Progress report on breeding activities of white lupin, cell fusion free brassica vegetables, apple, common bunt resistance in winter wheat and participatory tomato breeding

Deliverable number	D 3.8
Dissemination level	Public
Delivery Date	15.06.2021
Status	Final
Lead beneficiary	UPV
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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	22 March 2021	Adrian Rodriguez-Burruezo	First draft sent to subtask leaders for feedback and input.
2	30 March 2021	Adrian Rodriguez-Burruezo Paolo Annicchiarico Noemi Uehlinger Jelena Baćanović-Šišić Gunter Backes Anders Borgen Matteo Pettiti Sara Sestili Gabriele Campanelli	Integrating feedback from subtask leaders, document ready to be revised at the dedicated annual meeting session.
3	15-20 April 2021	Adrian Rodriguez-Burruezo Paolo Annicchiarico Noemi Uehlinger Jelena Baćanović-Šišić	Integrate feedback from annual meeting. Sent to subtask leaders for new feedback.
4	20 April/ 10 May 2021	Adrian Rodriguez-Burruezo Paolo Annicchiarico Noemi Uehlinger Kostas Koutis Niklaus Bollinger Jelena Baćanović-Šišić Matteo Petitti Sara Sestili Gabriele Campanelli	Send to reviewers for revision (Edwin Nuijten, Mariateresa Lazzaro).
5	15 May 2021-11 June 2021	Adrian Rodriguez-Burruezo Noemi Uehlinger Kostas Koutis Niklaus Bollinger Anders Borgen Matteo Petitti Sara Sestili Edwin Nuijten Mariateresa Lazzaro Monika Messmer Leone Ferrari	Integration of the most recent results and estimated parameters. Finishing Section 9 (discussion and conclusions) Summary
6	14 June 2021	Edwin Nuijten, Mariateresa Lazzaro	Check of final revisions and proof-reading.

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1. Executive Summary

Organic farming is increasing in Europe, and it is a key sector in the policies that promote the transition towards more sustainable farming systems in the European Union (Farm to Fork, Biodiversity Strategy). However, most organic production depends on organic quality seeds of cultivars which have been bred under conventional conditions or even sometimes are non-chemically treated conventional seeds. As a result, there is a remarkable lack of organic seeds of cultivars specifically adapted to organic farming. In this regard, LIVESEED Task 3.4 was aimed at **promoting organic breeding initiatives on several important crops as case studies**: white lupin (grain legume), winter wheat (cereal), apple (fruit tree), *Brassica* vegetables (mainly as winter vegetables) and tomato (mainly as spring-summer vegetable). On the whole, the main objectives of Task 3.4 were i) to determine the most important gaps and key factors which are limiting the availability of breeding of varieties adapted to low input/organic farming; ii) to propose solutions and alternatives; iii) to exchange knowledge and experiences among already existing (or newly created) organic breeding networks; iv) to develop, reinforce or give support to small organic breeding initiatives in Europe or even abroad and v) to provide new organic varieties and/or new breeding material for organic farming. This Deliverable D3.8 focuses principally on objectives iii to v and it is complementary to D3.4 (Report on breeding gaps and key factors for strengthening small breeding initiatives: Experiences on five crops). The main results are provided per crop (and subtask).

White lupin is an interesting crop for organic farming due to the high quality of its seeds (e.g. protein content, digestibility, high-quality oil), but also as a legume contributing to improve soil fertility. However, the known available diversity is very narrow. Hence, LIVESEED has contributed to widen the genetic base of this crop, working with crosses between bitter lupin landraces and elite lines sweet lines and composite cross populations (CCP). These materials were used to test efficient methods to differentiate sweet and bitter lupins, to identify superior candidate cultivars for biotic and abiotic stressors and low-input conditions (anthracnose disease, calcareous soils, drought, low winter temperatures) and for quality traits, as well as to perform genome-wide association study (GWAS) and genomic selection models that may help future selection work. All the activities were developed by a network of LIVESEED partners and other European centers, following a participatory approach.

***Brassica* vegetables** are one of the most relevant crops in Europe, used by their bulbs (kohlrabi), heads/leaves (cabbages) or inflorescences (broccoli, cauliflowers) and grown mainly in the winter and spring season in Southern Europe and in the summer to autumn season in Central and Northern Europe. Thus, countries like France, Italy, Portugal and Spain are the main producers for European markets in winter and spring. However, F1-hybrids derived from cytoplasm male sterile lines (CMS) that originated from cell fusion are predominant, which is a paramount concern for organic markets. In this subtask, a group of LIVESEED partners and other stakeholders have developed a network aimed at performing and coordinating participatory organic trials with cell fusion free cultivars and breeding lines from cabbage, cauliflower, broccoli and kohlrabi, with emphasis in the mentioned Southern countries to identify alternative varieties and to foster transnational collaboration in organic breeding for this project as well as for future initiatives.

Apple is the most important fruit tree in Europe, but their organic production is still based on varieties which have been bred for high input intensive growing conditions. Thus, there is a need of plant materials (both cultivars and rootstocks) specifically selected for and adapted to organic production that can perform well without copper treatments. The main aim of this subtask was to develop a European network of actors involved in apple organic breeding (e.g., research centers, universities, breeders, farmers, nurseries, retailers) and to stimulate joint organic breeding activities in this fruit tree.

Winter wheat is a world´s staple crop, particularly in Europe, which is used in a range of foods and culinary applications. Common bunt, caused by *Tilletia caries*, is a seed borne disease, which mainly affects the organic sector as it can increase from one year to the next when farm-saved seeds are not

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treated and not carefully screened for and, therefore, can affect the organic production of wheat significantly. Furthermore, there is a bottleneck in the current genetic pool used in organic farming in terms of the resistance to this disease. Here, farmers, breeders and scientists have worked in a participatory fashion with a wide collection of cultivars and breeding lines, encompassing several sources of resistances to *T. caries*, aimed at breeding for a wide resistance to this disease as well as to find molecular markers and QTLs linked to these resistances to allow pyramiding of the bunt resistance.

Finally, **tomato** is the most important fruit vegetable in Europe and particularly Italy and Spain are the main producers. In this regard, the use of organic seeds and organic varieties is very scarce in these two countries, although many small organic farmers and initiatives offer the opportunity of using a higher and improved varietal diversity based on organic seeds and local landraces and heterogeneous materials. In this subtask, several small initiatives for organic breeding of tomato have been supported in both countries by LIVESEED partners, creating new participatory networks or reinforcing already existing ones and using new materials (i.e., collections of landraces from seedbanks and community seed banks, CCPs from other EU projects and MAGIC experimental populations).

All the initiatives in these five crops mentioned above have contributed, according to the characteristics of each crop, i) to breed or identify cultivars adapted to organic farming or ii) to develop breeding materials for futures initiatives, iii) to check analytical or genetic techniques to increase the efficiency in organic breeding, iv) to create networks aimed at participatory organic breeding (or to promote already existing ones) for this project and even for future initiatives.

The five subtasks offered different approaches to build networks and to close breeding gaps in the context of organic farming. A range of technical and social aspects together make up the breeding process. Thus, the way breeding challenges can be dealt with depends on factors such as crop type, cultivars available, knowledge on different issues (agronomy, genetics, etc.), actors involved (e.g., farmers, breeders, researchers, consumers) and the available markets. From these activities, it can be concluded that there is not one single approach to build networks and to close gaps, but that the approach is shaped by many different factors. As a result, no single advice can be suggested to initiate breeding networks or close breeding gaps in other crops. Thus, comparative analysis of the five case studies described in this Deliverable must be done to develop an approach that fits best their own case. Policy makers are also advised to develop an enabling environment to stimulate the diversity in breeding approaches to close breeding gaps and to initiate different types of networks. Finally, promoting multi-actor approaches can be by itself an important and powerful tool for building networks and closing breeding gaps in an efficient way, and at the same time to stimulate the development of higher crop diversity and a more resilient organic farming sector.

2. Introduction

There is lack of suitable cultivars adapted to organic and low-input farming in Europe as most conventional breeding activities do not consider traits crucial for sustainable farming systems such as organic farming. The motivations are not only technical but institutional and socio-economic in nature for an important part as well (Van Loqueren and Baret, 2008). In this regard, there are several organic plant breeding (OPB) initiatives aimed to develop cultivars adapted to local conditions and diversity-based food systems. In addition, both the formal sector (universities, research centres, seed companies) and the informal sector (small breeders and farmer-breeders) still need to overcome knowledge gaps and build bridges among the actors involved in these activities, to achieve a more efficient interconnected organic breeding.

The main aim of Task 3.4 was to close major breeding gaps of five important crops by establishing breeding and knowledge networks. For this, it is important to develop a more comprehensive understanding of the socio-cultural, economic and technical factors that are important to mitigate the breeding gaps in these crops and to identify the most important approaches to stimulate the development of new initiatives and networks. The multi-actor methodology, applied in different degrees in the case studies, involves as many actors as possible in the evaluation of breeding materials and in the development of the connected value chains and food systems. Participatory breeding enables an efficient, inclusive, and comprehensive decision making during the breeding process.

The species worked with in this task are:

- White lupin (3.4.1, lead CREA-IT)
- *Brassica* vegetables (3.4.2, lead BNN 2017-2020, SATIVA 2020-2021)
- Apple (3.4.3, lead AEGILOPS-GR)
- Winter wheat (3.4.4, lead KU-DE)
- Tomato (3.4.5, lead UPV-ES)

Each species network (sub-task) provides examples of different approaches, socioeconomic factors and breeding objectives of the new network. One of the objectives of Task 3.4 was to work on identified breeding gaps and needs in various crops, as well as to identify the underlying technical and socio-economic key factors that play a role in these breeding gaps and as described in D3.4. The second objective was to boost small existing breeding initiatives and to start new collaborations, by developing common trials and experiments and learning platform for knowledge exchange and discussion among the actors involved (farmers, breeders, scientists, consumers, food processors, etc.). In this regard, the interaction, discussion and exchange of knowledge among the actors involved (from farmers to scientists, from retailers to consumers) is essential i) to boost the already existing small initiatives and ii) to stimulate further collaborations. Lessons learned in this research were translated into key-factors important to create and encourage new initiatives as described in D3.4.

Based on the results of the 48 months of activities, the objectives considered in D3.8 are:

1.- To describe, per subtask, the actors of the network, main approaches and methodologies applied and to describe the main results of breeding activities and exchange of knowledge performed within each breeding network.

2.- To provide a list of developed screening tools in order to achieve breeding goals efficiency

- White lupin: screening tool for anthracnose tolerance test in field and controlled conditions, molecular markers for resistance to anthracnose, drought, calcareous soil, sowing time, NIRS for protein, oil and low-alkaloid content selection.
- Apple: screening tool for scab and blotch resistance, marker-assisted selection (MAS) for resistance and fruit quality.
- Wheat: field testing against panel of different virulent strains of bunt, MAS for gene pyramiding of different bunt resistance genes.
- Tomato: network for participatory breeding in Italy and Spain, DNA fingerprinting to determine genetic diversity.

3.- To provide lists of improved plant material (populations, cultivars and breeding material) selected by the various partners of the five breeding networks with regard to the respective breeding goals:

- Genetically broadened white lupin populations (organic heterogeneous material) and breeding lines with improved tolerance to biotic (anthracnose) and abiotic (calcareous soil, drought, low temperature) stresses and low alkaloid content.
- Cell fusion free brassica cultivars (broccoli, cauliflower, kohlrabi, cabbage) open pollinated populations (organic populations) jointly tested for local adaptation in Central and Southern Europe.
- Apple candidates adapted to copper free organic production with robustness to pest and diseases, agronomic performance and good taste and storability.
- Winter wheat populations (organic heterogeneous material) and lines with improved bunt tolerance against several virulent races of common bunt.
- Locally adapted tomato populations (organic heterogeneous material) and lines derived from participatory breeding of heirloom tomato selected for high resilience and improved taste.

4.- To offer knowledge and experiences from the five case studies which can be extracted from the experiences detailed in this deliverable. Each of the subtasks has developed a specific approach to deal with its goals and breeding gaps. Hence, this document can be read as an advice upon which organic breeders, policy makers and other value chain actors may decide how to act in their specific situation.

In the next sections we will provide:
i) a brief background of the different breeding networks per crop (subtask) and across subtasks,
ii) the description of cultivars and plant populations, breeding activities, actors involved and methodologies on each subtask/species,
iii) the main breeding results in form of improved cultivars and populations, new screening tools, and selection parameters obtained at the end of the project.

3. Background of each subtask and aims

3.1. White lupin: Fostering collaboration in breeding and widening the genetic base for European breeding of the crop by population development and use of molecular approaches

Lead: CREA (Paolo Annicchiarico)

Project partners: FiBL-CH, LBI, UBIOS

New members/collaborators/stakeholders: James Hutton Institute (Dundee, UK), Institute of Industrial and Forage Crops (DEMETER, Larissa, Greece), Instituto Nacional de Investigación Agropecuaria (INIA, Carillanca, Chile), AssoSementi (Association of the Italian seed companies, Italy), Jouffray-Drillaud (France)

White lupin offers great potential as a high-quality food (high protein content; high-quality oil; high digestibility; heart-protecting and anti-diabetes properties) and feed crop. Hence, it is a very interesting crop to diversify organic production and, as a legume, to improve soil fertility, mitigate climate change and reduce crop energy costs via symbiotic nitrogen fixation.

However, **plant breeding has been very limited on this species and has largely relied on a few sweet-seed (low-alkaloid) genotypes that were discovered relatively recently** (1950-1960) and because of this, white lupin breeding relied on a quite limited genetic base.

The main objective of the white lupin subtask (T3.4.1) was to **widen the crop genetic base** by exploiting elite bitter-seed landraces and sweet-seed lines in order to produce a large number of sweet-seed lines. A large subset of these lines was genotyped and evaluated for key biotic and abiotic stressors, production and grain quality traits, in order to **identify superior candidate cultivars** and to perform genome-wide association study (GWAS) and genomic selection models that may help future selection work.

Related to the phenotyping, the usefulness of Near-infrared spectroscopy (NIRS) to predict grain quality traits and to separate lines with low-alkaloid seed content lines from lines with high-alkaloid content was tested.

Given the specific problem with the anthracnose disease (caused by the fungus *Colletotrichum lupini*), which is the biggest obstacle for cultivation in countries like Germany and Switzerland and can cause drastic decreases of yield and often complete loss of the crop, another objective of the project was to develop field and controlled condition screening protocols for monitoring the susceptibility to this disease.

As well, LIVESEED worked on high-diversity cultivars for white lupin by testing for 3 years a composite cross population initiated by FiBL in 2015 and by **developing a new composite cross population** by

CREA, to be used as a starting point for the development of various evolutionary populations adapted to specific environments.

3.2. *Brassica*: Initiate a European network for evaluation of cell-fusion free *Brassica* vegetables

Lead: BNN (K. Arp, T. Kimmel, J. Zellfeder, H. Scharpenberg; June 2017-June 2020), SATIVA (N. Uehlinger, since June 2020)
Project partners: Sativa (CH), Vitalis (NL), Kultursaat (DE), LSSV (PT), UPV (ES), ITAB (FR), BNN (DE)
New members/collaborators/stakeholders: **France:** P.A.I.S. (Plateforme Agrobiologique d´Experimentation de Suscinio), GRAB (Groupe de Recherche en Agriculture Biologique), **Spain:** Félix Esteve & Sons, LA UNIÓ de Llauradors, AgrologicVal, La Verde Coop., SURINVER farmers Assoc., **Italy:** Daniele Perina, Sativa Bio Sementi, **Germany:** Kattendorfer Hof, Biolandhof Müller-Ölbke, Gärtnerei Walsegarten, Oldendorfer Saatzucht, Gärtnerei Tempelhof, Gärtnerei Obergrashof, **The Netherlands:** De Beersche Hoeve

Brassica oleracea vegetables are produced from spring to autumn in Northern Europe and from autumn to spring in Southern Europe, from where they are also exported to Northern European countries in the winter season. In terms of surfaces, F1-hybrid cultivars make up the main part of *Brassica* vegetable production, both in organic and conventional systems. A large part of these F1-hybrid cultivars have been developed with cytoplasmatic male sterility (CMS) derived from cell fusion. Cell fusion is rejected by the organic movement because of its direct interference at DNA level. Therefore, **there is a need for identifying superior cell fusion free *Brassica* vegetables cultivars adapted to organic cultivation**.

Until now, the breeding activities of organic initiatives have focused on the development of open-pollinated (OP) varieties. Population improvement and cultivar development of *Brassica* vegetables is a challenging work, especially when it comes to homogeneity and marketable yield. Until now, organic breeding activities have concentrated on Northern Europe, but there is a need for exchanging breeding material and methodologies between organic breeding initiatives across the whole of Europe. In order to have a year-round supply of cell fusion free *Brassica* produce, we emphasized the activities in this subtask (T3.4.2) in Southern countries (France, Italy, Portugal and Spain), where export-oriented organic production supplies the market in central and northern countries during the winter-spring season.

