring genotypes for WSA and WTA could help drive breeding for weed competition (although the authors warn that results might only be valid for wild oats, the model weed species adopted). Cosser et al. (1997) showed that the historic wheat Maris Widgeon, known as being one of the most competitive wheats, is actually a very good weed-tolerant crop for organic systems *"when high weed infestations were anticipated, but could not be relied upon to suppress weed development, and in some circumstances could actually encourage certain species"*. Similarly, Fradgley et al. (2017) found that taller oat varieties exhibit better weed tolerance rather than suppressive ability. These studies suggest that the widely accepted association between long straw in cereals and weed suppression needs to be revisited, as the difference between WSA and WTA might have important implications for integrated weed management. In fact, weed tolerant cultivars can limit crop yield losses in the presence of weeds, but not necessarily help reduce weed seed return and the build-up of seed banks in the soil. In addition, tall cereal cultivars can prevent farmers from using weed surfers, i.e. mechanical tools that remove weed biomass emerging from the crop canopy, which mostly contain reproductive structures (e.g. wild oat panicles), in an attempt to control weed seed return for the following season (personal observation Ambrogio Costanzo ORC).

In conclusion, adaptation to the local pedo-climatic and management environment seem to be the most relevant breeding goal, in an integrated weed management framework. Whilst it seems unlikely to frame crop breeding around a "weed-proof" ideotype, several works have highlighted which traits can help improve weed suppressive ability or weed tolerance (Hoad et al. 2012). Variation for these traits has been relatively unexplored in modern breeding but is present in heritage germplasm and can be harnessed with an interdisciplinary approach bridging agroecology and genetic studies (Lazzaro et al. 2019). Furthermore, crop interference against weeds can be optimised with different tools besides competitive varieties, including mixed cropping (Liebman and Dyck 1993), which can in turn alter weed community composition (Poggio 2005). This underlines the importance of breeding for, and possibly into, a target overall cropping system, including its weed community and the weed management strategies adopted.

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LIVESEED is funded by the European Union's Horizon 2020 under grant agreement No 727230 and by the Swiss State Secretariat for Education, Research and Innovation (SERI) under contract number 17.00090.

Topic 13) Challenges and perspectives of root stock and scion in organic fruit tree breeding

Introduction

In order to produce perennial crops (fruit trees, grapevine) under organic conditions ensuring ecological sustainability and economic viability, varieties specifically selected for organic farming are needed. In order to obtain a productive orchard, both the scion and the rootstock need to be suitable for growing under organic conditions. Breeding of perennial crops under organic conditions needs to take into consideration the strong changes in management due to no use of chemical fungicides, insecticides, herbicides and mineral fertilisers of organic *vs* conventional orchards. Minimising the use of plant protection is a general objective of organic breeding and this holds even stronger in the context of perennial crops for which organic production still relies on quite high use of allowed plant protection substances (e.g. copper). The general robustness of the cultivar, meaning broad tolerance against pests and diseases and physiological disorders is therefore very important under organic conditions. Regarding the tolerance to diseases, both the scion and the rootstock have influence and should be appropriately selected for broad range of resistance and tree growth. Grafting is an ancient agricultural practice that joins the root system (rootstock) of one plant to the shoot (scion) of another. It is most commonly employed in woody perennial crops to indirectly manipulate scion phenotype. However, research and breeding on rootstock is largely neglected as described by Warschefksy et al. (2016). Preservation and enhancement of biodiversity is another major focus in organic breeding because due to a reduced diversity in parents used in conventional breeding programmes, many commercial fruit cultivars (e.g. in apple production) are closely related, resulting in narrow genetic diversity in cultivated fields (Kienzle and Kelderer, 2017). Because of this, for a truly resilient fruit production system under organic conditions, it is necessary to broaden the genetic basis by increasing the number of more distantly related varieties that are cultivated in large fruit-growing areas (Kienzle and Kelderer, 2017). In this context, local varieties that are very well adapted to specific regions should also become more important (Kienzle and Kelderer, 2017). There are several initiatives for the conservation of local cultivars in different European countries and those should be more connected with the breeding programs. The diversity of varieties should be part of the development of quality concepts that include also adjusted standards for external fruit quality (Kienzle and Kelderer, 2017).