3.3. Apple: Initiate European organic apple/rootstocks breeder network

Lead: AEGILOPS (Kostas Koutis)
Project partners: ITAB (FR), FiBL (CH), AEGILOPS (GR), POC (POMA CULTA, CH), GRAB (FR)
New members/collaborators/stakeholders: IPC (PT), SEAE (ES), CRAW-W (BE), AGROSCOPE (CH), SERIDA (ES), KOB (DE), BIOFRU (GR), Copenhagen University (DK), APFEL GUT (DE), LWVO (DE), UASVM (RO), State Research Institute for Pomiculture (DE), Gavle University (SE), Holovousy Institute of Pomology (CZ), Pomology Institute-HAO DEMETER (GR), Vienna University for Natural Resources (AT), VZ Lainburg (IT), Ministry of Agriculture (RS), Luke Natural Resources Institute (FI), Fruit Growing Institute (BG), University Hohenheim (DE), Farm Hellas Nursery (GR), CRPV (IT), University Oldenburg (DE), AgroBio47(FR), AUTH (GR)

Compared to annual crops, the market introduction of new apple cultivars is much more difficult, as the trees usually have a lifetime of 15 to 20 years. Thus, planting new cultivars is a considerable and risky investment for organic growers as they do not know how the cultivar will perform under their conditions and whether the traders and consumers will accept the fruits of the new cultivar. Another challenging factor to introduce and promote new apple cultivars in the organic market is the fact that consumers are rather conservative in purchasing new cultivars and often expect to obtain the same cultivars from both conventional and organic production.

The main objective of LIVESEED Subtask 3.4.3 was to improve breeding for organic apple production through developing an **apple breeding** network to share efforts, knowledge and genetic resources.

Besides networking, the LIVESEED apple task included also **test cultivation** of advanced breeding lines in biodynamic farms in different growing regions of Europe **to optimise the market introduction of candidate cultivars from the biodynamic breeding** organization POC (Poma Culta).

Additionally, FiBL conducted in collaboration with POC (i) screenings for apple diseases, (ii) application of molecular markers for selection in POC breeding program, (iii) assessment of sensory qualities and shelf life of apples from POC candidate lines, and developed a marketing strategy for new organic apple varieties.

3.4. Winter wheat: Supporting organic breeders by providing modern breeding tools to improve resistance to seed borne diseases - the case of *Tilletia caries*

Lead: KU (Gunter Bakes, Jelena Baćanović-Šišić)
Project partners: AGROLOGICA (DK), CULTIVARI and DOTTENFELDERHOF as German wheat breeders

Wheat is a major crop, and it is widely cultivated in organic farming in Europe. However, there is a serious bottleneck in the current genetic pool to face *Tilletia caries*, a seed-borne fungal disease which mainly affects wheat grown for consecutive years from farm-saved seed without seed treatment and careful seed management (e.g., making sure that the seed stock is free from *Tilletia caries*), typical for organic farmers growing wheat cultivar mixtures, conservation varieties and populations.

In this regard, this subtask (T3.4.4) focused in **supporting the use of modern breeding tools to broaden the resistance of organic wheat to *T. caries***. The starting point was about 450 winter wheat lines: i) 420 F8 lines derived from a diverse range of lines as sources of new resistances to *T. caries* and ii) 30 lines from the German breeders CULTIVARI and DOTTENFELDERHOF. These lines were chosen in order to study seven resistance genes (Bt1, Bt2, Bt5, Bt7, Bt13, BtZ and Quebon resistance), distributed more or less equally among them.

This task involved different activities:

a) on-field resistance phenotyping of breeding lines to 10 highly virulent *T. caries* strains (2 years, AGROLOGICA);

b) genotyping with genotyping by sequencing (GBS) method of 274 breeding lines (KU), among the ones included in the on-field resistance phenotyping;

c) perform GWAS to identify marker-trait associations (MTAs) for resistance to common bunt (KU);

d) test the most promising lines from on-field resistance phenotyping in the facilities of CULTIVARI and DOTTENFELDERHOF, including agronomic behaviour and baking quality.

3.5. Tomato: Support participatory tomato breeding in Italy and Spain

Lead: UPV (Adrián Rodríguez-Burruezo)
Project partners: RSR (and LTP Arcoiris), CREA-OF (Monsampolo del Tronto, AP),
New members/collaborators/stakeholders: ITALY: Organic farms for PPB trials Azienda Agricola Celestino Benetazzo (Padova, PD), Azienda Agricola Malavolta, (Campofilone, FM), Azienda Agricola Corrado Salvatore (Metaponto, MT), Associazione Diversamente Bio (Rubano, PD), Azienda Agricola Alle Fontanine (Sestola, MO), Azienda Agricola Di Leo Silvano (Castronuovo S.A., PZ), Azienda Agricola Battezzato Vincenzo (Campobasso, CB), Azienda Agricola Petacciato (San Giuliano di Puglia, CB),

Azienda Agricola Primo Sole (Montagano, CB), Azienda Agricola Sperimentale Didattica Pollino (ALSIA Rotonda, PZ); SPAIN: La Verde Coop. and Viviendo en el Campo (Cadiz, Andalusia) and LA UNIÓ de Llauradors and Vivers Peris (Valencia), supporting the main field trials and PPB in Spain.

Tomato is the most important fruit vegetable in Europe, Italy and Spain being the main producers of organic tomatoes. However, apart from small initiatives aimed at diversifying the genetic basis of this crop (e.g., cultivating, multiplying and exchanging landraces and ecotypes), most varieties are modern F1-hybrids, which were bred under high input conditions, with a **considerable bottleneck in terms of genetic diversity and fruit quality and taste**. Thus, on the one hand, there is a **need of high-quality cultivars adapted specifically to organic production and stress conditions**. And, on the other hand**, small initiatives aimed at preserving, recovering and improving landraces must be promoted and supported** to achieve more sustainable and resilient farming.

This subtask (T3.4.5) has included different populations and approaches in three projects, having in common organic conditions and participatory evaluation of populations. Thus, in Spain the UPV team has evaluated a **collection of 270 landraces and local varieties** in different agroclimatic conditions. In **Italy, two projects were conducted: 1) inbreeding lines from a MAGIC population under construction** (Multiparent Advanced Generation InterCross; from crossing 8 divergent parents and encompassing a range of traits) and 2) **Composite Cross Populations** (CCP) from the FP7 SOLIBAM Project based on the “Cuore di Bue” tomato type (i.e. Ox-heart), have been submitted to participatory evaluation and selection by farmers for a range of traits under organic condition in different locations across the country.

The task has involved, among others, a number of objectives:

a) To evaluate a large and diverse collection of landraces in Spain during three seasons, based on PPB for agronomic value (e.g. yield, management, farmers´ preferences, incidence of diseases) and fruit quality (mainly sugars and acids)

b) Each year, in the second half of the trials, to perform shelf life and taste evaluations on a pre-selection of landraces resulting from objective a) with consumers and retailers

c) In Italy, to evaluate with participatory approaches under organic conditions: i) the collection of MAGIC advanced lines so that these breeding lines are being selected during their breeding for adaptation to organic conditions as well as to other traits of interest (fruit quality, resistances, etc.) (CREA-OF) and ii) to evaluate and select on an experimental CCP “Cuore di Bue” (RSR and Arcoiris)

d) To develop, select and release for the organic sector seeds of improved and competitive cultivars and populations with improve fruit quality for consumers from the genetic pool of landraces and other experimental populations, contributing to the preservation of agrobiodiversity.

e) Based on the MAGIC population from objective c) DNA genotyping to find DNA fingerprinting of the best cultivars and to exploit heterosis for further breeding by planning in the near future crossings between genetically low-related parents.

f) To promote and/or reinforce participatory networks with a wide range of socio-economic actors.

4. White lupin

4.1. Methodologies

4.1.1. Original plant material used

CREA developed a new genetic base by the factorial crossing of four elite bitter-seed landraces (from Italy, Greece or Madeira) with four elite breeding lines or cultivars (from France, Italy and Morocco). Based on earlier results from previous works, the parent germplasm encompassed material that displayed adaptation to severe drought, moderately calcareous soil and low winter temperatures, favourable grain quality traits (high protein and oil; high gamma-conglutin), other positive features (e.g. good lodging tolerance), and different phenology. This genetic base was used to develop a large set of inbred lines evaluated by CREA, LBI and other non-partner institutions, as well as a composite cross population distributed to several partners at the end of the project.

Research by FiBL-CH, which focused specifically on tolerance to anthracnose, also included an additional different genetic base formed by a large set of landraces (ca 100 new accessions every year) and several cultivars and breeding lines belonging to FiBL's own genetic base, including a CCP. This CCP derives from 22 crossings between 14 parents performed at FiBL in 2015. The CCP was then developed based on the selection of 10 genotypes in the F1 and by mixing amounts of seed proportional to seed yield of each genotype.

Finally, UBIOS planned to evaluate a small set (about 20) of cultivars and breeding lines provided by CREA and Jouffray-Drillaud in a small on-farm experiment repeated for two years.

4.1.2. Breeding activities, locations and years

The inbred lines and the composite cross population developed by CREA were multiplied in non-organic fields without the use of chemical treatments (fungicides, herbicides) in the last generations of multiplication (year 2017 for inbred lines; years 2019 and 2020 for the population). Due to the number of different experiments and initiatives, test years and test locations, the methodological details are described in the corresponding results subsections. Most inbred line evaluations performed by CREA were performed in non-organic conditions, but without using chemical treatments. Inbred evaluations performed by LBI were conducted under organic conditions. The small on-farm evaluation of cultivars and breeding lines performed by UBIOS faced various problems; agronomic management problems in 2018 and 2019 because white lupin was a completely new crop to the farmers involved and COVID-19 related restrictions in 2020 because of which the trials could not be sow in due time. The anthracnose tolerance research conducted by FiBL on a different set of landraces and cultivars was performed under growth chamber and on-field conditions. The on-farm evaluations by FiBL were conducted in certified organic fields.

4.2. Main results

4.2.1. Generation of plant material (CREA and FiBL-CH)

In order to increase the genetic diversity of European white lupin breeding materials, crosses were made between 4 bitter landraces and 4 sweet breeding lines. Over 600 F_5 inbred lines, about 40 for each of the 16 crosses, were developed by CREA under insect-proof conditions to avoid outcrossing (Figure 1), with selection for low-alkaloid content performed at the F_3 stage (see below). This segregation ratio of 180 sweet lines out of the 600 (3:1) suggests that there is only one major mutant gene present. Up to 180 of these lines underwent evaluation for various traits. Concurrently, a large and constant number of F_3 sweet-seed lines per cross were used to develop a CCP with low initial level of alkaloids. However, some shift towards increasing high alkaloid content may occur for any sweet-seed population as a consequence of mutation and natural selection due to greater fitness of high-alkaloid material.

The CCP was advanced by CREA for two generations before distributing it to various partners for further adaptation to specific conditions:

- Rete Semi Rurali (for evolution under autumn sowing in farms of Tuscany, Sardinia and Sicily, in Italy);
- AGROLOGICA (for adaptation to spring-sown environments of Denmark);
- FiBL-CH (for adaptation to spring-sown environments of Switzerland);
- LBI (for adaptation to calcareous soils and spring-sown environments of the Netherlands);

- UBIOS (for developing two evolutionary populations adapted to French autumn-sown and spring-sown environments, respectively).

CREA is also using this material for producing one CCP adapted to pure stand and another CCP adapted to intercropping with cereals under autumn sowing in Northern Italy.

The distributed CCP is in general useful for breeding and/or further selection under specific stress pressures in different pedo-climatic conditions across Europe.

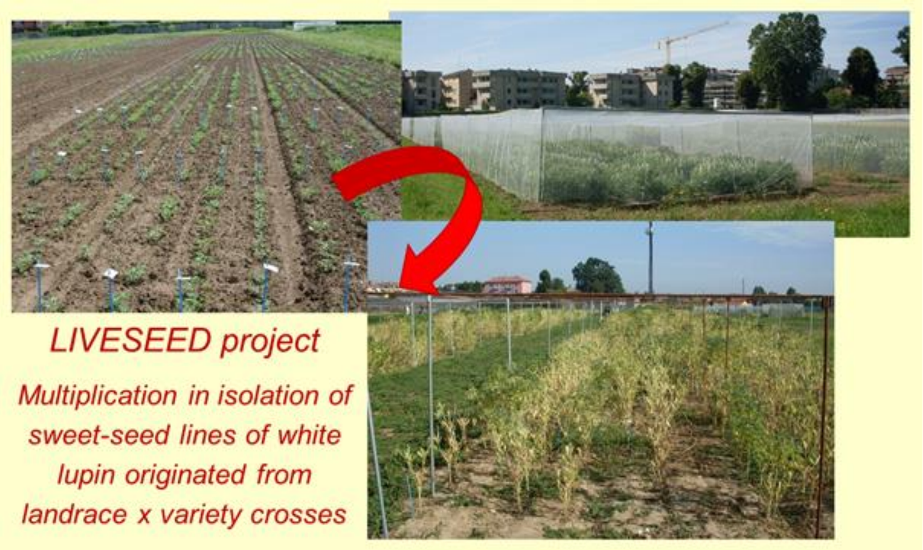

Figure 1 - Development and multiplication of inbred lines in Northern Italy

Between 25 and 60 new crosses were performed by FiBL-CH each year for an ongoing pedigree breeding program. From the F1 to the F5, the progenies are grown under tunnel for insect isolation. From F4, single plant selection is performed on-field. Field selection of breeding lines under high anthracnose disease pressure was performed each year (2017-2020). Additionally, from 2020 onwards selection under controlled conditions (Figure 2) is also included to select for lines with a reduced infection compared to the best variety currently available for cultivation in Switzerland (cv Frieda) following the methodology developed in the framework of this subtask by Alkemade et al. (2021).

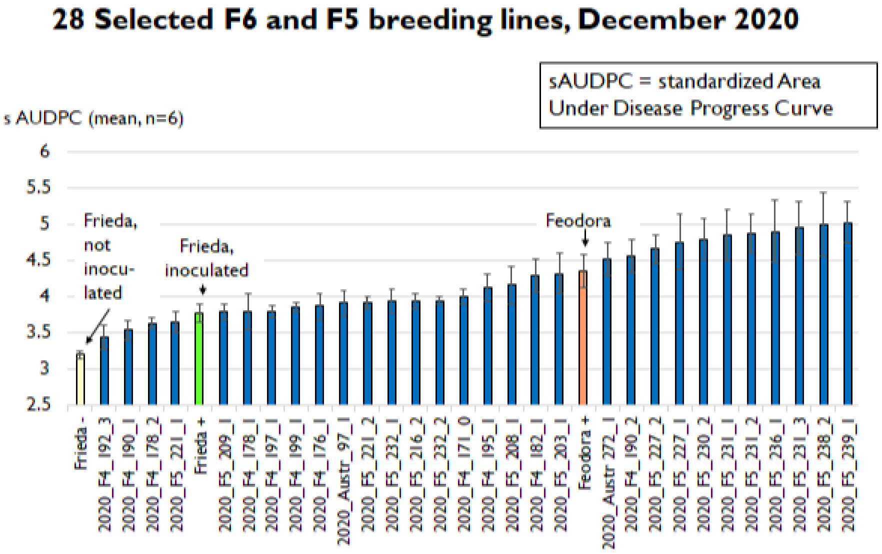

Figure 2: Test for anthracnose resistance under controlled conditions with artificial infection of 28 breeding lines from FiBL-CH pre-breeding program (cv Feodora is the susceptible check and cv Frida the resistant check)

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Currently, 14 advanced breeding lines (F5 and F6) from FiBL have been selected that will be further evaluated and multiplied in collaboration with the Swiss organic breeding organization GZPK (linked third party of FiBL-CH). In 2-3 years, the best breeding lines will go for multiplication and variety registration. The system always allows to include new genetic resources for crossing in the rolling trial and to advance with the field test of the best advanced lines in a coordinated manner between the pre-breeding project at FiBL-CH and the commercial breeding organization GZPK.

The CCP developed by FiBL-CH was cultivated on two sites in Switzerland in 2018, 2019 and 2020. On this population, selection for sweet grains was applied each year. Both yield and disease resistance in the CCP decreased over years at both locations (data not shown). This CCP derives from crosses effectuated in 2015 with little information on the anthracnose resistance of the parental lines, as the pre-breeding program on lupin started at FiBL-CH in 2014 and only 1 year of genetic resources screening was available at that point in time. A new CCP will be initiated in 2021 derived from crosses from the best genetic resources' accessions and promising advanced breeding lines selected within LIVESEED. Furthermore, given the problematic introgression of bitter types into the population derived from the 2015 crosses, the new CCP will be developed from sweet parental lines only, and will serve also as a reservoir for line selection, where it is easier to control the low alkaloid levels.

Specific considerations for CCP development of partially outcrossing species as white lupin

In contrast to CCPs developed for cereals, we faced several challenges for white lupin as a strategy for fast adaptation to local conditions. Both CCPs of FiBL and of CREA were created without prior knowledge on anthracnose resistance. Unfortunately, both CCP have no sufficient genetic diversity to allow for local adaptation and for spring sowing in Switzerland when infection pressure is high. Thus, the screening of large sets of genetic resources for anthracnose tolerance and the development of crosses with adapted sweet breeding lines was essential for developing a new CCP for Switzerland, whereas the one from CREA works fine in Italy and The Netherlands where there is no anthracnose.