Considering the specific management strategies under organic conditions, a very important aspect, which strongly diverges from the requirements in conventional farming is the functioning of the rootstock in order to guarantee a balanced nutrient supply in the absence of mineral fertilisers use (Atkinson 2018). Rootstocks are used to modify the growth of a scion variety and usually to achieve a predictable control of the growth and of the relationship between growth and crop production (Atkinson 2018). Organic fruit growers have to deal with more difficulties in regulating the crop load because of higher yield alternation and higher production costs (e.g. manual and mechanical thinning). The use of size controlling dwarfing rootstocks can support in adjusting the crop-load, but for other objectives (anchoring in the soil, nutrients and water absorption, management of above and belowground competition) more vigorous rootstock and even own-rooted plants might be useful (Lauri et al. 2020). More knowledge on the behaviour of different types of rootstock under organic conditions is needed to support farmers with best choice for the specific farm conditions. For example, results of trials on rootstocks for apple under organic cultivation are available (Pfeiffer et al., 2014, 2016; Ruess 2006), but currently the demand for rootstocks more suited to organic farming is much higher of what offered by the market (Kienzle and Kelderer, 2017). Moreover, only a few studies have investigated the interaction of rootstock x scion genotypes. Weibel et al. (2008) tested 10 rootstocks with 3 cultivars under organic management and found that productivity on rootstock Supporter 2 was up to 64% higher than on rootstock M.9 Fleuren 56 which is usually a productive rootstock under conventional conditions. Tolerance to fire blight and replant diseases are traits increasingly desired

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from rootstocks. The rootstock has a high potential to improve and stabilise the yield performance in modern organic fruit production, but it is also very difficult and time-consuming to develop and introduce new rootstocks into practice (Weibel et al. 2013).

The limitations of the current conventional system are not only agronomic but also related to socio-economic aspects. Wolter and Sievers-Glotzbach (2019) describe for the case of apple breeding in Germany how market and legal aspects have actually a strong impact in the possibility to deliver resilient cultivars on the market. The concept of club variety with restricted access to club members is widespread in the sector. New varieties are provided only to selected farmers that agree to join the club variety and follow production rules decided by the club. This strategy determines the privatizations of the new cultivars and in practice farmers and breeders have very limited access to these club varieties (Wolter and Sievers-Glotzbach 2019). In the case of club varieties, theoretically, the breeder's exemption holds true, but in practice breeders outside the club hardly have access to bud wood and pollen of club varieties, since they need to be members of the club to get this material (Wolter and Sievers-Glotzbach 2019). A further barrier to market entry for organic plant breeding initiatives is the fees for variety registration (Bruszik et al., 2020). In Switzerland, variety registration costs 10,000€ and the application fee represents a financial risk in case the new variety is not successful on the market. This is problematic especially for small-medium OPB initiatives that are often non-profit organizations with limited financial resources (see Topic 3, Topic 5). Given the long cycle of this type of crops and the need to focus on both rootstock and scion, breeding new varieties of fruit trees is cost- and labour-intensive, and takes many years. For these reasons, the set-up of perennial crop breeding initiatives under organic conditions is still a major challenge.

Examples

The first steps for new breeding programmes dedicated to produce varieties can be observed in different countries, for example, Apfel:Gut Germany, Poma Culta Switzerland, and Novafruits in Belgium (Koutis et al. 2018). LIVESEED activities allowed to connect existing and new organic apple breeding initiatives to join forces and share knowledge and genetic resources via an active network among apple breeding initiatives across Europe (https://www.liveseed.eu/tools-for-practitioners/maps/). From the networking activities in LIVESEED has emerged that still many issues need to be tackled by organic breeders in order to be able to provide sufficient reproductive material on the market of apple, one of the major fruit crops in Europe.

Challenges

In addition to the already challenging context of developing sufficient breeding initiatives to work on the scion, major challenges regarding the rootstock to be addressed by the perennial crops breeding community are the following:

- Select rootstocks to maximise the positive interactions with the living soil;
- Provide farmers with diverse rootstock options (dwarf, semi-vigorous and vigorous) tailored to cope with organic management peculiarities;
- Research on optimised scion-rootstock interactions under organic conditions;
- More knowledge on the plasticity of plant architecture and ecophysiology in response to the more complex biotic and abiotic environment (Lauri et al. 2020);
- Deliver cultivars with increased general robustness to facilitate the decrease of plant protection use under organic conditions.

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TOPIC 14 Efficient breeding methods: Decentralized-Participatory Breeding for Organic Agriculture based on Dynamic Management of Evolutionary Populations

Introduction

From a plant breeder's perspective, organic agriculture represents a heterogeneous target population of environments (TPE) fundamentally different from the more homogenous TPE typical of conventional agriculture. In the latter, the use of chemical fertilizers and pesticides has a powerful effect in smoothing most of the differences between agronomic environments even when they are geographically distant, except for those associated with climate. Therefore, the breeding strategies used to deliver varieties for conventional agriculture and the centralized seed systems associated with them, which are both based on a negative interpretation of genotype x environment interaction (GEI) (Ceccarelli, 1996) are not well suited to serve organic agriculture.

To serve such a heterogeneous TPE, characterized by different climates, soils, landscapes, farming practices, clients and markets, a highly flexible and dynamic breeding strategy is needed, fundamentally different from corporate breeding. This strategy is based on decentralized selection, namely selection conducted *independently* in each target environment, combined with participation, namely selection conducted in collaboration with the users. Decentralized selection represents the implementation of a positive interpretation of GEI based on the experimental assessment of the repeatability of genotype x location (GxL) interaction. This interpretation assumes the recognition of the fundamental difference between GxL interaction and genotype x year within location (GxY_L) interaction (Singh et al., 2006), a difference that is still seldom mentioned in most of the GEI literature, even the most recent. Yet already 50 years ago, Allard and Hansche (1964) specified that GxY_L and GxL interactions cannot be combined into GxE interactions, because the former is largely unpredictable while GxL interactions can be, to some extent, predictable. The distinctions make also possible to understand why GxL interactions are significant, whether because of interactions between genotypes and locations-specific social factors (GxL_S) or between genotypes and location-specific management factors (GxL_M) or a combination of the two.

Before considering the role of participation in a breeding strategy for organic agriculture, we need to consider another implication of decentralized selection, which was also recognized by Allard and Hansche (1964). While decentralized selection can make a positive use of GxL interactions by selecting for specific adaptation, varieties well buffered against unpredictable weather fluctuations are the solution to GxY_L interactions. This can be achieved through individual and population buffering. While individual buffering is a property of specific genotypes, and particularly of heterozygotes, population buffering arises by the interactions among the different genotypes within a population, beyond the individual buffering of the specific genotypes. Therefore, **the advantage of heterogeneous populations is that they can exploit both individual and population buffering**.

Challenges

The recognition that heterogeneous populations, such as evolutionary populations (EPs) and mixtures, are the ideal genetic materials for a breeding strategy addressing a heterogeneous TPE (target population of environments) is particularly important today as a way to cope, at the same time, with the extraordinary complexity of climate change (Ceccarelli and Grando, 2020). Climate change is a complex breeding objective because:

1. Changes in temperature and rainfall are likely to vary from location to location, thus adding to the heterogeneity of the TPE represented by organic farms;
2. Climate change is not only about temperature and rainfall, because these changes also affect the distribution and outbreak of pests (Heeb et al. 2019), particularly the spectrum of insects

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(Zavala et al. 2008; Deutsch et al. 2018), including pollinators such as bumblebees (Kerr et al. 2015), diseases (Newton et al. 2011; Pautasso et al. 2012) and weeds (Ziska and Dukes 2010; Colautti and Barrett 2013; Matzrafi et al. 2015);

3. Extreme weather events can influence the interactions between crops and pests in an *unpredictable* way (Rosenzweig et al. 2001).

All this evidence points at climate change as an extremely complex and evolving problem, which requires an evolving solution such as evolutionary populations and mixtures (Ceccarelli and Grando 2020). There is a large body of research, spanning from the seminal paper of Harlan and Martini (1929) to the most recent work (Wolfe et al., 1992; Goldringer et al., 2006; Raggi et al., 2017; Brumlop et al., 2017) showing that EPs and mixtures are able to evolve, adapting their phenology, increasing their disease resistance, yielding ability and yield stability. There is considerable anecdotal evidence of the ability of EPs to controlling weeds, a major problem in organic agriculture. More information is needed to understand under which conditions EPs have improved ability to control weeds and when EPs do not have this ability.

Of particular relevance to organic agriculture is that the type of resistance of EPs and mixtures is much more durable than the type of resistance obtained by single genes or gene stacking using conventional breeding or genetic engineering, which accelerates the evolutionary changes in agricultural pests (Palumbi 2001; Ceccarelli 2014).

Interestingly, most of the research on EPs and mixtures has been conducted on self-pollinated species such as wheat, barley and rice, suggesting an even greater evolutionary potential by EPs and mixtures of cross-pollinated crops. Because of their crossing ability, the same EP planted in *n* different locations and propagated with the seed produced in each location, with time breaks down in *n* populations each adapted to its own location (including management). Therefore, they are the ideal breeding material to dynamically respond to the challenges of climate changes while adapting to the heterogeneous TPE represented by organic agriculture. An improved understanding of so-called pedo-climatic zones in organic agriculture could help improve the efficiency of this approach. For example, it is clear that a country like Italy is much more heterogeneous in terms of TPE compared to a country like the Netherlands.