However, selecting then for only sweet cultivars (homozygous recessive mutant), will reduce the progeny to 25%. Thus, large populations are needed to start selection. Since white lupin shows up to 15% outcrossing (depending on presence of insects), it is important that for further multiplication and selection of CCPs and for participatory breeding with farmers, the population is kept in geographic isolation from bitter cultivars, to avoid that the population becomes bitter. Also, populations derived from crosses of sweet lines based on different mutants will segregate in bitter and sweet progenies. Therefore, it is very important to know the genetic background of the sweet lupin lines used for CCP development (see below).

4.2.2. Drought tolerance of 140 inbred lines in a phenotyping platform (CREA)

The drought tolerance trial was sown in mid-February 2019. The evaluation of dry grain yield and aerial biomass and onset of flowering in a large phenotyping platform (Figure 3) was conducted under: (a) severe drought [from April 15, 10 mm irrigation when reaching wilting point, replenishing 15% of the maximum available water; 20 mm in total] and (b) relatively moisture-favourable conditions [from April 15, 20 mm irrigation when soil water content decreased below 60% of the maximum available soil water in the upper 40 cm, replenishing > 80% of the maximum available soil water; 330 mm in total]. The experiment was arranged in RCB design with 4 replicates for each cropping environment.

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Figure 3 - One of the two modules of the phenotyping platform used for grain yield evaluation of 140 lines under drought stress and moisture-favourable conditions (Lodi, 2019)

A large genetic variation was observed for grain yield, aerial biomass, and for onset of flowering under low and severe stress level (Table 1).

Table 1 – Mean and range values for production and phenology traits of 140 inbred lines under different drought stress levels.

	Dry grain yield (t/ha)			Arial biomass (t/ha)			Onset of flowering (dd Apr. 1)	
Stress level	Mean [a]	Range [c]		Mean [a]	Range [c]		Mean [b]	Range [c]
Low	2.28	0.48 - 4.68		9.15	3.04 - 17.88		41.5	31.3 - 52.3
Severe	0.88	0.12 - 1.63		3.15	1.08 - 5.38		38.7	30.5 - 50.5

[a] Difference between stress level means significant at $P < 0.01$. [b] Difference between stress level means significant at $P < 0.10$. [c] Difference between lines significant at $P < 0.01$.

Phenotypic and genetic correlation values indicated only moderate genotype × environment interaction for grain yield across conditions (Table 2). This was probably due to the fact that the late-winter sowing was followed by high spring temperatures resulting in poor adaptation to these conditions in those lines more adapted to autumn sowing, regardless of mild or severe stress.

Table 2 - Broad sense heritability under low and severe drought stress (H2), and phenotypic and genetic correlation between stress conditions (value ± SE), for 140 inbred lines.

	$H^2 \pm$ SE			
Trait	Low stress	Severe stress	r	$r_g \pm$ SE
Grain yield	0.69 ± 0.04	0.70 ± 0.04	0.60 **	0.84 ± 0.07
Aerial biomass	0.71 ± 0.04	0.65 ± 0.05	0.66 **	0.93 ± 0.07

** Different from zero at $P < 0.01$

Grain yield responses under severe stress could be due to stress escape responses, indicated by the regression line as a function of onset of flowering, and intrinsic stress tolerance responses indicated by high positive deviations from regression (Figure 4). The plant type needed for autumn sowing in several Mediterranean regions should combine intermediate flowering (implying moderate tolerance to low winter temperatures) with high intrinsic drought tolerance, as the inbred lines highlighted in Figure 4.

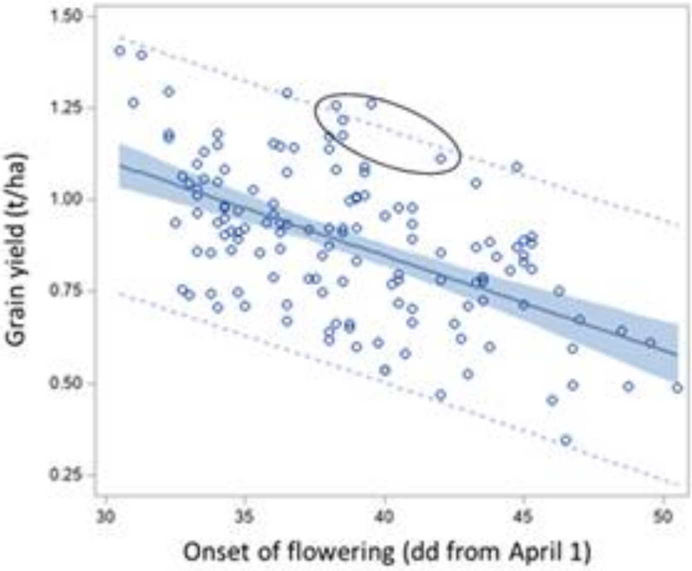

Figure 4 - Yield responses of 140 inbred lines under severe drought stress. Stress escape responses are indicated by the regression as a function of onset of flowering expresses (R2 = 0.31), whereas intrinsic stress tolerance responses are indicated by deviations.

4.2.3. Tolerance to moderately calcareous soil of 144 inbred lines under field conditions in the Netherlands and Greece (LBI; IIFC Demeter, Larissa; CREA)

The experiments were sown in autumn 2018 in Greece [with own resources] and spring 2019 in the Netherlands. The Greek environment (in conventional crop management) featured soil total lime = 6.1% and soil pH = 7.6. The Dutch environment (an organic farm) featured soil total lime = 6.4% and soil pH = 7.2. The experiment was arranged in RCB design with 3 replicates in both sites. In the Netherlands, non-ripe grains from late maturing plots were harvested all the same, to highlight tolerance responses. A 9-level visual susceptibility score, and onset of flowering, were also recorded.

On average, lupin yield was lower in Greece (where also soil pH was quite suboptimal for lupin) than in the Netherlands (Table 3). Both test sites displayed large variation among the lines for all recorded traits (Table 3).

Table 3 - Mean and range values for grain yield, lime susceptibility score (1 = no damage; 9 = high damage) and phenology traits of 144 inbred lines in two regions

	Dry grain yield (t/ha)		Susceptibility score		Onset of flowering (dd Apr. 1)	
Region	Mean	Range	Mean	Range	Mean	Range
Greece	1.01	0.08 - 1.87	6.5	3.0 - 8.2	26.5	-8.3 - 32.8
The Netherlands	2.89	1.39 - 4.90	2.7	1.0 - 5.0	85.4	75.0 - 111.3

Difference between lines significant at $P < 0.05$

The correlation between grain yield and susceptibility score was high (−0.69, $P < 0.01$) in Greece, and moderately low (−0.35, $P < 0.01$) in the Netherlands. There was large inconsistency for yield responses of the lines across the two sites (Figure 5), with no correlation between line values ($P > 0.05$), probably

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because of different climatic adaptation of the lines. However, two putatively lime tolerant lines showing high yield response in both locations could be identified.

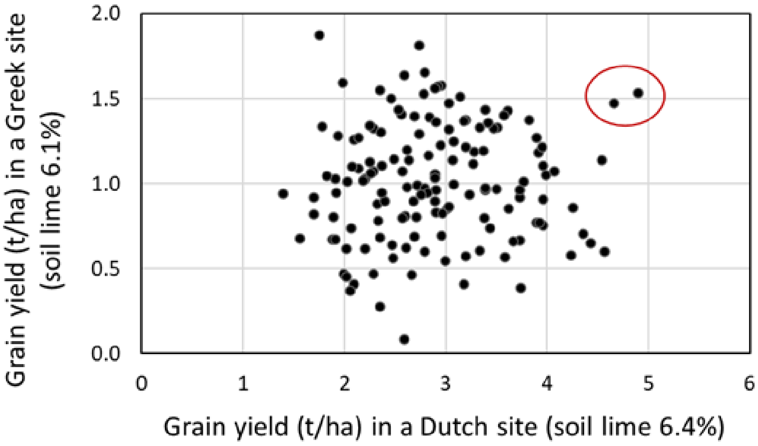

Figure 5 - Yield responses of 144 inbred lines in moderately calcareous soils of Greek and Dutch locations.

4.2.4. Grain yield of 180 inbred lines under spring and autumn sowing in Northern Italy (CREA)

The experiments were sown in February 2018 for the late-winter sowing (RCB experimental design with three replications) and in October 2018 the autumn sowing (RCB experimental design with two replications). The trials were sown on non-organic fields, and managed without chemical pesticides and fertiliser. Criteria recorded were grain yield and onset of flowering.

A large genetic variation was observed for both traits (Table 4). However, the adaptive responses of the lines across sowing times were highly inconsistent (hence, there was a large genotype × environment interaction), with no genetic correlation (Table 5), despite the occurrence of a mild winter for the autumn sowing that caused no winter plant mortality. Late-winter sowing emphasized the phenological differences, with material that needed vernalization that flowered very late and underwent severe terminal drought (implying early flowering to be important to escape terminal drought).

Table 4 – Mean and range values for grain yield and phenology traits of 180 inbred lines under different drought stress levels

	Dry grain yield (t/ha)		Onset of flowering (dd Mar. 1)	
Sowing time	Mean [a]	Range [b]	Mean [a]	Range [b]
Autumn	4.18	0.16 - 9.38	33.80	29.0 - 41.5
Spring	2.61	0.04 - 6.00	28.30	15.7 - 45.0

[a] Difference between sowing times significant at $P < 0.05$. [b] Difference between lines significant $P < 0.01$

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Table 5 - Broad sense heritability under autumn and spring sowing, and phenotypic and genetic correlation between sowing times (value ± SE) for grain yield of 180 inbred lines

	H^2 ± SE			
Trait	Autumn sowing	Spring sowing	r	r_g ± SE
Grain yield	0.43 ± 0.07	0.70 ± 0.05	-0.04	0.00 ± 0.13

Correlation of grain yield with onset of flowering highly negative (−0.66, $P < 0.01$) under spring sowing, nil (0.01) under autumn sowing.

The results highlighted not only the need for specific material selected for the two sowing times but also the large variation in adaptive responses of the test lines – variation that is expected also within the composite cross population (which derived from the same genetic base) and which is very important to obtain CCPs specifically adapted to sharply different climatic regions. This is the reason why the composite cross population was distributed to different pedoclimatic and growing conditions.

4.2.5. NIRs results relative to protein and fat grain content of 180 inbred lines under spring and autumn sowing in Northern Italy (CREA)

The test material for this experiment included the best-yielding lines that were evaluated for grain yield under both spring and late-winter sowing in Northern Italy (see 4.2.4). The analysis was performed on grain samples collected in each plot, analysing 20 seeds per plot. Additionally, 145 grounded seed samples underwent chemical analysis of protein content (Dumas' method) and oil content (Soxhlet's method). All seed samples underwent NIRS evaluation (by a NIRFlex N500 equipment) performed by averaging results of spectra for the individual seeds. NIRS predictions were highly reliable for protein content and moderately reliable for oil content (Table 6). Calibrations were applied to all seed samples, to estimate their values for quality traits.

Table 6 – R2 value based on cross-validations and Ratio of Prediction to variation (RPD) of NIRS calibrations for two grain quality traits.

Trait	R^2	RPD
Protein content	0.911	11.2
Oil content	0.780	4.5

Grain protein content and oil content were characterized by large genetic variation (Table 7), described by high broad-sense heritability in single experiments (Table 8), and high consistency of line response across contrasting environments (as displayed by high genetic correlation values: Table 8). All of these results are favourable for selection of these traits.

Table 7 - Mean and range values for grain protein content and oil content of 155 inbred lines averaged across two sowing times.

Trait	Mean	Range
Protein content	39.6	32.8 - 43.2
Oil content	9.3	7.3 - 11.5

Difference between lines significant at $P < 0.01$

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Table 8 - Broad sense heritability under autumn and spring sowing, and phenotypic and genetic correlation between sowing times (value ± SE), for two grain quality traits of 155 inbred lines.

	$H^2 \pm SE$			
Trait	Autumn sowing	Spring sowing	r	$r_g \pm SE$
Protein content	0.72 ± 0.04	0.79 ± 0.03	0.56 **	0.80 ± 0.06
Oil content	0.81 ± 0.03	0.92 ± 0.01	0.80 **	0.94 ± 0.03

** Different from zero at $P < 0.01$

Also favourable from a breeding point of view was the finding that protein content and oil content were not correlated genetically neither between themselves nor with grain yield (Table 9). On the whole, the combined selection for grain yield and grain quality seems quite feasible, especially for protein content (whose prediction by NIRS was highly reliable).

Table 9 - Phenotypic and genetic correlation (value ± SE) between grain protein content, grain oil content and grain yield of 155 inbred lines under different sowing times.

Traits	Sowing time	r	rg ± SE
Protein content – Oil content	Autumn	0.13	0.19 ± 0.10
Protein content – Oil content	Spring	-0.02	0.09 ± 0.10
Protein content – Grain yield	Autumn	0.15	0.25 ± 0.15
Protein content – Grain yield	Spring	0.12	0.12 ± 0.11
Oil content – Grain yield	Autumn	-0.11	-0.11 ± 0.12
Oil content – Grain yield	Spring	0.18 *	0.22 ± 0.10

* Different from zero at $P < 0.05$

4.2.6. NIRs results relative to separation of (bitter-seed) landraces and (sweet-seed) breeding lines (CREA)

A study was performed on seed samples of: (i) two to four seed samples for each of the 97 landraces (known to have high alkaloid content from earlier studies) and 12 commercial cultivars, with two replicates per seed sample; (ii) 182 breeding lines evaluated in 2018 and 163 lines evaluated in 2018-2019 (all initially selected for low alkaloid content), with separate seed samples for primary and secondary inflorescences and two replicates per seed sample.

The results indicated good NIRS-based discrimination of high-alkaloid landraces (in red) from expected sweet-seed lines (in green) (Figure 6). Interestingly, the samples in grey colour in Figure 6, related to seed samples of the old variety *Multitalia*, which is known to have become partly bitter after many cycles of multiplication, were partly classified as bitter-seed material. Sweet seed breeding lines above the threshold line of Figure 6 will undergo chemical analysis, to verify whether they really have at least some seed with alkaloids. Such increased level of alkaloids in supposed sweet genotypes could result from i) outcrossing, ii) the effect of minor or modifier genes, and/or iii) environmental growing conditions, which can influence the absolute alkaloid content.

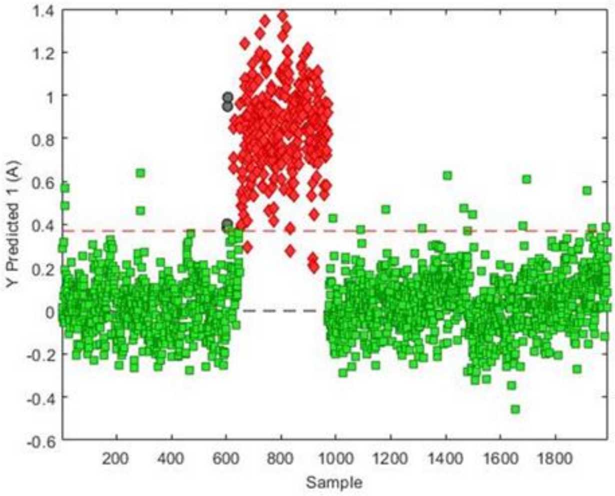

Figure 6 – NIRS-based discrimination of bitter-seed landrace grains and sweet-seed breeding line and variety grains.

Present recommended threshold for alkaloids in food (0.02%) and feed (0.05%) cannot always be met. Until now, the exact quantification by chemical analysis is very cumbersome and expensive. Therefore, it would be a great advantage if the differentiation for low alkaloid content within sweet lupin cultivars could be achieved with NIRS.

Due to the covid situation, an unexpected delay occurred, and the work is still in progress to analyse chemically the alkaloid content of 170 breeding lines and 10 parent cultivars or varieties that were evaluated under spring sowing in 2018. This would also allow to map the major and minor genes for alkaloid content and confirm if the sweet parental lines are derived from the same mutant, or if pyramidizing different mutants are necessary to meet the thresholds. In any case, outcrossing must be avoided between sweet and bitter cultivars but also between different sweet cultivars or populations carrying different types of mutants to avoid shift towards bitterness. Cost efficient NIRS measurements allow to monitor the alkaloid level of populations and lines across generations and locations.

4.2.7. Screening of genetic resources for anthracnose resistance (FiBL-CH) in field and controlled conditions

From 2017 till 2020, each year ca 100 new white lupin accessions from different seedbanks were tested in Switzerland in a certified organic farm for anthracnose resistance in single rows plots between infection rows of the susceptible cultivar Amiga (Figure 7). The relative disease scoring was assessed and used to select the most tolerant accessions to be included in a pool of 100 to 200 selected accessions also tested in the same set-up and updated each year to include the best accessions.