The word dynamic refers not only to how the EPs are developed – for example using parents selected for traits useful in organic agriculture (Messmer et al., 2012) or to generate as much diversity as possible – and used, but also to the final products of an evolutionary-participatory plant-breeding program. In fact, from the same EP it is possible to obtain either heterogeneous populations and/or uniform varieties at different times or for different markets. Eventually "dynamic" also refers to the process and mode of collaboration between scientists/breeders and farmers (or more generally clients). For example, the degree and the type of scientists' involvement may vary between locations and, with time, within locations: in the former, the mode of collaboration is shaped according to local habits, traditions, knowledge, socio-economic conditions and use of the crop, to mention some, while the latter reflects a continuous and reciprocal fine-tuning of roles as the collaboration evolves.

Figure 1 shows a general model of Decentralized-Participatory Breeding for Organic Agriculture (the number of farms is purely indicative). Note that the EP does not need to be the same for all the organic farms representing a given TPE. In addition, the responsibility for assembling the evolutionary population can be of both a formal as well as of an informal institution or association. Instead of a single organic farm, a community of farms can manage the evolutionary population, to allow exploiting the evolutionary ability of the population to adapt to different environments.

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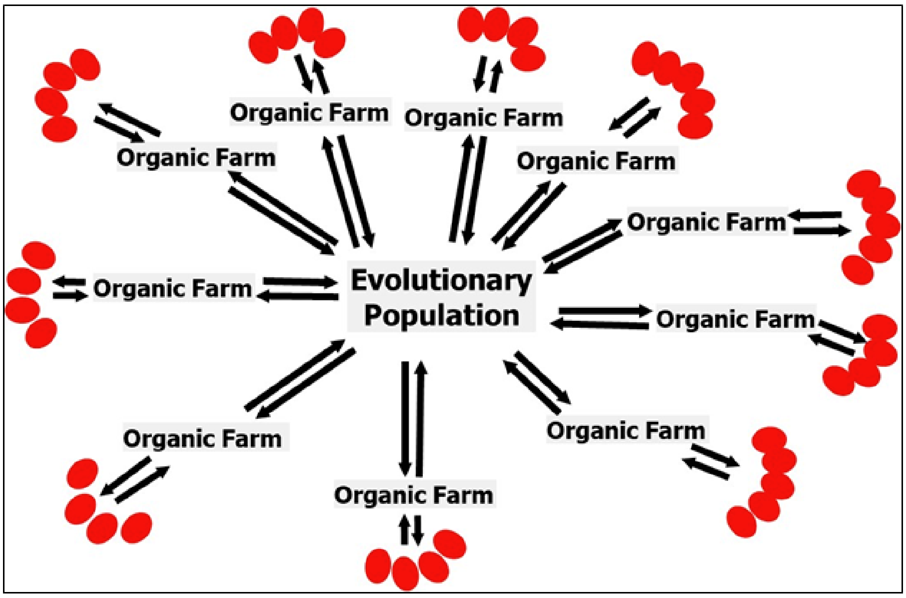

Figure 14.1. A general model of Decentralized-Participatory Breeding for organic agriculture. The red circles represent the selection sites (see also Figure 2). The arrows indicate the flow of both material and information.

Figure 2 shows the details of the selection process within each organic farm or community of organic farms. While the EP evolves over time (pathway A), farmers themselves, or in collaboration with scientists (pathway B), do selection. Selection can be done in different ways depending on the type of variety the farm or the community is aiming at. For example, it is possible to select for uniform material for certain types of markets, uses or seed systems, and for heterogeneous material for other types of markets, uses and seed systems. Participation continues (pathway C) resulting in the development of varieties from the initial selection through Multi Environment Trials (MET) conducted in a number of neighbouring farms or in different farms of the community.

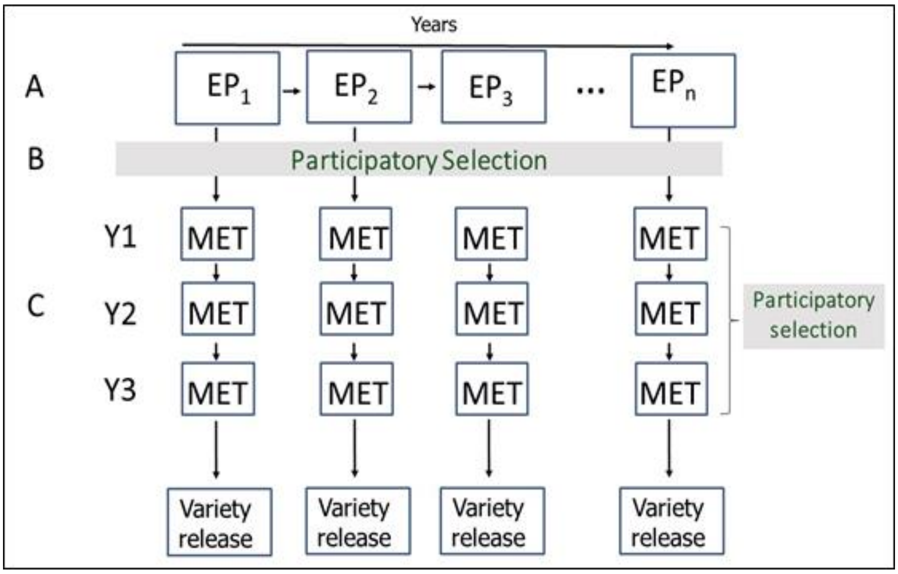

Figure 14.2. The management of an evolutionary population within a single organic farm or within a community of farms following different pathways (A: farmers themselves maintaining populations, B: in collaboration with scientists, or C: developing uniform varieties using participatory selection).

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The model shown in Figure 2 can be replicated in every TPE. If necessary, the model can accommodate the use of molecular tools to increase the speed and the precision of selection. In Figure 2, the last step, variety release and /or notification of organic heterogeneous material (EPs) in the scope of organic regulation that will be active from 2021, can follow after elaborated testing with other farmers. The new EU organic regulation 2018/848 allows in future the commercialisation of seeds of such organic heterogeneous material.

Example

An example of pathway C is an initiative started a few years ago by the organic breeding company Sativa to collaborate with farmers to develop horizontal resistance against mildew in lettuce. An example of pathway B, in which so far more experience is available is described below.

A Participatory Plant Breeding (PPB) programme on cereals in which a network of local farmers' associations belonging to Réseau Semences Paysannes (RSP) and Institut National de la Recherche Agronomique (INRA) work together started in 2005 (Dawson et al., 2011). The aims of the programme are to develop new cereal population-varieties adapted to organic agriculture free of intellectual property to provide healthy flour and bread and enhance the autonomy of farmers' organisation in breeding and management of cultivated biodiversity by creating methods, tools and training sessions. The PPB programme follows a recurrent process in three steps: i) create diversity, ii) breed new varieties following agronomic and organoleptic criteria, and iii) produce grain. The whole process is co-constructed: internal rules have been set up in order to work together (data and seed access, decision process, etc).

The first step of the process is to create diversity. Farmers can choose new varieties coming from gene banks, varieties tested in the breeding network, or based on mixtures or crosses. Regarding crosses, farmers choose the parents, and the research team can give technical support to realise the crosses.

Then, the second step: the breeding process. To do selection, a dedicated organisation has been set up. The experimental design is based on a satellite/regional farm network: all farmers agree on a common control that is sown in each farm of the network and farmers choose the varieties they want to sow (landraces, mixture of landraces, new germplasm coming from crosses …). Qualitative measures are taken on specially developed sheets by farmers themselves. Quantitative measures are done by the research team that receive samples of spikes of each of the varieties of each farm of the network. All data are recorded into a database (De Oliveira et al, 2020) and analysed with a tailor-made statistical method dealing with high disequilibrium (Rivière et al, 2015 ; Van Frank et al, 2019) using the R software (Rivière et al, 2020). Based on the analysis, farmers can get information on the varieties on their farm (mean comparisons) and on the network of farms (genetic, location, interaction effects). In addition, every year organoleptic analyses are conducted on a subset of populations grown in some regions. Based on this information and knowledge exchange through meetings, farmers can carry out breeding regarding their objectives. Several meetings are organised at the regional or national level to discuss results and exchange seeds.

Finally, the third step: grain production. When a variety behaves well on a farm, the farmer can multiply it to sow it on the field. For almost all cases, farmers create mixtures with breed varieties. After 10 year of PPB programme, PPB varieties are of great agronomic interest, combining relatively good performance and good robustness. The PPB varieties also tend to show a good temporal dynamic stability and appeared promising for the farmers involved (Goldringer et al, 2020). Moreover, the PPB populations have a wide adaptive potential (van Frank et al, 2020).