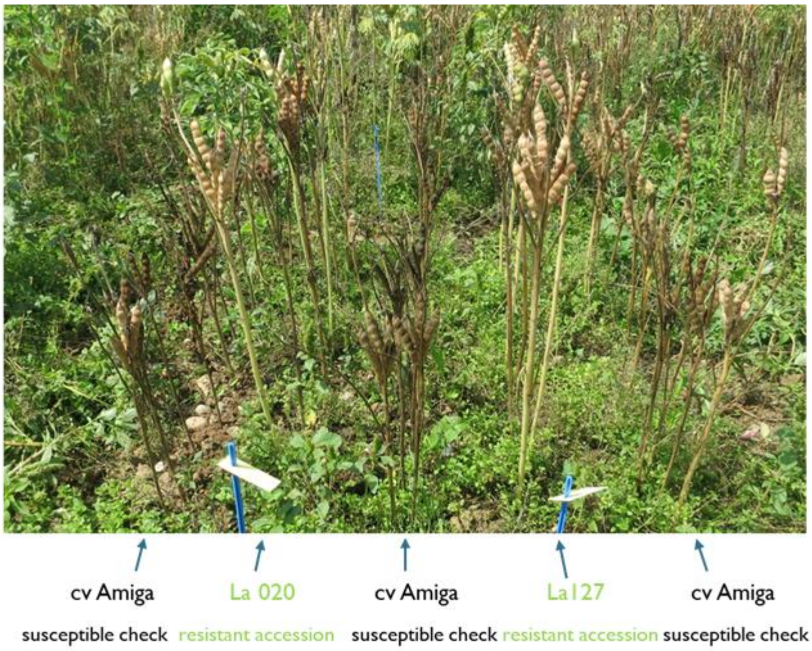

Figure 7 – Example of small plot on-field anthracnose resistance scoring in Switzerland. In black the susceptible check cultivar Amiga and in green two of the genetic resources accessions included in the trial that resulted resistant in the test

This screening allows to develop a pool of accessions to be used as a base for the crossings in the breeding program at FiBL-CH and to be exchanged and shared with other organic breeders working on white lupin (Figure 8). For example, from the comparison of the relative anthracnose disease score (relative to the 0 value of the susceptible cv Amiga, a positive value means a more susceptible cultivar and a negative value means a more resistant cultivar) in 2019 between the new genebank accessions and the selected pool from the previous years (Figure 8), it emerged that the selected genepool includes more lines with increased resistance (negative value).

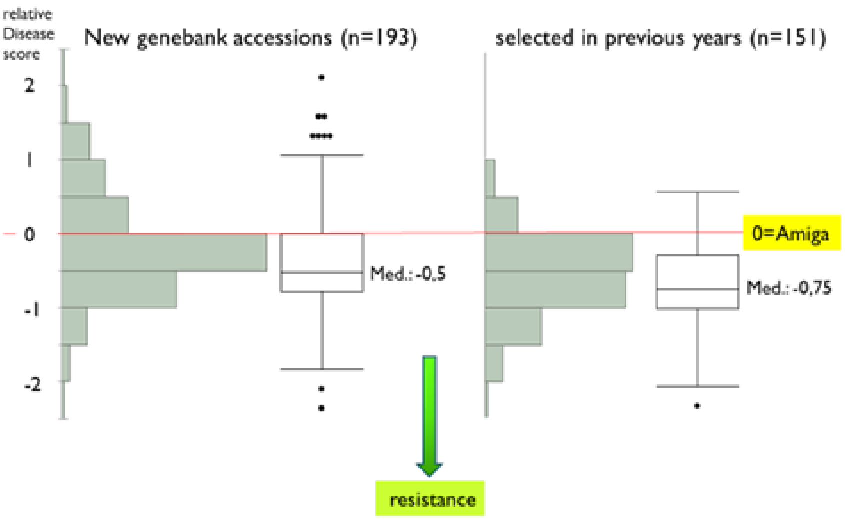

Figure 8: Screening of genetic resources for anthracnose resistance at FiBL: comparison of the distribution of the relative disease score (0= to value of susceptible cultivar Amiga) between the new genetic resources pool (accessions cultivated for the first time in 2019) and the selected pool of accessions (genetic resources pool selected from the previous years)

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In order to speed up the disease scoring and increase the reliability of the data collected, FiBL-CH developed a high-throughput phenotyping method to identify anthracnose resistance in white lupin accessions. The methodology is a stem inoculation-based disease phenotyping under controlled conditions which allows for time-effective identification of field-relevant resistance which can now be applied to further identify sources of resistance and their underlying genetics. With this phenotyping method, the plants are classified into 5 classes of susceptibility (Figure 9). Susceptibility responses in the artificial conditions displayed high correlation with responses under field conditions observed in a subset of genotypes (Alkemade et al., 2021). The methodology was used in the project and it is promoted for further use by other organizations that work on anthracnose resistance in white lupin. A tutorial video has been produced to promote this new phenotyping methodology.

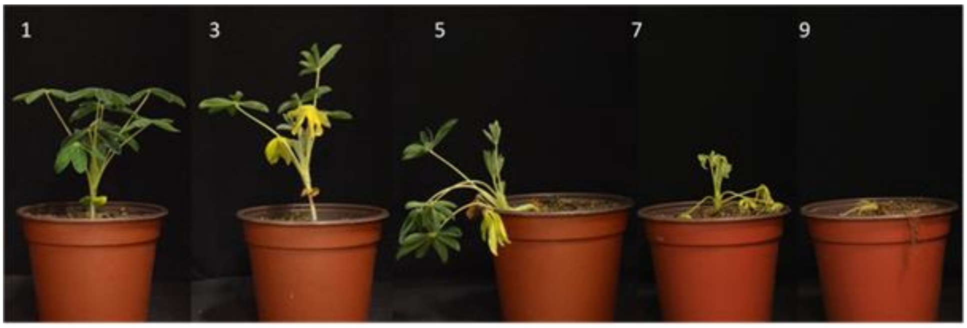

Figure 9 – Anthracnose susceptibility responses ranging from tolerant (1) to highly susceptible (9)

The 8 parental lines used by CREA to develop the 180 inbred lines and CCP were tested for anthracnose resistance with this methodology. The results showed that they were very susceptible to the disease. Due to this lack of genetic variation of the 8 parental lines by CREA for anthracnose tolerance, CREA provided genetic resources to FiBL with expected resistance based on genotypic data (QTL selection and genomic selection based on a recent study (Książkiewicz et al. 2017) to see if these QTL could be validated. However, there was no correlation when tested under controlled and field condition (Alkemade et al. 2021). Therefore, FiBL tested the diversity and virulence of different *Colletotrichum* strains of Switzerland compared to global strains to see if that was an explanation (Alkemeade et al. under review). In order to identify resistance genes relevant under the Swiss conditions, FiBL phenotyped 200 genotypes (159 of CREA and 41 of FiBL) for anthracnose resistance under controlled conditions. These lines showed good segregation for the trait. The phenotypic data collected in this last trial was used for the GWAS analysis to identify molecular marker-trait associations for anthracnose resistance.

4.2.8. Genotyping (CREA and FiBL-CH)

Overall, 598 DNA samples were genotyped with GBS using *Apek*I as the restriction enzyme. These samples included:
- 400 of the 640 sweet-seed inbred lines by CREA;
- 12 inbred lines from LBI;
- 20 inbred lines from INIA (Chile);
- 125 landrace genotypes;
- 41 additional lines by FiBL-CH.

The genotypic analysis of the 41 additional lines by FiBL-CH was delayed due to COVID-19 by half a year. The SNP calling was performed on the recently published lupin genome. Over 4,000 polymorphic SNP markers have been obtained for further GWAS and genomic selection studies (Table 10).

Table 10 – Number of SNP markers for two data sets (for the threshold of 10% genotype missing data).

Data set	No. of SNP markers
Sweet-seed lines (for on-going analysis of inbred lines of up to 180 genotypes by the CREA)	4,309
Landraces and varieties (for anthracnose analyses by FiBL of 174 genotypes)	4,849 [a]

[a] Reduced to 4,603 after various quality filter steps

4.2.9. GWAS analysis for anthracnose resistance (FiBL-CH and CREA)

The GWAS analysis was conducted on 174 genotypes of the lines screened under controlled conditions with artificial infection by a Swiss strain of the pathogen with the method developed by Alkemade et al. (2021) and genotyped with GBS. The GWAS identified significant associations of three SNP markers with disease score and relative shoot fresh weight. The identified SNP markers were found within candidate genes suggested to be involved in biotic and abiotic stress in the family *Fabaceae.* A scientific paper including all the information about the MTAs detected will be submitted before the end of the project.

The SNP markers for quantitative trait loci (QTL) determined in this GWAS analysis will be converted into KASP markers for marker-assisted selection in segregating populations derived from tolerant parental lines by FiBL-CH to allow marker assisted selection (MAS) on anthracnose resistance in white lupin.

4.2.10. Genomic selection and GWAS for production and grain quality traits (CREA)

This activity was delayed due to COVID-19 and is expected to be completed by the end of June and, in any case, before the end of the project. Identification of QTLs for low alkaloid content of different mutants will help to test if parental lines used for CCP are based on the same mutation. This will help to minimise segregation for bitter types in successive generations of sweet CCPs. In addition, it has to be controlled that the CCP is grown in geographic isolation to bitter landraces and sweet cultivars with other genetic background to avoid cross pollination into the evolutionary population.

4.3. New cultivars & breeding material for potential further use

The following white lupin breeding materials are planned to be released and/or further used for by the partners in LIVESEED subtask 3.4.1 (Table 11)

Table 11. Summary of exploitation plans for breeding materials from the LIVESEED subtask 3.4.1 on white lupin (Lupinus albus)

Partner	Planned cultivar release	Planned year of release	Further use of the material
CREA	Inbred lines: few inbred lines have high drought tolerance, and two have good adaptation to calcareous soil (to be confirmed). All selected inbred lines have low alkaloid content	By 2023-24: identify one or two elite inbred lines to be proposed for variety registration	Further evaluation and selection (for lines already tested or never tested yet). Definition and validation of molecular marker-based breeding tools (e.g., genomic selection for specific traits)

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CREA	CCP: it has broad geographic base; inter-crossing between traditional landraces and improved cultivars; low alkaloid content selection.	2020: distribution of CCP to several organizations in Europe and Chile	Developing CCP adapted to pure stand and mixed stand in Northern Italy (CREA, by core budget), and other populations under development by other institutions. At least for Italy, a registration as OHM of few populations (in collaboration between CREA and Rete Semi Rurali) is envisaged after a period of further assessment of their agronomic value
LBI	Inbred lines: sweet lupin candle types, suitable for mixed cropping and suitable for calcareous soils under Dutch short season conditions. The activities of LBI on plant breeding are limited to the pre-commercial stages. For the introduction of the lupin lines as new cultivars, LBI cooperates with the breeder company Nordic Maize Breeding. This small breeder organization will use lines to develop options for mixed cropping with maize. The mixed lupin / maize crop is used as whole crop silage for ruminant animal feed.	2021-2023: follow-up evaluation By 2025: at least 1 line ready for registration	Continue the field evaluation of the lines to develop one or more lupin varieties that are suitable for mixed cropping with maize
FiBL-CH	14 advanced breeding lines of spring-sown white lupin with good anthracnose resistance have been developed. Among the developed lines, 1 is very sweet and promising for food production use. These advanced breeding lines will be further developed together by GZPK (third linked party of FiBL-CH) and common submission for registration of first candidate is foreseen in 2-3 years.	By 2025: obtain 1-2 lines ready for formal registration By 2028-2030: start seed marketing of new varieties	Further use of the advanced breeding lines developed in the ongoing pre-breeding programme for the development of inbred lines and CCPs

4.4. Multi-actor activities: networks developed and participatory activities

In this subtask the networking activity was prominent at the level of research institutes. The evaluation trials were carried out not only by project partners but also by various non-partner public or charity research institutes, such as the James Hutton Institute (Dundee, UK), the Institute of Industrial and Forage Crops of DEMETER (Larissa, Greece) and the Instituto Nacional de Investigación Agropecuaria (INIA, Carillanca, Chile). In the network both organic and conventional oriented research institutes were involved.

Joint research was set up or genetic material was provided for anthracnose screening by several national genebanks and breeders (e.g. Eric von Baer in Chile, LfL and DSV in Germany).

Although participatory evaluation with farmers was not included in the white lupin activities, FiBL-CH and GZPK conducted several farmers field days to promote lupin as a new or reintroduced crop in Switzerland and produced articles on farmers' magazines and dedicated videos (on lupin cultivation and lupin as alternative grain legume).

5. *Brassica* crops

5.1. Methodologies

5.1.1. *Brassica* crops evaluated in trials

Over the four years of the project, the *Brassica* crops kohlrabi, broccoli, cauliflower and different types of head cabbage were evaluated in trials. These crops were selected according to the following criteria: (1) the availability of advanced breeding material from organic breeding initiatives and (2) the need for identifying a set of cell-fusion free varieties that allows a year-round production in Southern Europe (during winter) and Northern Europe (during summer). Besides organic population varieties, cell fusion-free F1-hybrids were used as commercial standard varieties. The complete list of F1-hybrids and open pollinated (OP) cultivars tested from autumn 2018 to spring 2021 is included in Table 12.

Table 12 - List of cultivars and corresponding breeder tested within the Brassica trial network (for convenience, only the second part of Kultursaat's labels is used later in the report).

Species	Cultivar	Breeder	Species	Cultivar	Breeder
Kohlrabi	**Azur Star**	Sativa	Cauliflower	**Skywalker F1**	Bejo
Kohlrabi	**Dario (Sat22)**	Sativa	Cauliflower	**Liria F1**	Bejo
Kohlrabi	**Enrico (Sat21)**	Sativa	Cauliflower	**Nuage**	Kultursaat
Kohlrabi	**Fridolin** (KS-KOK-**JJ-Tre37)**	Kultursaat	Cauliflower	**Amabile** (KS-KOB-**MG-AMB)**	Kultursaat
Kohlrabi	**Korist F1**	Bejo	Cauliflower	KS-KOB-**JJ-SG**	Kultursaat
Kohlrabi	**Kordial F1**	Bejo	Cauliflower	**Daniel** (KS-KOB-**JJ-REV)**	Kultursaat
Kohlrabi	KS-KOK-**CHE-Soko**	Kultursaat	Cauliflower	**Tabiro**	Kultursaat
Kohlrabi	**Lippe F1**	Rijk Zwaan	Cauliflower	**Daniel**	Kultursaat
Kohlrabi	**Orinoko** (KS-KOK-**JJ-NOR)**	Kultursaat	Cauliflower	**All-Year Cauli**	Organic Seed Alliance
Kohlrabi	**Quickstar F1**	Sakata	Pointed cabbage	**Berns**	Kultursaat
Kohlrabi	**Rasko**	Kultursaat	Pointed cabbage	**Cape Horn F1**	Beringmeier
Kohlrabi	**Sat79**	Sativa	Pointed cabbage	KS-KOW-**UB-Bernhard-8G**	Kultursaat
Kohlrabi	**Sat80**	Sativa	Pointed cabbage	KS-KOW-**UB-Charleston Wakefield-8G**	Kultursaat
Broccoli	**Belstar F1**	Bejo	Pointed cabbage	KS-KOW-**UB-Sappemeerse-7G**	Kultursaat
Broccoli	**Calinaro**	Kultursaat	Pointed cabbage	**Eersteling**	BSAG
Broccoli	KS-BRO-**CHE-Balimo B**	Kultursaat	Red cabbage	**Resima F1**	Rijk Zwaan
Broccoli	KS-BRO-**CHE-GRE-A-niedrig**	Kultursaat	Red cabbage	Rodynda	Kultursaat
Broccoli	KS-BRO-**CHE-GRE-A-aufrecht**	Kultursaat	Red cabbage	KS-KOR-**CHE-Autoro NB**	Kultursaat
Broccoli	**Covina F1**	Bejo	Red cabbage	KS-KOR-**SV-MalaBa**	Kultursaat
Broccoli	**Marathon F1**	Sakat	Red cabbage	KS-KOR-**SV-Rouge gros**	Kultursaat
Broccoli	**Parthenon F1 CMS**	Sakat	Red cabbage	KS-KOR-**JF-Dän.Steinkopf**	Kultursaat
Broccoli	**Rasmus**	Kultursaat	Red cabbage	KS-KOR-**SV-Braso**	Kultursaat
Broccoli	**Sat32**	Sativa	Red cabbage	**GranitexRedDrumHead F4**	Organic Seed Alliance
Broccoli	**Sat62**	Sativa	Red cabbage	**RedDrumHeadxGranite F4**	Organic Seed Alliance
Broccoli	**sg531**	saat:gut	White cabbage	**Kalorama F1**	Rijk Zwaan
Broccoli	KS-BRO-**TH-Lim 20/68**	Kultursaat	White cabbage	KS-KOW-**CHE-KleinerWeisskohlF6**	Kultursaat

Broccoli	KS-BRO-**TH-Lim 37/59**	Kultursaat	White cabbage	KS-KOW-**CHE-MarnerLagerElite19**	Kultursaat
Broccoli	**Thunder Dome F1**	Takii	White cabbage	**Dottenfelder Dauer**	Kultursaat
			White cabbage	KS-KOW-**CHE-Lennox NB**	Kultursaat
			White cabbage	KS-KOW-**JF-Holt**	Kultursaat
			Savoy cabbage	**Smaragd**	Kultursaat
			Savoy cabbage	KS-KOI-**TH-Marner Grünkopf**	Kultursaat
			Savoy cabbage	KS-KOI-**TH-Alpwirsing**	Kultursaat

5.1.2. Methods

The main activities in this subtask included the field evaluation of the different *Brassica* crops, often on a participatory basis together with farmers. For each crop, evaluation templates were developed collectively by the project partners. The templates included parameters to be recorded after germination and planting, during vegetative growth and at harvest. They also included the possibility to differentiate between different types, as is sometimes necessary for open pollinated varieties. As an example, the evaluation scheme for broccoli is shown in Table 13, the full sampling templates for each crop are in Annexes 1-4.

Table 13 – Example (integrated scheme) of the traits included in the evaluation templates of brassica: broccoli. Parameters in green were compulsory, whereas those in yellow were optional.

At sowing/planting	During vegetative development	At harvest	
Number of seeds seeded	**Vigor/ vegetative development** *(1-5 vigorous)*	**Beginning of harvest** *[date]*	**Number of heads harvested**
Number of seedlings germinated	**Homogeneity of foliage** *(1-5 homogeneous)*	**50% of harvest** *[date]*	**Weight of heads harvested (g)**
Remarks on seeds	**Homogeneity of heads** *(1-5 homogeneous)*	**End of harvest** *[date]*	**Number of heads (1st choice)**
Number of plants planted	**Colour** *[light lettuce green to dark cucumber green, with shade of blue or grey]*	**Nb of harvests**	**Weight of marketable heads (1st choice) (g)**
	Leaf shape *(1 spread to 5 erected)*	**Pictures** *(recommended at 50% of harvest)*	**Number of marketable heads (2nd choice)**
	Leaf surface *(1 smooth to 5 curly)*	**Colour (light, middle, dark green, with shade of blue or grey)**	**Weight of marketable heads (2nd choice) (g)**
	Disease/Pest Description *(disease/pest name if present)*	**Shape** *(1 flat to 5 round, ideal=3)*	**Number of non marketable heads**
	Score *(1 very infested to 5 nothing)*	**Regularity** *(1 irregular, like 10 ping pong balls to 5 very regular, like one balloon)*	**Weight of non marketable heads (g)**
	Disease/Pest Description *(disease/pest name if present)*	**Ramification** *(1 low to 5 high)*	**Why not marketable?**
	Score *(1 very infested to 5 nothing)*	**Compactness** *(1 large to 5 short)*	
		Buds *(1 very small to 5 very big, ideal=3)*	

An important addition were various analyses for traits related to flavour (Figure 10), conducted by Universitat Politècnica de València (UPV):
- HPLC analytics for the levels of sugars in kohlrabi;
- GC/MS volatile profile in cultivars of different crops, as a key factor in the flavour of *Brassica*;

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- Organoleptic taste panels in kohlrabi and cabbages (e.g. sweetness, flavour intensity, pungency, juiciness and texture).

Figure 10 – Preparation of brassica samples for taste panel and analytics at UPV labs.

The main partners involved in the participatory activities were:

- Organic breeding initiatives and companies: Kultursaat, Sativa, Sementes Vivas/Living Seeds, Vitalis;
- Universities and Research Institutes: UPV, ITAB;
- A range of organic vegetable producers and farmers in Germany, Italy, Spain, Portugal, France, Switzerland and The Netherlands;
- The German umbrella organization of organic traders: BNN.

Locations involved several farms in the areas indicated in Figure 11, in seasons 2018/19 (to a lesser extent), 2019/20 and 2020/21, with most trials conducted in France, Italy, Portugal and Spain.

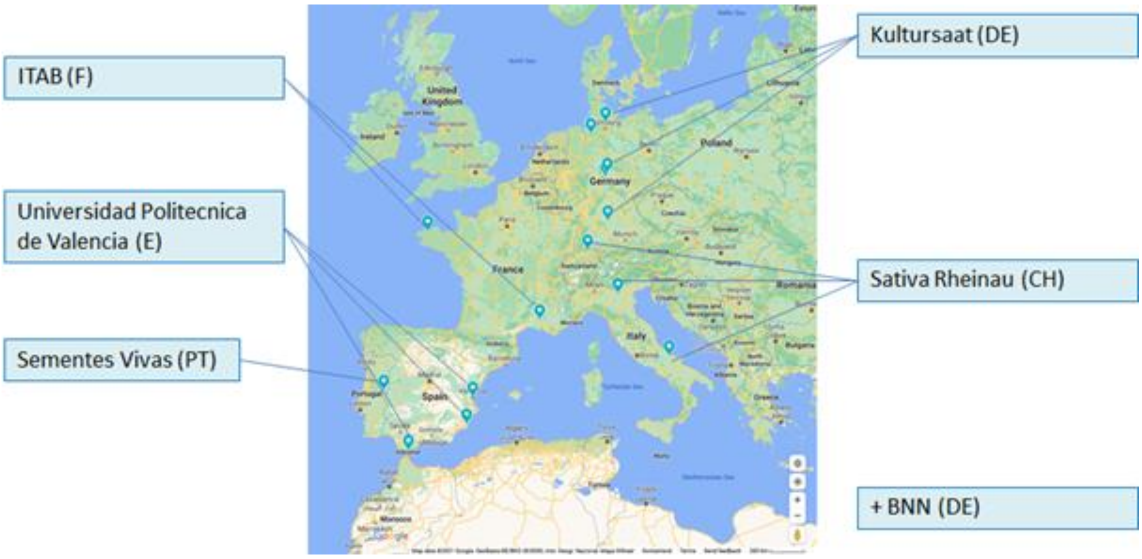

Figure 11 – Countries and locations involved in the Brassica participatory trial network

5.2. Main results

5.2.1. Kohlrabi trials

In the kohlrabi trials, 10 organic OP cultivars originating from Sativa's and Kultursaat's breeding pipelines were compared to different cell-fusion free F1-hybrid cultivars. These F1-hybrids were chosen as they are the standard cultivars used in organic production in the respective locations.
The partners have trialed the kohlrabi cultivars across a broad range of environments: the trials took place at different locations, with different planting times and under both plastic tunnel and open field conditions. Of the 14 trials, only two were discarded because of bad trial conditions. It was shown that kohlrabi is very much suited to production under plastic tunnel where the growing conditions can be well controlled. Indeed, high variations in rainfall can lead to splitting, making the bulbs unmarketable.

Figure 12 – Kohlrabi field trials 2020-21, evaluations at harvest and lab pictures in Valencia (UPV).

Regarding kohlrabi's performance, growing time is an important criterion: the faster the variety (e.g., vigorous growth) and more concentrated production (e.g. short harvesting window), the better for producers. Weight plays a minor role, as kohlrabi is usually sold per piece. Nevertheless, too big diameters should be avoided as some big producers, focused on export markets, prefer medium size bulbs for marketing reasons. This illustrates the importance of a uniform growth for some growers and traders. Throughout the trials, the following patterns could be identified (Table 14):

- Most of the time, even if not always, the (cell-fusion free) F1-hybrids showed a vigorous growth, a fast development and a high marketable yield. *Lippe F1* (Rijk Zwaan) was the cultivar with the highest marketable yield in most of the locations. *Korist F1* (Bejo) performed quite differently over the locations.
- Some of the OP cultivars competed quite well with the cell fusion free F1 hybrids. The more promising OP cultivars were *Enrico* (Sativa), *Dario* (Sativa), *Sat79 Hetero* (Sativa) and *Fridolin* (Kultursaat). Indeed, they showed a quite consistent performance over all sites. Soko (Kultursaat) and Rasko (Kultursaat) performed poorly over all sites. They were reported to germinate less and, probably, this issue partly provoked that they showed a lower vigour and were much slower than the other cultivar

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Table 14 – Overall performance of the kohlrabi cultivars, expressed as part of marketable yield (% of evaluated plants) or as ranking among the cultivars included in the specific trial (PT: plastic tunnel, OF: open field)

	Partner	**Sativa**	**LSSV**	**Sativa**	**UPV**	**LSSV**	**LSSV**	**UPV**	**GRAB**	**PAIS**	**PAIS**	**Sativa**	**UPV**	**LSSV**	**LSSV**
	Planting	13.09.2018	22.09.2018	10.09.2019	15.10.2019	22.10.2019	29.10.2019	28.11.2019	02.02.2020	27.02.2020	06.05.2020	24.08.2020	25.11.2020	14.10.2020	28.10.2020
	Harvesti	08.11.2018 (56 DAP)	08.02.2019 (139 DAP)	13.11.2019 (64 DAP)	04.-25.01.2020	05-26.02.2020	13.02-12.03.2020	17.02-02.03.2020	13.04.2020 (70 DAP)	28.04-18.05.2020	09.07-15.07.2020	8-9.09.2020	05-23.03.2021	18.02-04.03.2021	25.02-04.03.2021
	Location1	Verona (PT)	Idanha-a-Nova (OF)	Verona (PT)	Coop. SURINVER, Murcia (OF)	Idanha-a-Nova (OF)	Idanha-a-Nova (OF)	Coop. SURINVER, Murcia OF)	GRAB, Provence (PT)	PAIS, Bretagne (PT)	PAIS, Bretagne OF)	Verona (PT)	Pobla Vallbona (valencia) (OF)	Idanha-a-Nova (OF)	Idanha-a-Nova (OF)
	Country	Italy	Portugal	Italy	Spain	Portugal	Portugal	Spain	France	France	France	Italy	Spain	Portugal	Portugal
	Evaluation type	1-day	1-day	1-day	whole harvest (2-3x)	whole harvest (4x)	whole harvest (4x)	whole harvest (2-3x)	1-day	whole harvest (4x)	whole harvest (2x)	1-day harvest	whole harvest (3x)	whole harvest (3x)	whole harvest (2x)
	Trial quality	5	2	5	5	4	4	5	5	5	2	5	5	4	4
Variety	**Origin**														
Korist F1	Bejo	53%(1)	X(3)		1(2)	84%	72%	3	1	88%	X		63%		
Kordial F1	Bejo				1			1							
Lippe F1	Rijk Zwaan	64%	X	96%		92%			2	93%	X	1	89%	89%	93%
Quickstar F1	Sakata	71%	X			59%	57%		1	87%	X		69%		
Enrico (Sat21)	Sativa	63%	X	87%		97%	77%		3	100%	X	2	72%	76%	80%
Dario (Sat22)	Sativa	63%	X			72%			2	99%	X		72%		
Rasko	Kultursaat	61%	X		*discarded*	48%	29%	4	2	88%	X		0%		
Orinoko	Kultursaat	33%	X		3	56%		2	1	50%	X		31%		
Fridolin	Kultursaat	34%	X		3	71%	66%	2	2	98%	X		40%	73%	81%
Soko	Kultursaat	27%	X		4	32%		3	4	65%	X		0%		
Sat79 hetero	Sativa	61%		83%	2	82%		1	3	98%	X	3	68%		
Sat79 homo	Sativa			82%	4	91%		3	1	100%	X				
Sat80	Sativa			85%								2	65%		
Azur Star	Sativa			82%								2	67%		

1 For a better and more simple comparison among cultivars within each trial the percentage of marketable yield is showed as an accurate measurement of the cultivar´s utility. 2 In some trials, marketable yield data were not available or could not be recorded and, therefore, a comparative scale based on the different parameters evaluated is showed (with 1= best of trial,2= good performance, 3= acceptable performance, 4= suboptimal performance, 5= worst in trial, not usable). 3 X means that despite trials were conducted up to harvest, they performed very poorly because of difficult conditions (weather, soil, etc.) and, therefore, data were not included to ensure that overview comparison was based on well performed trials.

In the analyses performed at UPV, the cultivars evaluated also showed a remarkable variation in terms of sugar composition, which can be considered an added value. In all the cultivars, monosaccharides are predominant, with levels 2/3-fold the levels of sucrose. In all the cultivars, glucose was the most abundant sugar, ranging from 25 to 34 g/L, with slightly lower levels of fructose, which were comprised between 22.4 and 31.2 g/L (Table 15). Levels of sucrose were considerably much lower, ranging from 8.1 to 13.5 g/L. In comparison, the cultivars *Soko*, *Rasko* and *Kordial F1*, followed to a lesser extent by *Sat79 hetero*, showed in general the highest levels, with total sugars > 70 g/L (Table 15).

The analysis of volatiles performed by GC/MS confirms this variation. The set of cultivars analysed also showed a considerable diversity in the composition of the volatile fraction (Figure 13), which is key for the aroma but also for the perception of "taste" (actually flavour) during mastication and eating as observed in the panel tests (Table 16). The studied varieties showed a wide variation in their profiles of volatiles, including derivatives from isothiocyanates, as expected in *Brassica spp* and *Brassicaceae* crops in general, which are the main responsible for the sulphurous, sometimes pungent and mustard flavour in these crops.

These results indicate that a wide variation in sugars and volatiles can be found in kohlrabi, whose combination is key for consumers´ preferences. In the next steps, comparative analyses with the results from our taste panels may offer some clues about consumers´ preferences and, therefore, which compounds must be paid attention to for a selection more adapted to consumers´ preferences.

Table 15 – Content in the main sugars by HPLC from eight kohlrabi varieties from the 2019/20 evaluations in Spain (grown in San Javier, Murcia, Coop. SURINVER)

Variety	Fructose (g/L)	Glucose (g/L)	Sucrose (g/L)	Total sugars (g/L)
Total Mean	25.5	28.2	10.2	63.9
Min. mean	22.4	24.9	7.9	55.8
Max. mean	31.2	33,6	13.5	76.5
Cultivar significance	P< 0.05	P< 0.05	P< 0.05	P< 0.05

For each parameter, the total mean of the evaluated cultivars is displayed, as well as the minimum mean (i.e. the lowest variety mean), the maximum mean value (i.e. the highest variety mean) and the significance or non-significance of the cultivar effect (ANOVA-based on P< 0.05).

Table 16 – Results from pilot taste panels in the collection of kohlrabi varieties (n 0 10 persons/variety, both genders, age 22-60 years)

Variety	Sweetness1 low 5 high	Cabbage flavor 1 low 5 high	Pungency 1 nil 5 wasabi	Texture 1 tender 5crunchy	Juiciness 1 dry 5very juicy	Fibrosity1 low 5 hard	Global 1 poor 5 good
Total Mean	2.4	2.6	1.3	3.9	3.3	1.9	3.2
Min. mean	1.6	2.1	1.1	2.7	2.8	1.7	2.8
Max. mean	4.2	3.2	2.0	4.5	3.9	2.2	4.0
Cultivar effect significance	P< 0.05	P< 0.05	NS	P< 0.05	P< 0.05	NS	P< 0.05

For each parameter, the total mean of the evaluated cultivars is displayed, as well as the minimum mean (i.e. the lowest cultivar mean), the maximum mean value (i.e. the highest cultivar mean) and the significance or non-significance (NS) of the cultivar effect (ANOVA-based on level of significance at P< 0.05)

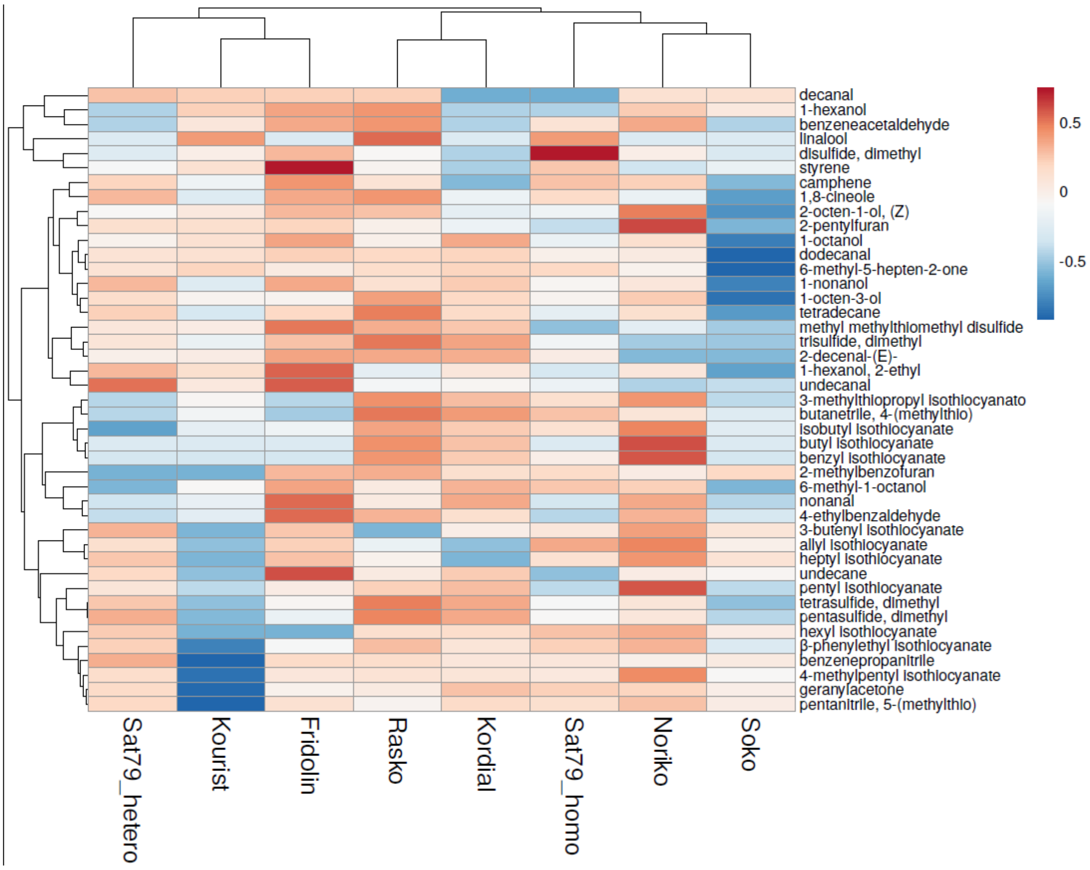

Figure 13 – ClustVis hierarchical heatmap based on the volatile profile of kohlrabi varieties

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5.2.2. Broccoli trials

In the broccoli trials, 10 organic OP cultivars originating from the breeding pipelines of Sativa' Kultursaat and Saat:gut were compared to different cell-fusion free and cell-fusion based F1-hybrid cultivars. These F1-hybrids were chosen as they are standard cultivars used in organic commercial production in the respective locations.