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General Discussion

In this review we have described what organic breeding is and how it can contribute to organic agriculture and sustainable agriculture in general using new innovative perspectives from a systems-based approach. A systems-based approach is a holistic approach and helps understand how various technical and socio-cultural aspects are interrelated (see Topic 1), and how this helps to understand how intellectual property rights can be organised in different ways (see Topic 2). Awareness that there can be different approaches means also a broadening in concepts, such as collaborative approaches in breeding (i.e. multi-actor and participatory approaches, see Topic 3) and to foster breeding for diversity based on agro-ecological principles (e.g. fostering the benefits of GxExS interaction instead of minimising them). Moreover, resilience does not only include the agro-ecological sphere but also the socio-economic sphere (see Topic 4). This also implies developing novel financing approaches that fit to the different breeding approaches based on agro-ecological principles, e.g. that plant breeding is an integral part of society and as such the whole food system can be involved in different ways in financing organic breeding (Topic 5).

In order to foster organic breeding approaches means further developing new effective organic breeding methodologies and a better understanding of the various processes in plant growth and how they interact, such as the actual existence of trade-offs between resilience, yield and quality in the breeding process (Topic 6). Organic breeding aims to emphasise quality as much as yield as resilience. It also means developing guidelines for breeding for complex systems such as mixed cropping and agroforestry (see Topic 7). More crop diversity is considered as key to improve yield and yield stability of organic farming systems. Lessons learned about breeding for mixed cropping may be a starting point for breeding for agroforestry. Moreover, organic breeding emphasises a better understanding of the relationships between plants and microbes (see Topic 8). Evidence is gradually growing that balanced plant – microbe interactions can contribute significantly to crop resilience and in managing pests and diseases. In combination with breeding for complex systems, this could prove to make organic food systems more robust.

The development of novel breeding methodologies also means developing further existing tools and/ or developing new tools. For example, Topic 9 provides examples on how direct and indirect selection can be used for complex traits like Nutrient Use Efficiency (NUE) and Water Use Efficiency (WUE) which are not only important for organic agriculture, but for agriculture in general in order to mitigate the effects of climate change. For particular issues, molecular marker tools may be used, as in the case of pyramiding resistance genes to develop more durable resistance against phytophthora in potato (Topic 10). However, organic breeding also seeks to develop holistic systems-based selection methods to develop durable resistance based on for example, increasing crop diversity and benefiting from beneficial plant-microbe interactions either targeting specific pathogens or by improving the robustness of the farming system as a whole (Topic 11). When it comes to breeding for weed suppressiveness, also a holistic perspective is used by understanding how both breeding and improved farming practices can reduce weed problems, e.g. so-called breeding for integrated weed management. Here we have to differentiate between weed tolerance and weed suppression ability (Topic 12). Not only new breeding methods need to be developed for annual crops. Topic 13 deals with the challenges and perspectives in the case of perennial crops, like rootstock and scion organic fruit tree breeding. And finally, Topic 14 describes decentralized-participatory breeding approaches based on dynamic management of evolutionary populations and how diversity-based breeding methods can be used to optimise GxE interactions instead of minimising them. This approach underlines the possibility of developing organic heterogeneous material and uniform varieties in the same process.

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Together these fourteen topics describe how organic breeding can use a holistic perspective in successful ways, e.g. that innovations in organic breeding are well connected with innovations in other knowledge fields such as agro-ecology, micro-biology, weed and disease management, sociology and economy (e.g. re-arrangements of the market) and law and governance (such as seed legislation and development on Intellectual Property rights). This means that to find the best solution for specific problems one need to consider knowledge of different fields in an integrated and transdisciplinary way. In the context of conventional agriculture, it is often suggested that molecular techniques are needed for further progress. This review shows that in the context of organic agriculture there is still much scope for improvement at various levels: at plant level (Topic 6, 8, 11, 12), crop or field level (Topic 7, 9, 11, 12, 14) and at value chain or food system level (Topic 1, 2, 3, 4, 5). This means that molecular marker tools that comply with the principles of organic agriculture can be useful (Topic 10), but only in an integrated way with other solution pathways.

Overall recommendations

Part of the systems-based breeding approach described by Lammerts van Bueren et al. (2018) are twelve key elements that need to be carefully addressed to achieve a holistic perspective on plant breeding. In addition to knowledge development in science and practical breeding, also action is needed at policy and at value-chain level. In LIVESEED Milestone 3.5 various practical questions and issues are described that need to be addressed to achieve organic systems-based breeding (Verrière et al 2019). An overall conclusion of various discussions is that all these questions and various action-points are interrelated. This makes it very difficult to define simple concrete action points.