Figure 14 – Illustrative pictures from broccoli trials 2020-21 in Valencia. Upper: field trials evaluations and pictures to evaluate uniformity within cultivar, heterogeneous flower formation in the head, cases of fast flowering. Lower: lab pictures of Belstar F1 (left) and Rasmus (right).

The trials took place at different locations and to different planting times. The trials in Spain, Portugal and Italy were harvested from February to March, whereas the trials in France were harvested in autumn (Table 17). Only one trial was planted in a tunnel, while all other trials were cultivated in open field, which is the standard practice for broccoli.

Table 17 – Overall performance of the broccoli cultivars, expressed as part of marketable yield (% of evaluated plants) or as ranking among the cultivars included in the specific trial (OF: open field, GH: greenhouse)

	Partner	**Sativa**	**LSSV**	**UPV**	**LSSV**	**GRAB**	**PAIS**	**UPV**	**LSSV**
	Planting	16.10.2018	21.11.2018	Nov 19	22.+29.10.2019	03.09.2020	08.07.2020	22.11.2020	14.10.2020
	Harvest	25.02.2019 (133 DAP)	20.02.2019 (90 DAP)	March 2020	Feb-March 2020	Nov-December 2020	Sept-October 2020	March 2021	February 2021
	Location 1	Termoli (OF)	Idanha-a-Nova (GH)	Coop. SURINVER, Murcia (OF)	Idanha-a-Nova (OF)	GRAB (OF)	PAIS, Bretagne (OF)	Valencia (OF)	Idanha-a-Nova (OF)
	Country	Italy	Portugal	Spain	Portugal	France	Fance	Spain	Portugal
	Evaluation type	1-day	whole harvest	whole harvest	whole harvest	whole harvest	whole harvest	whole harvest	whole harvest
	Trial quality	4	5	4	4	5	4	5	4
Variety	**Origin**								
Parthenon F1 CMS	Sakata	1[1]	2	1	55%[2]	1		3	
Marathon F1	Sakata	1	3		75%	1	95%	3	39%
Belstar F1	Bejo	1	1		57%	1	57%	1	44%
Covina F1	Bejo	2	1				92%		
Rasmus	Kultursaat	2	3	*3*	13%	*4*	60%	3	46%
Calinaro	Kultursaat			*3*	12%	*4*	46%		24%
TH-Lim 20/68	Kultursaat	5	4			*4*			
CHE-Balimo B	Kultursaat	4	2	*3*		*4*	23%		
Sat32	Sativa	4	5						
Sat62	Sativa	3	5						
Thunder Dome F1	Takii						53%		
TH-Lim 37/59	Kultursaat			2			50%	1	
CHE-GRE-A-niedrig	Kultursaat							1	32%
CHE-GRE-A-aufrecht	Kultursaat							3	20%
sg531	saat:gut							3	30%

[1] For a better and more simple comparison among cultivars within each trial the percentage of marketable yield is showed as an accurate measurement of the cultivar´s utility. [2] In some trials, marketable yield data were not available or could not be recorded and, therefore, a comparative scale based on the different parameters evaluated is showed (with 1 being "best of trial",2="good performance", 3="acceptable performance", 4="suboptimal performance", 5="worst in trial, not usable")

As shown in Table 17, the cultivars' performance differed throughout the different locations. As an example, *Belstar F1* ranked at some sites as the best cultivar, whereas at others it showed a quite mediocre marketable yield of ca. 50% of the evaluated plants. This strong genotype x environment interaction applies to most broccoli cultivars. Non-adapted broccoli cultivars tend to stay small and flower quickly. However, *Covina F1* seemed to show a more stable performance than all other cultivars.

Regarding the organic OP cultivars, none of them really convinced in the trials (Table 17). In two trials, they were reported inadequate. The main deficits were too small, light and heterogeneous heads, a poor head quality, as well as a too long harvest window. However, *Rasmus*, *CHE-Balimo-B* and *CHE-GRE-A-niedrig* showed good performances at some sites. These results should be confirmed in further trials at these locations. Further, they indicate that it is not impossible to develop interesting cultivars in Northern Europe but that evaluation under the target environments during cultivar development is inevitable.

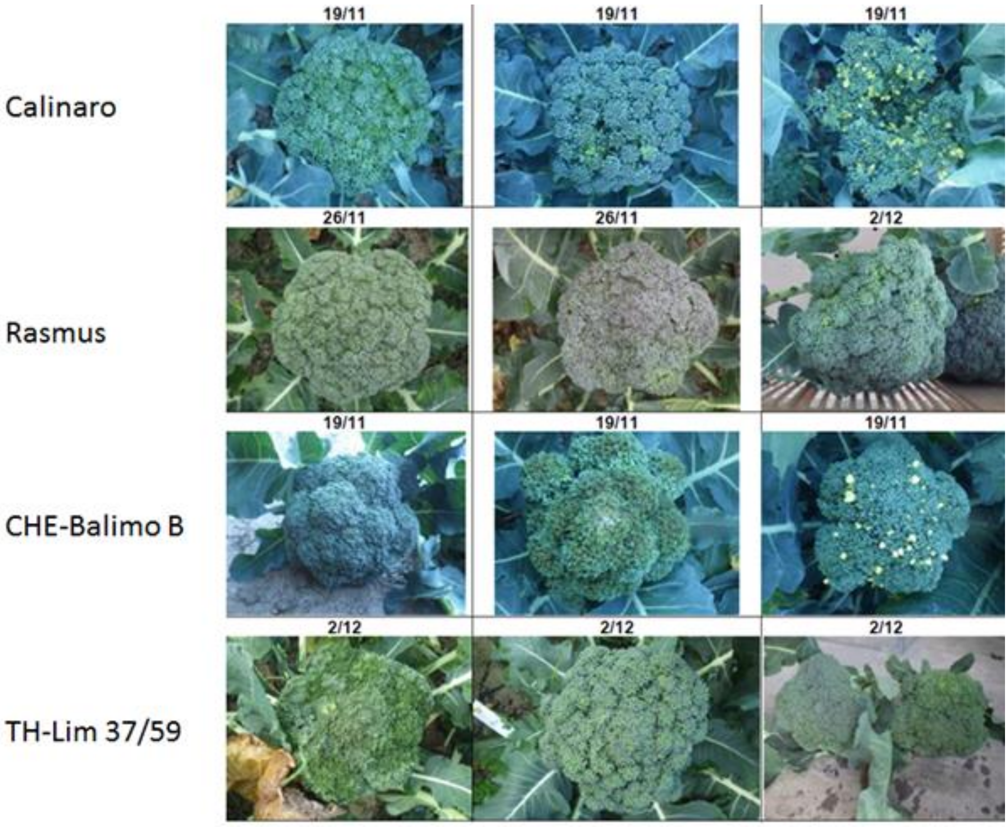

Figure 15 - Pictures of the OP varieties trialled by GRAB in autumn/winter 2020

5.2.3. Cauliflower trials

In the cauliflower trials, 7 organic OP cultivars originating from the breeding pipelines of Kultursaat and Organic Seed Alliance were compared to two standard cell-fusion free F1-hybrid cultivars (Table 18). The trials took place at very different locations and with different planting times. The trials in Spain were harvested from March to May, whereas the trials in the Netherlands and in France were harvested in autumn. The quality of the trials in the Netherlands and in Germany were suboptimal due to hot weather at transplanting.

Table 18 - Overall performance of the cauliflower cultivars, expressed as a comparative ranking.

	Partner	**Kultursaat**	**Kultursaat**	**UPV**	**PAIS**	**UPV**
	Planting	01.07. 2019	Early July 2019	October 2019	08.07.2020	22.11.2020
	Harvest	*8.10.2019*	24.09-22.10.2019	March-April 2020	24.09-30.10.2020	April-May 2021
	Location	BSAG	De Beersche Hoeve	La Verde Coop., Cadiz	PAIS, Bretagne	Pobla de Vallbona, Valencia
	Country	Germany	The Netherlands	Spain	France	Spain
	Evaluation type	one harvest *ranking*	whole harvest *ranking*	whole harvest *ranking*	whole harvest *ranking*	whole harvest *ranking*
	Trial quality	3	3	4	4	5
Variety	**Origin**					
Skywalker F1	Bejo			1	1	1
Liria F1	Bejo	1	1			
Nuage	Kultursaat	1	2	5	4	
Tabiro	Kultursaat					1
Amabile (MS-AMB)	Kultursaat	3	5	5	2	1
Daniel (JJ-REV)	Kultursaat	3	4	5	3	2
JJ-SG	Kultursaat	5	2	4	3	1
All-year-round	OSA					5

For a better and more simple comparison among cultivars within each trial a comparative scale based on the different parameters evaluated is showed (with 1 being “best of trial”,2=“good performance”, 3=”acceptable performance”, 4=”suboptimal performance”, 5=”worst in trial, not usable”)

As depicted in Table 18, the cauliflower cultivars performed very differently throughout the locations. Only *Skywalker F1* and *Liria F1* showed good performance across the trials they were planted. Regarding the OP cultivars, they showed an acceptable and comparable performance in one location in Spain only. At the two other sites, they were reported to be less vigorous, to grow more heterogeneously and to produce smaller and lighter heads than the reference *Skywalker F1*. The absence of sufficient self-coverage was also noted for almost all varieties, making the heads yellow. At each site, another OP cultivar was reported to be better than the others. These results indicate a strong genotype x environment interaction as depicted for broccoli too and suggest that a selection under the target environment is essential for variety development.

Figure 16 – Cauliflower trials 2020-21 in Valencia. Left: field trials evaluations in april and pictures to evaluate uniformity within cultivar (here the JJ-SG). Center: case of fast flowering (upper) and harvesting. Right: lab pictures of JJ-SG

5.2.4. Head cabbage (pointed, round, savoy and red) trials

In the head cabbage trials, several organic OP cultivars and breeding lines that originated from the breeding pipelines of Kultursaat and Organic Seed Alliance were compared with each other and with various F1-hybrid cultivars. The trials took place in Germany and Spain, the Spanish site being an interesting complement to evaluate the potential of the material in a different pedoclimatic environment as the one the lines/cultivars were bred in.

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Figure 17 – Cabbage trials 2020-21 in Valencia. Upper block: field trials and evaluations. Left: field trials in late February with white, pointed and savoy (upper), savoy and red (middle) and detail of slowly developed red cabbages (bottom). Center: beginning of participatory evaluation and harvest in late March (upper), detail of early flowering and harvested pointed cabbage (bottom). Right: On-the-field measurement of size at harvesting, pointed cabbages (upper, March 23rd), white cabbages (middle, end of April) and red cabbages (bottom, beginning of May). Lower block: mix of lab pictures of cultivars.

In contrast to cauliflower and broccoli, the overall performance of the organic OP material was much higher. Indeed, in terms of marketable yield, many cultivars showed values above 85% (Figure 18). In the red cabbages, the established organic variety *Rodynda* as well as the standard cultivar *Resima F1* showed a high marketable yield and a high stability over all locations. In the Savoy cabbages, the *Albwirsing* showed a slightly higher marketable yield than *Marner Grünkopf*. Finally, in the white cabbages, the organic variety *Dottenfelder Dauer* and the organic breeding line *Holt (JF)* were both superior to the standard cultivar *Kalorama F1*.

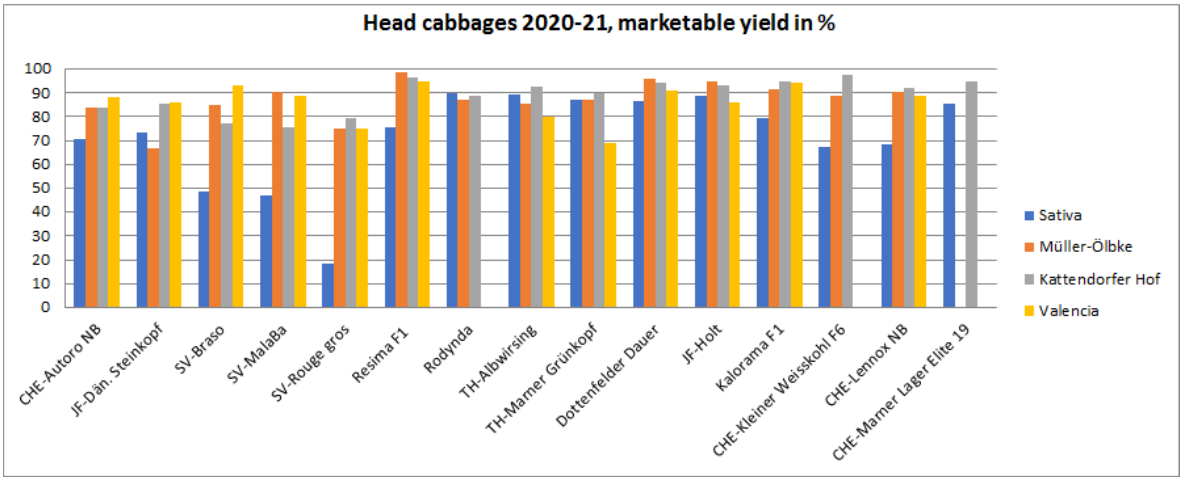

Figure 18 - Overall performance of the head cabbage cultivars, expressed as part of marketable yield (% of evaluated plants)

In the pointed cabbage trials, several organic OP cultivars and breeding lines originated from Kultursaat's breeding pipeline were compared with each other. The trials took place in Germany and Spain, the Spanish site being an interesting complement to evaluate the potential of the material in a different pedo-climatic environment as the one the lines/cultivars were bred in (in Germany).

As shown in Figure 19, the overall performance of the 6 cultivars was quite good. The trial site Walsegarten showed comparatively low values, which were due to dry and hot conditions at planting. However, despite the difficult conditions, the standard hybrid variety *Cape Horn F1* showed a relatively high marketable yield. The performance of the cultivars at the Spanish trial did not differ from the German sites, indicating that there are several organic pointed cabbage varieties with potential for cultivation in Spain.

In addition, a considerable variation among cultivars was found for flavour-related parameters in the taste panels performed at UPV, in particular in "cabbage flavour" and "pungency", which also contributed to report differences in terms of "global value" (Table 19). This issue indicates that there are ample opportunities to select cultivars adapted to different consumers´ preferences in flavour and taste as well as to reinforce participatory networks.

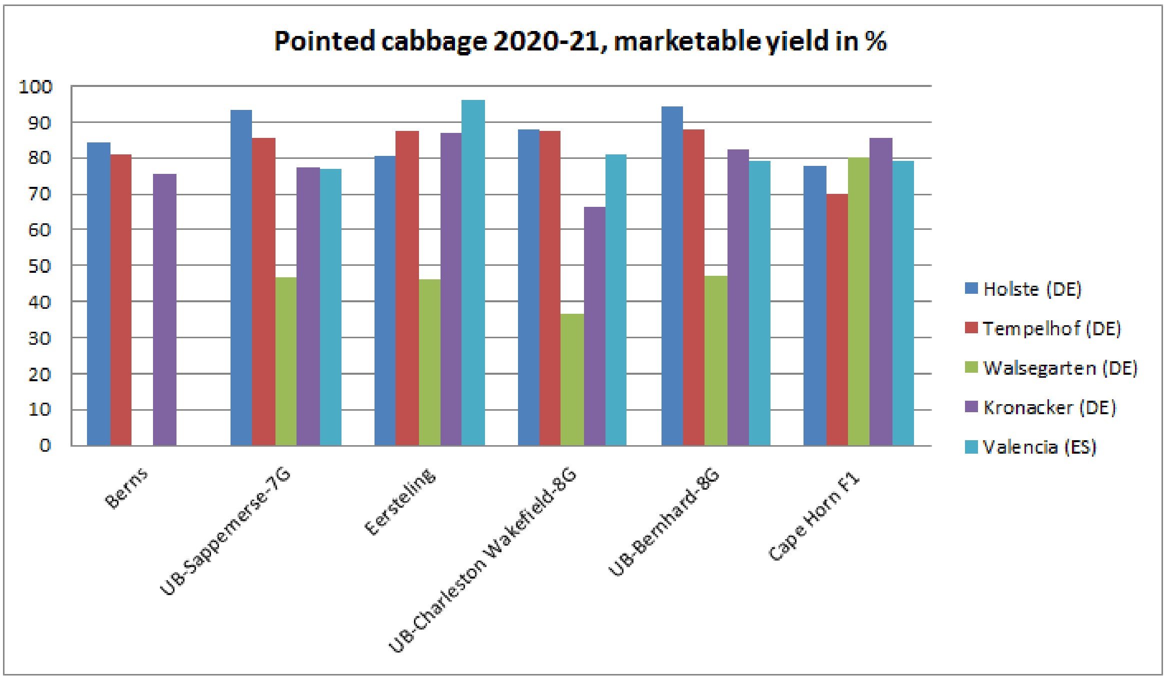

Figure 19 - Overall performance of the pointed cabbage cultivars, expressed as part of marketable yield (% of evaluated plants)

Table 19 – Results from pilot taste panels in the collection of cabbages (N = 12 persons/variety, both genders, age 22-60 years)

Variety	Sweetness1 low 5 high	Cabbage flavour 1 low 5 high	Pungency 1 nil 5 wasabi	Texture 1 tender 5crunchy	Juiciness 1 dry 5very juicy	Fibrosity1 low 5 hard	Global 1 poor 5 good
Total mean	2.6	3.1	2.3	3.6	3.1	2.5	3.2
Min. mean	2.0	2.4	1.4	3.1	2.4	1.8	2.2
Max. mean	3.4	4.1	4.2	4.4	3.6	3.9	4.0
Cultivar effect significance	P< 0.05	P< 0.05	P< 0.05	P< 0.05	P< 0.05	NS	P< 0.05

For each parameter the total mean of the evaluated cultivars, as well as the minimum mean (i.e. the lowest cultivar mean) the maximum mean value (i.e. the highest cultivar mean) and the significance of the cultivar effect (ANOVA-based on significance at P< 0.05) are displayed.

5.3. New cultivars & breeding material for potential further use

The following *Brassica* crop cultivars and breeding lines are planned to be released and/or further used for by the partners in LIVESEED subtask 3.4.2 (Table 20)

Table 20. - Summary of exploitation plans for breeding materials from the LIVESEED subtask 3.4.2 on Brassica crops

Partner	Planned cultivar release	Planned year of release	Further use of the material
Kultursaat	Open pollinated, CMS-free breeding lines of pointed cabbage adapted to the conditions of organic farming. Selected	By 2024 – 2025: submission for registration of at least	In Task 3.4.2, Kultursaat cultivars and breeding lines of kohlrabi, broccoli, white cabbage, pointed cabbage, red cabbage,

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	for early and medium-early ripening and for organoleptic quality. Kultursaat is responsible for cultivar development and submits the cultivars for registration. Sales and distribution of seeds marketing is handled by distribution partners such as Bingenheimer Saatgut or Sativa Rheinau.	one cultivar. Parallel seed production.	cauliflower and savoy cabbage have been tested at different Central and Southern European locations in 2018, 2019 and/or 2020. The pointed cabbage breeding lines are the most advanced in the breeding program. Most of the *Brassica* breeding lines tested in LIVESEED at different locations in Europe will undergo further selection steps in the next years. The results of the LIVESEED trial helped to assess the status of the respective variety development, to characterize the breeding lines and to identify further development needs.
LSSV	Promising advanced breeding lines of *Brassica rapa*, *B. napus* and *B. Oleracea.* Traditional Portuguese cultivars of *Brassica* crops improved specifically for cultivation under organic farming conditions and with improved uniformity and improved production compared with the landraces.	By 2023: at least 1 cultivar ready for registration	Further on-farm testing of varieties for better improvement and selection of potential cultivars. LSSV sends seeds back to INIAV (Instituto Nacional de Investigação Agrária e Veterinária) in Portugal and the material will be used for further crossings for plant breeding.
Sativa	Cell fusion-free, open-pollinated broccoli varieties, adapted to production both in Northern and Southern Europe and kohlrabi varieties, adapted to the whole harvest season in Southern Europe (November-May). The varieties will be certified by the association Bioverita. The entire breeding programme has been conducted under organic conditions.	2021-2024: further on-farm evaluation and shortlist of candidate cultivars By 2025: at least 1 broccoli variety submitted for registration By 2026: at least 1 kohlrabi variety submitted for registration (regular or as amateur variety depending on results of homogeneity test)	As for most long-term breeding programs, Sativa will put effort in maintaining and continuously improving different broccoli and kohlrabi populations. Further, Sativa is planning to build up a trial network across Europe in order to get substantial results on adaptiveness and performance of breeding lines at different locations.

5.4. Multi-actor activities: networks developed and participatory activities

The network developed in this subtask included various types of actors: organic and biodynamic breeders, universities and research institutes, organic advisory services and organic farmers in seven European countries. In most countries, farmers were involved in the evaluation of the trials and all the partners involved worked on a common pool of cultivars, which enabled comparisons among them and the identification of cultivars with a wide adaptation to different pedo-climatic conditions Additional quality analyses were performed by a university, allowing a more in-depth understanding

of the quality of the cultivars tested related to consumers´ taste preferences.
Towards the end of the project, also traders were involved in determining which kohlrabi cultivars can be suitable for large scale cultivation.
The established network has a strong collaborative and co-ordinated nature, which transpires from the joint protocol development and joint decision making in the trial set-ups. As also traders were involved, the experiences in this subtask may help the development of new financial models for organic plant breeding. In addition, cultivars and testing protocols were shared with Horizon2020 project BRESOV for their on-farm testing network of broccoli.

6. Apple

6.1. Methodologies

The main objective of LIVESEED Subtask 3.4.3 was to improve breeding for organic apple cultivation through a network that shares knowledge, protocols, methods and genetic resources (Deliverable 3.4). In order to develop this network, many workshops and on-line meeting were organised.
The task included also practical breeding and evaluation activities by POC (Poma Culta), a biodynamic apple breeder, and FiBL-CH. Breeding lines (segregating populations from key crossings and advanced candidate lines) from POC program were used as material in the trials and experiments.
POC organized and coordinated the testing of six of their own candidate breeding lines on six commercial biodynamic apple farms in different growing regions of Europe, to facilitate and speed up the market introduction of cultivars from biodynamic breeding (Figure 20).

Figure 20 – Trees and harvested apples from POC (Poma Culta) breeding program (Photo by N. Bolliger)

In parallel to the on-farm cultivar testing, FiBL-CH has conducted several research and testing activities with the aim to support the overall breeding program at POC and to provide new tools to all small-scale organic apple breeders. The following activities were conducted in collaboration with POC and using plant material from its breeding program:

- screenings for resistance to apple diseases (blotch caused by *Marssonina caronaria* and scab caused by *Venturia inaequalis*)
- application of molecular markers for biotic and abiotic stressors, and quality traits
- sensory and storability assessments
- development of marketing strategies for organic varieties.

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6.2. Main results

6.2.1. Development of common protocols and sharing of knowledge (AEGILOPS)

LIVESEED activities coordinated by AEGILOPS allowed to map the initiatives working in organic apple breeding (www.liveseed.eu/tools-forpractitioners/maps/) and to connect existing and new organic apple breeding initiatives via an active network across Europe. This activity included an online survey, several workshops, the organization of informative and demonstration events and meetings, participation to conferences and personal contacts. This broad range of activities was needed for setting up the network and connecting the different initiatives. The major events that gave visibility to the network were: (1) Organic apple breeding in Europe- Common strategy and networking to face coming up organic sector challenges and market opportunities, at the biggest fair of the organic sector, Biofach, in 2019 and (2) LIVESEED project: Opportunities for a European participatory organic (apple) fruit breeders' network, at the International Conference on Organic Fruit Growing organized by University of Hohenheim (Germany) in 2020. From the networking activities in LIVESEED it 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.

The discussion focused on elaborating a **European Participatory Organic Fruit Breeding Network**. This would need a broad platform of shared information and material exchange connecting different ongoing and existed projects.

Information should include:

- testing protocols/methodologies;
- lists of pre-breeding materials (e.g. parental lines);
- candidate selections with relevant testing results.

Information and data should be customized into open, easily and freely accessible forms. A permanent institution was suggested to undertake the organization and creation of such a database collaborative and platform information exchange system. Organizing responsibility, also, should be shared among existing institutions and people involved. The database could be enriched by and integrate information from existed networks or institutions (e.g. European cooperative Programme for Plant Genetic Resources, ERCPGR).

Based on these interactions facilitated by LIVESEED, the **European Participatory Organic Fruit Breeding Network** was launched. This network is now working on preparing a common cooperation principles statute and a knowledge-sharing road map (16 participants from 10 countries). As well, the EURO FRUITS NETWORK (**EUropean RObust FRUITS**) was created.

This initiative is open to organisations that work on organic breeding of all fruit species, but it is based on the apple breeding network created under LIVESEED.

Additionally, a **Balkan network on conservation and sustainable use of Plant Genetic resources** (also apple and fruit) was created to strengthen organic breeding in this region. This cooperation initiative includes breeders, public seed bank and research institutes, seed savers and organic farmers' associations' representatives (29 participants from 10 countries). This network is developing a memorandum of understanding, based on common principles and priorities and setting up a roadmap for cooperation on plant genetic resources conservation, information and material exchange, organic breeding, training and other activities.

6.2.2. On-farm cultivar trial (POC)

Before the start of the trial, 720 trees were grown centrally by the same nursery. At each of the 6 sites, 20 trees per candidate line were planted for a total of 120 in each location. The trees were planted in spring 2017 at five sites (Site_2 – Site_6) in Europe. One site (Site_1) could only be planted in spring 2018. The trials started with 6 candidate lines in 2017 that were increased to 7 in 2018 and to 10 in 2020 (Table 21).

Table 21 - List of sites, regions of cultivation, year of trial plantation and remarks for the on-farm apple cultivar trial coordinated by POC

Site code	Region	Year of Plantation	Remarks
Site_1	Altes Land (D)	2018	Younger trees
Site_2	Zeeland (NL)	2017	Yield and Storage Survey
Site_3	Bodensee (D)	2017	Mice Damage
Site_4	Thurgau (CH)	2017	Yield Survey
Site_5	Nantes (F)	2017	Damage of *Neonectria ditissima*
Site_6	Südtirol (I)	2017	Hailstorm 2019

For each farm, test trees were selected and were entered into the POC barcode-based software system for in-depth annual assessment. As part of the project, in 2018 and 2019, a visit was made to all the farms, during which various evaluations were carried out. At the same time, the data from harvesting, carried out directly by the farms, were discussed with the farm managers.

The development of the trees differed among the sites. The trees developed most vigorously at Site_4, as this was a new plantation on a clover-grass fallow. The orchard at Site_6 was severely damaged by a hailstorm in 2019. At Site_3, there were crop failures due to mice damage and at Site_5 the plants became more heavily infested by the pathogen fungus *Neonectria ditissima every year*.

In 2019, based on the results of the first three years of cultivation, it was decided to replace the three poorer breeding lines with new ones and to focus further evaluations on the more promising ones.

From all the evaluations collected on the trees and fruits from 2018 to 2020, it resulted that two (PoC_ 1399 and PoC_3800) of the six candidates were rated as good to very good in terms of suitability both for cultivation, and fruit quality and storability.

The storability of the lines PoC_1399 and PoC_3800 was investigated at Site_4, in the Netherlands and the optimal harvesting point was determined. The farm of Site_2 in The Netherlands was able to offer fruits for tasting in the farm shop. Positive feedback resulted from this informal taste panel with consumers.

The result of the on-farm trial can be considered a success. By testing the candidate varieties on the different farms, PoC_1399 can be considered to have potential for market introduction and registration as a Europe wide organic apple cultivar. In addition, PoC_3800 is a candidate line as an early autumn cultivar and PoC_3301 as a cultivar for direct marketers have been identified (see Table 22).

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Table 22 - Summary of the results across sites with overall decision about the 6 six apple lines included in the on-farm trial from 2017 to 2020

Candidate PoC-No.	Vigour tree architecture	Robustness	Yield potential	Regularity	Fruit quality	Storability	Overall scoring	Overall evaluation
1399	9	9	9	8	8	9	9	Suitable as storage cultivar
3800	8	8	8	7	8	3	9	Suitable as early autumn cultivar. Not suitable for long storage
3301	7	7	7	5	8	8	8	Relatively small fruit-size, suitable for direct marketing
1381	7	7	6	6	6	5	6	
3999	5	6	9	3	8	4	5	
1403	3	6	4	3	5	5	3	

*scoring values: 9= best, 5=acceptable, <5=insufficient

From the European on-farm trials organized by POC, it is evident that the test cultivation under real farm conditions on commercial farms provides valuable results. For the producers it is very valuable to have the opportunity to assess the cultivation value of the candidates as well as the quality of the fruit. Normally, variety testing is done on station and under conventional growing condition. Involving organic farmers in the testing procedure is very important as it allows testing under organic conditions and can speed up the variety development process. Based on this experiment, a protocol can be developed on how to test potential apple varieties on-farm. This protocol may also be used for other fruit crops.

6.2.3. Screenings for resistance to apple diseases (FiBL-CH)

<u>Susceptibility testing of varieties and breeding lines to blotch disease caused by *Marssonina caronaria*</u>

In 2019 and 2020, 15 accessions (including POC advanced variety candidates, heritage varieties conserved by POC and the two reference varieties "Topaz" and "Elstar") were tested for resistance to the blotch disease caused by *Marssonina caronaria*. The reference varieties Topaz and Elstar resulted to be very susceptible (Topaz) and susceptible (Elstar).

Of the 13 breeding lines and cultivar tested for susceptibility to *Marssonina caronaria*, four were found to be very tolerant and four moderately tolerant. None of the cultivars showed complete resistance. However, the degree of tolerance or partial resistance of the four best varieties is promising to be durable under field conditions.

The methodology used for this susceptibility testing trial is useful to support in the screening of candidate varieties and possible crossing parents.

<u>Scab screening of POC cross progeny by FiBL</u>

In 2020 a screening for scab susceptibility was conducted on seedlings of POC progeny from 2019 crosses, as early-step selection. The population seedlings were grown in seed trays up to the cotyledon stage on the POC farm and then transferred to the greenhouse at FiBL, where they were potted and cultivated under standard conditions (20°C, 16 h light) up to the 3-leaf stage (approx. 10 days). At this stage, inoculation and incubation in the laboratory and climate chamber were performed. The evaluation took place after 10-12 days. Scab-susceptible individuals were excluded, and the scab-

resistant seedlings were sent back to the POC nursery for further cultivation in pots
and then planted in the field.

The procedure requires some effort (transfer to FiBL and potting of individual plants) but allows the resistance reaction of the seedlings to be assessed individually. It also allows the individual progeny to be observed over a quite long period before planting. Future adjustments of the procedure will be discussed between FiBL and POC, but the scoring system proved to be useful for scab resistant selection on breeding material before planting in the field.

6.2.4. Application of molecular markers for biotic and abiotic stressors, and quality traits (FiBL and POC)

Molecular marker analysis was conducted to check for disease robustness and fruit quality of the POC materials in 2017-2018. A collection of 13 candidate cultivars and 57 potential parental lines were tested for 11 SSR markers for traits related to resistance to pests, diseases, and improved quality (Table 23). For the genetic analysis with molecular markers (SSRs) leaf samples were taken that were sent for analysis to the lab of the company Ecogenics.

Table 23 - List of markers and associated traits used for selection on POC (Poma Culta) breeding material

SSR marker	Associated trait
Rvi2	scab resistance
Rvi4	scab resistance
Rvi6	scab resistance
Pl1	powdery mildey resistance
Pl2	powdery mildey resistance
PlD	powdery mildey resistance
FbF7	fire blight
Md-ACS1	storability, low ethylen production
Md-ACO1	storability, low ethylen production
Md-PG1	fruit quality, firmness and texture
Dp-fl	resitance against mealy apple aphid (*Dysaphis plantaginea)*

Of the 70 apple genotypes, 49 revealed the presence of Rvi6. In 16 cases, the QTL for fire blight was detected, in 33 cases the QTLs for low ethylene production and 15 times the QTL for fruit firmness. The MAS investigations showed that only little monogenic resistance can be detected in the breeding material of POC. With regard to scab, only Rvi6 is present, but this has largely lost interest with the Europe-wide breakthrough in resistance. Powdery mildew occurs sporadically, with Pl1 found a few times. It is likely that polygenic resistance is involved in the actual high disease tolerance of POC lines. MAS testing is not very useful in these cases. In the case of fire blight (QTL FbF7 present), MAS can give clues for parental selection, as no phenotypic field observations can be made. The markers for quality traits showed a good agreement with the observations made in the field, but do not bring any significant advantages in the breeding process of POC, as the parent selection is based on phenotypic observations of several years.

6.2.5. Sensory and storability assessments (FiBL and POC)

Initial fruit quality assessment of the lines included in the on-farm cultivar trial was done at or immediately after the harvest each year. The appearance of the fruit and the sensory value (aroma, juiciness, bite) were evaluated as an overall score (Table 22). The healthy fruits, which were also promising with respect to the other parameters, were subsequently evaluated for storability. The fruits from the site in Switzerland were submitted to a tasting panel for sensory evaluation organised by FiBL-CH. FiBL-CH conducted degustation each year with a semi-trained panel of FiBL-CH employees and an un-trained panel of the participant to the annual event “Organic Fruit Conference” organized by FiBL. The semi-trained panel included 30 to 40 FiBL-CH researchers and the untrained panel included 80 to 100 participants of the annual “Organic Fruit Conference” organised by FiBL-CH in Frick (Switzerland) where different actors of the organic fruit sector gather (including organic fruit growers, advisors, organic fruit sellers, BioSuisse delegates, retailors, nurseries managers, researchers).