In this context, the new organic regulation can be a driver for change at multiple levels. For example, more diversity in cultivars is considered important for organic agriculture, but breeders till today had to meet strict criteria in terms of Distinctness, Uniformity and Stability to be able to register new cultivars. With the new organic regulation, it is possible to register organic varieties with higher levels of diversity or to register organic heterogeneous material from 2022 onwards. For organic breeders, this means new opportunities in order to develop new types of organic cultivars well adapted to various organic conditions in different socio-ecological contexts as described in Topic 14. For policymakers, this is a new alternative breeding approach to get acquainted with. As described in various topics more collaboration is needed at various levels to develop organic breeding further (e.g. breeders to collaborate with farmers, traders, processors, researchers, policy makers and citizens). This was also the outcome of various workshops on systems-based breeding (Nuijten et al. 2019, Verrière et al. 2019). Another driver to promote organic breeding is the EU farm to fork strategy (EU Commission 2020) aiming to scale up organic production to a 25% share of farmland, as well as a 50% decline in pesticides and 50% reduction in nutrient losses by 2030.

In this report we have underlined the fact that social, cultural and ethical aspects shape technology development pathways. Departing from the IFOAM principles of care, ecology, fairness and health, this means that organic breeding is a holistic and transdisciplinary approach, aiming to involve all actors in the breeding process. Together, all relevant actors can discuss the relevant tasks or first steps for researchers, policy makers, practical breeders and other relevant value chain actors. This in itself can be considered a first task for all. In this review we have described examples, opportunities and challenges for the further development of organic breeding. In terms of opportunities, new effective holistic breeding approaches and methods are to be further developed and to be scaled up. Common elements of these effective holistic breeding approaches are 1) collaborative approaches, e.g. working with farmers and the whole value chain, also in terms of financing; 2) benefiting from plant – microbe interactions; 3) benefiting from plant diversity in complex systems, and 4) decentralised evolutionary breeding approaches**.** This should not be only a task of plant breeders but should be conducted together with researchers and other value-chain actors. A better understanding is also needed of the

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following areas: potential trade-offs between traits in plants, the potential of plant – microbe interactions and other forms of organising the value chain and financing organic plant breeding. It is a task for researchers to take the lead, again in close collaboration with other actors, like practical breeders, farmers, value-chain players and policy makers. Policy makers may have the best background to develop, in close collaboration with the value chain, enabling policy for new effective organic breeding approaches and methods. The first policy field to think of is the field of cultivar registration, enabling new organic approaches that take common heritage as a point of departure. Other policy fields could be at the level education and dissemination, e.g. to make citizens aware of the potential benefits of organic breeding and organic agriculture at large (amongst others departing from the Farm to Fork strategy). Practical examples could be that organic breeding should be conducted in the field, with plants growing in soil and that plants should maintain their reproducing capacity. Another element is the understudied field of food quality. In that respect, the new Farm to Fork strategy could provide an important impulse at various levels.

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Annex 1 Current definitions of Organic Plant Breeding, Breeding for Organic and Cultivar

In LIVESEED Milestone 3.1, definitions were provided for Organic Plant Breeding and Breeding for Organic, and definitions for the term 'cultivar' and 'variety'. These definitions are listed below, supplemented with the definitions of organic heterogeneous material and organic varieties according to the EU new organic regulation effective *2018/848*.

Definition of Organic Plant Breeding (OPB)

Organic Plant Breeding is defined by the IFOAM International norms of 2014, **Article 4.8 Breeding of organic varieties.** Organic cultivars are obtained by an organic plant breeding program which fulfil following requirements:

- 4.8.1 To produce organic varieties, plant breeders shall select their varieties under organic conditions that comply with the requirements of this standard. All multiplication practices except meristem culture shall be under certified organic management.

- 4.8.2 Organic plant breeders shall develop organic varieties only on the basis of genetic material that has not been contaminated by products of genetic engineering.

- 4.8.3 Organic plant breeders shall disclose the applied breeding techniques. Organic plant breeders shall make the information about the methods, which were used to develop an organic variety, available for the public latest from the beginning of marketing of the seeds.

- 4.8.4 The genome is respected as an impartible entity. Technical interventions into the genome of plants are not allowed (e.g. ionizing radiation; transfer of isolated DNA, RNA, or proteins).

- 4.8.5 The cell is respected as an impartible entity. Technical interventions into an isolated cell on an artificial medium are not allowed (e.g. genetic engineering techniques; destruction of cell walls and disintegration of cell nuclei through cytoplast fusion).

Most important characteristics of OPB programs is that **all breeding steps** from crossing till final selections take place **under organic conditions** and that the applied breeding techniques are in accordance with the techniques listed in the Annex of the position paper of **IFOAM International for organic breeding** from November 2017.

Moreover, cultivars derived from OPB shall also not be patented.

Definition of Breeding for Organic (BfO)

Breeding programs for organic are more product oriented and have a special focus on the breeding goals which are specific for organic agriculture (e.g. tolerance against seed borne diseases, weed tolerance, nutrient use efficiency), they do not use critical breeding techniques and selection occurred at least partially under organic conditions. BfO programs fulfil following requirements:

- Plant breeders shall select their cultivars **at least in the final selection steps under organic conditions**. All multiplication practices except meristem culture shall be under certified organic management.

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· Organic plant breeders shall develop organic varieties only on the basis of genetic material that has not been contaminated by products of genetic engineering.

· The genome is respected as an impartible entity. Technical interventions into the genome of plants are not allowed (e.g. ionizing radiation; transfer of isolated DNA, RNA, or proteins).

· The cell is respected as an impartible entity. Technical interventions into an isolated cell on an artificial medium are not allowed (e.g. genetic engineering techniques; destruction of cell walls and disintegration of cell nuclei through cytoplast fusion).

Most important characteristics of OPB programs is that **derived cultivars are suited for organic production** and that the applied breeding techniques are in accordance with the techniques listed in the Annex of the position paper of **IFOAM International for organic cultivation** from November 2017.

Further information on OBP and BfO can be found in the position paper on organic plant breeding of the European Consortium for Organic Plant Breeding (ECO-PB (2012).

Definition of Cultivar:

The term "cultivar" is used within LIVESEED in a much broader sense than the UPOV definition of "variety" and it includes DUS varieties, landraces, CCP, populations, farmers selections.

"Cultivar" = general term for officially released varieties, land races, less homogeneous populations, niche varieties, etc.

"Variety" = defined term for officially registered cultivars according to the international UPOV definition for the protection of new varieties which meet the DUS criteria

Definition of Organic Heterogeneous Material (EU new organic regulation 2018/848)

Article 3 - Definitions
(18) 'organic heterogeneous material' means a plant grouping within a single botanical taxon of the lowest known rank which:

a) presents common phenotypic characteristics;
b) is characterised by a high level of genetic and phenotypic diversity between individual reproductive units, so that that plant grouping is represented by the material as a whole, and not by a small number of units;
c) is not a variety within the meaning of Article 5(2) of Council Regulation (EC) No 2100/94 (1);
d) is not a mixture of varieties; and
e) has been produced in accordance with this Regulation

Article 13 - Specific provisions for the marketing of plant reproductive material of organic heterogeneous material
Plant reproductive material of organic heterogeneous material may be marketed without complying with the requirements for registration and without complying with the certification categories of pre-basic, basic and certified material or with the requirements for other categories, which are set out in Directives 66/401/EEC, 66/402/EEC, 68/193/EEC, 98/56/EC, 2002/53/EC, 2002/54/EC, 2002/55/EC, 2002/56/EC, 2002/57/EC, 2008/72/EC and 2008/90/EC or acts adopted pursuant to those Directives.

Definition of Organic Varieties suited for organic production (EU new organic regulation 2018/848)

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Article 3 – Definitions

(19) 'organic variety suitable for organic production' means a variety as defined in Article 5(2) of Regulation (EC) No 2100/94 which:

a) is characterised by a high level of genetic and phenotypical diversity between individual reproductive units; and
b) results from organic breeding activities referred to in point 1.8.4 of Part I of Annex II to this Regulation

Annex II: 1.8.4. For the production of organic varieties suitable for organic production, the organic breeding activities shall be conducted under organic conditions and shall focus on enhancement of genetic diversity, reliance on natural reproductive ability, as well as agronomic performance, disease resistance and adaptation to diverse local soil and climate conditions.

All multiplication practices except meristem culture shall be carried out under certified organic management

Preface

(39) In order to meet the needs of organic producers, to foster research and to develop organic varieties suitable for organic production, taking into account the specific needs and objectives of organic agriculture such as enhanced genetic diversity, disease resistance or tolerance and adaptation to diverse local soil and climate conditions, a temporary experiment should be organized in accordance with Directives …. 66/401/EEC, 66/402/EEC, 68/193/EEC, 2002/53/EC, 2002/54/EC, 2002/55/EC, 2002/56/EC, 2002/57/EC, 2008/72/EC and 2008/90/EC for a term of seven years, should involve sufficient quantities of plant reproductive material and should be subject to yearly reporting. It should help to establish the criteria for the description of the characteristics of that material and to determine the production and marketing conditions for that material.

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