6.2.6. Development of marketing strategies for organic varieties (FiBL-CH and POC)

In order to facilitate the market introduction of new apple varieties in organic production in Switzerland, FiBL-CH has developed a “variety testing concept” consisting of on-station and on-farm trials and a stakeholder platform “organic apple variety team”.

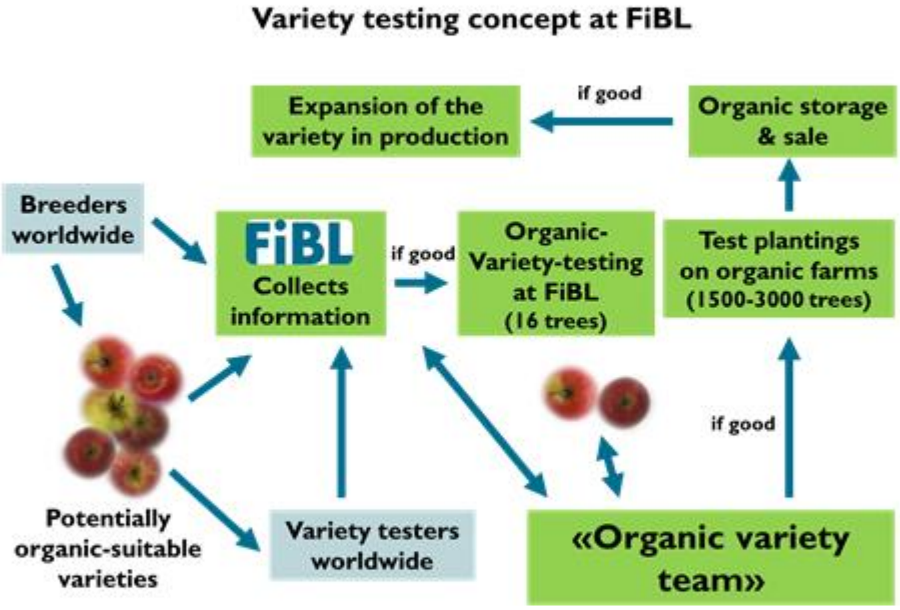

Figure 21 - Apple variety testing concept at FiBL-CH. Work-flow from potentially interesting genotypes for organic production to variety testing under organic condition, then test-planting on organic farms and finally market introduction of the variety

The “Organic variety team” consists of the public apple breeder Agroscope, the organic apple breeder POC, organic apples growers, organic nurseries, wholesalers (e.g., Biofarm, Tobi-fruits, Fenaco) and retailors (e.g., Coop). The most two promising candidates of POC have been integrated in this Swiss organic variety testing selected by the “organic variety team” and has been tested the last years on station and in pilot production in Switzerland coordinated by FiBL-CH. Results of these trials are broadly discussed among the team and disseminated to organic apple growers in annual fruit meetings and farmers' journal. Fruits of these trials were also used for sensory testing (see above). As it is very difficult to integrate new cultivars in retailor shops, the flavour-colour concept (yellow for sweet, red for slightly acidic and green for predominantly acidic types) has also been applied (presented at the apple workshop at Biofach 2019). In close collaboration with Bioverita (www.bioverita.ch) an association certifying products derived from organic breeding, several exhibitions in Switzerland (e.g., Vielfaltsmarkt 2018) and at the European Biofach (2018, 2019) with degustation of the promising candidates have been conducted to create awareness of upcoming organic apple cultivars.

6.3. New cultivars & breeding material for potential further use

The following apple cultivars and breeding lines are planned to be released and/or further used for by the partners in LIVESEED subtask 3.4.3 (Table 24).

Table 24 - Summary of exploitation plans for breeding materials from the LIVESEED subtask 3.4.2 on apple breeding

Partner	Planned cultivar release	Planned year of release	Further use of the material
POC (Poma Culta)	Candidate varieties selected under on-field organic conditions, that show reduced susceptibility to key apple diseases. The robustness of the selected genotypes is not based on individual resistance genes but on selection for complex traits. Introduction of new varieties by involving all stakeholders in the value chain.	2022: submit first varieties for registration 2026 and 2027: test sales	From the rest of the POC breeding programme, about 20 interesting genotypes with special characteristics in terms of disease tolerance and/or fruit quality (firmness, storability) are available for further testing and use as breeding parents. Particularly interesting genotypes from the remaining breeding material will be used by POC for further crosses and made available also to other breeders.

6.4. Multi-actor activities: networks developed and participatory activities

The need for long term coordination among actors and projects and long-lasting networks to save, spread and exchange knowledge, data as well as genetic material was expressed from various breeders. While such network exists for conventional fruit production like EUFrin (European Fruit Research Institutes Network) connecting institutes in Europe on fruit research, which involves working subgroups in interaction with EU Commission and common agreements between breeders on variety tests, a similar network was missing for the organic sector. Therefore, LIVESEED initiated the discussion and started the **European Participatory Organic Fruit Breeding Network**. This can become the basis for trustful collaboration for the benefit of all actors. Especially the exchange of vegetative planting material of new candidates will only be possible through personal relationships and trust built up via the participation to the network.

The protocol for on-farm testing of candidate lines developed and tested by POC and FiBL is important as it provides a relatively simple collaborative tool for on-farm testing of new candidate lines of fruit crops.

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.

7. Wheat

7.1. Methodologies

7.1.1. Plant materials used

A collection of 450 breeding lines (including 30 lines from the German breeders CULTIVARI and DOTTENFELDERHOF) and varieties with 12 different resistance genes (Table 24) were grown and evaluated in two field trials at AGROLOGICA (Denmark) in 2018/19 and 2019/20. After selection, a set of selected lines were multiplied and grown for selection for other agronomic traits and baking quality in the final part of the project. A total of 100 breeding lines of winter wheat from AGROLOGICA with resistance to bunt, lodging tolerance, resistance to leaf diseases and with high gluten index were mixed and multiplied together and denominated “Popkorn”. In 2021, 60 ha are sown with this “Popkorn” multi-line mixture. In 2020, 14 new mixtures were composed with different agronomic and quality traits in respect seed colour, gluten structure, earliness. In total, 50 ha were sown with these additional mixtures for seed multiplication.

Based on the setup developed for winter wheat, a similar setup has been developed for spring wheat. A total of 50 ha was sown with one bunt resistant multi-line mixture.

Table 24 - Number of lines that, based on phenotypic evaluation, is expected to have a specific resistance gene.

Resistance gene postulation	Number of lines
Bt5	75
Bt7	59
Bt13	32
Bt1	30
Bt2	30
BtZ	26
Quebon resistance	16
Bt6	2
Bt10	2
Bt+	2
Bt0	1
Bt9	1

7.1.2. Research and breeding activities

AGROLOGICA (Denmark)

The first field trials were performed in 2018 and 2019 in Denmark, to identify resistances to common bunt in wheat as well as agronomic traits and to perform selection. Lines were tested for their reaction to inoculation with spores of isolates of *Tilletia caries* (collected in Denmark except for one that was collected in Germany) showing different virulence pattern (Table 25). Common bunt was evaluated based on artificial inoculation and visual disease incidence in the field using a protocol described in Borgen et al. (2019). Agronomic traits, including plant height, mildew and rust susceptibility, lodging tolerance and earliness, were also evaluated in the field. In the second year, selected breeding lines were grown without bunt infection for multiplication and for re-evaluation of agronomic and quality traits. Plot trials for agronomic and quality traits evaluation were conducted also by CULTIVARI and DOTTENFELDERHOF. Baking quality tests included NIT-analysis for protein and gluten-index with the Perten Glutomate and Farinograph.

Some of the lines selected during this experiment have been also used by CULTIVARI and DOTTENFELDERHOF in their wheat breeding program to improve common bunt resistance in their materials.

Development of bunt resistant wheat mixtures at AGROLOGICA is done in cooperation with 10 millers and a total of 50 farmers that are involved in the participatory network Landsorten (landsorten.dk).

Table 25 - List of the Tilletia caries isolates used in field trials at AGROLOGICA in 2018/19 and 2019/20

Isolate	2018	2019	Virulent against	Comment
Vr0	●			no virulence detected
Vr1	●		Bt-1, Bt-2, Bt-7	= Vr2
Vr2	●	●	Bt-1, Bt-2, Bt-7	
Vr3	●	●	Bt-2, Bt-3	
VrR	●	●	Bt-2, Bt-3	=Vr3
Vr4	●			= Vr341
Vr5	●	●	Bt-7	
VrG	●	●	Bt-7	=Vr5
Vr8	●	●	Bt-7, Bt-11	
Vr10	●	●	Bt-7, Bt-11, Bt-Z	
Vr13	●	●	Bt-13	
Vr341	●	●	Bt-Z	spore collected from infected wheat line Pi554341
Vr3540	●		Bt1	spores collected from infected wheat line PG3540
VrDot	●		Bt-2	spores by DOTTENFELDERHOF in Germany = Vr2
VrP	●	●		

Uni Kassel (KU Germany)

Out of the 450 breeding lines and varieties phenotyped at AGROLOGICA in Denmark, 274 selected lines have been genotyped with a 25K chip at TraitGenetics GmbH. Obtained data were used in the genome-wide association study (GWAS). Several statistical methods for association have been tested: general linear model (GLM), mixed linear model (MLM), multi-locus mixed model (MLMM), fixed and random models circulating probability unification (FarmCPU) using the R script GAPIT version 3, as well as a network-based model using the R package *netgwas* for detecting epistatic selection. The network based GWAS has been selected as the most appropriate method because the infection rate (%) is the relative frequency of the occurrence of disease symptoms and is mostly Beta distributed. Most GWAS methods are developed for normally distributed data and in contrast, network-based GWAS, can handle ordinal data, non-Gaussian continuous data, and mixed discrete-and-continuous data. It also adjusts for the effect of all other SNPs and phenotypes while measuring the pairwise associations between them, and therefore accounting for population structure by definition. The resulting genotype-phenotype network is a complex network made up of interactions among: (i) genetic markers, (ii) phenotypes, and (iii) between genetic markers and phenotypes.

Maximum value of the standardised and log+1 transformed data of both experimental years have been used in the GWAS. Lines represented in just one of the two experimental years well as those virulence races that are tested on a small number of lines in both years were excluded from the calculation, leaving only five virulence strains for the analyses – Vr13, Vr10, Vr5, Vr2 and Vr3.

Missing genotypic data have been imputed based on machine learning approaches for genetic imputations (local XGBoost models, Privé et al. 2018). Genetic maps of Allen et al. (2017) and Wang et al. (2014) have been used to map genomic regions for gene loci affecting the resistance to common bunt.

For capacity building at AGROLOGICA, KU has assisted AGROLOGICA in the development of a simpler analytical method based on spread sheet analysis of markers with specific focus on the analogue knowledge of the individual breeding lines, and in particular NILs of the variety Starke-II included in the core material (Christensen and Borgen, 2021).

7.2. Main results

AGROLOGICA

The infection rates of the selected wheat lines are summarised in Table 26 and presented in detail for the season 2018/19 in Annex 5, where the level of infection is shown by colour gradient (from green=low infection rate to red=high infection rate). In Annex 5, the wheat lines are sorted in the table based on a subjective evaluation of similarity in reaction to the different virulence races. The infection rates ranged from 0 to 100% infection with some being resistant to all virulence races, and others being susceptible to all races. Lines with zero infection to all races are not presented and are hypothesised to carry multiple genes.

Table 26 - Infection rate (%) of wheat averaged across lines with 7 different postulated resistance genes infected with 11 different virulence races of Tilletia caries in seasons 2018/19 and 2019/20.

2018/19	Bt1	Bt2	Bt5	Bt7	Bt13	BtQ	BtZ
Vr1	25.7	35.3	0.7	27.2	1.3	1.3	0.1
Vr2	15.6	28.4	0.8	29.2	0.7	0.9	0.0
Vr3	0.0	32.5	0.4	0.3	0.1	1.5	0.0
Vr4	0.1	0.4	0.1	0.2	0.0	0.0	0.0
Vr5	0.0	2.5	21.4	7.0	0.0	0.0	0.0
Vr8	8.9	0.5	3.1	4.1	0.0	0.0	0.0
Vr10	0.5	0.4	1.7	16.3	1.5	0.1	32.1
Vr13	0.1	0.0	0.2	1.3	12.7	0.0	0.1
VrP	0.0	34.8	5.8	8.5	2.3	0.0	0.0
Vr341	0.0	0.0	0.0	0.0	8.9	0.0	0.0
Vr3540	1.5	4.7	0.2	18.7	0.0	0.0	0.0
2019/20	Bt1	Bt2	Bt5	Bt7	Bt13	BtQ	BtZ
Vr0	0.1	5.0	0.4	1.1	0.1	0.0	0.2
Vr2	59.5	57.3	2.3	74.6	0.1	1.8	0.0
Vr3	0.3	54.2	2.6	0.8	0.1	0.2	0.0
Vr5	3.1	17.3	50.8	44.9	0.3	7.6	0.5
Vr8	2.6	1.3	0.4	33.2	5.6	0.0	0.0
Vr10	3.1	5.0	4.5	54.9	2.4	0.7	29.2
Vr13	0.2	0.5	0.0	15.9	51.3	0.6	0.0
VrP	1.9	4.1	0.5	37.3	5.2	0.3	35.3
Vr341	0.1	3.8	1.5	0.5	0.6	0.0	12.9

VrDot	10.1	42.8	0.7	0.4	4.8	2.1	0.0

The line PI 181463 (Thule III) and a few of the lines with this line as parent showed low infection rates when contaminated with isolate Vr13. This is surprising, since in previous years this isolate has demonstrated high virulence against Bt13. This may indicate that the spores in 2017 may have been of low vitality and/or been applied in low quantity.

Most differential lines were infected by one or more of the virulence races. This shows that virulence is present against most of the known Bt-resistance genes. However, lines with Bt9 or Bt11 were not infected by any of the races (data not presented).

Lines with the resistance gene Bt12 were infected with the isolate Vr341 (data not presented), which is surprising since virulence to Bt12 has not previously been described in Europe. Also, lines with the resistance gene Bt6 were infected by this isolate, which is also surprising, as *Tilletia leavis* has never been observed in Denmark, and virulence against Bt6 has so far only been observed in Eastern Europe in areas where *Tilletia leavis* is present (Mascher *et al.* 2016). However, isolate Vr341 has not been identified at species level.

Based on the sorting presented in Annex 5, it seems likely that some lines react in a similar way to the different isolates and it is hypothesised that lines with similar reaction have the same resistance gene. Since each group has one or more differential lines with known resistance genes (lines marked in blue in the Annex 5), it is hypothesised that the groups represent lines with the same resistance genes as the differential line in the group. However, Bt10 and BtZ react in a similar way to the different isolates. Differential lines with Bt10 and BtZ have been assessed with the genetic marker identified for Bt10 (Laroche *et al.,* 2000), and only Bt10 had this marker which supports the fact that Bt10 and BtZ are indeed two different genes and that isolate Vr10 in this study apparently is virulent to both Bt10 and BtZ. Therefore, the distinction between BtZ and Bt10 in this study is based on information about the parental lines.

In this study, lines with multiple resistance genes were not infected by any of the races. However, some combinations of dual resistance are relatively easily overcome through the development of new virulence strains of the pathogen, given that virulence against the parent resistance gene are present in pathogenic strains in the region (Hoffman 1982). Therefore, a safer strategy is to combine resistance genes where virulence against at least one of the genes are rare. In Europe, virulence is frequently found against Bt7 and relatively common also against Bt1, Bt2 and Bt5. Our study shows that these genes are also found in several commercial varieties in Europe, and this is likely the reason for the virulence. Combining these resistance genes alone can therefore not be used as the only strategy to control the disease but must be combined with other control measures or at least be followed with seed analysis for the presence of spores prior to sowing. Knowledge about local virulence patterns and the presence of different resistance genes allow an improved cultivar management to avoid resistance breakdown.

Phenotypic results of the first-year field trial were published in Borgen et al 2019. The phenotypic evaluation of breeding lines has not only provided basic knowledge for association with genotypic results but has indeed also been used for breeding at AGROLOGICA, CULTIVARI and DOTTENFELDERHOF. Several varieties and mixtures have been selected and marketed as bunt resistant varieties or organic heterogeneous material. Material and information have also been exchanged with the Horizon 2020 project ECOBREED.

Based on the genotyping, AGROLOGICA has built up a GWAS lab to be able to use marker assisted breeding in the future. Parallel to the GWAS done at KU, AGROLOGICA has identified linkage-groups associated with bunt resistance and has located the physical position of most of the major Bt-genes searched for in the study (Table 27).

Table 27 - Mapping and intervals of Bt-genes

Bt-genes	Mapping Chromosome	Mapping interval (location on Chromosome, bp)	Information from literature
Bt1	2B	789,867,236 -801,253,554	
Bt5	1B	285, 345,287 - 285,608,205	Corresponding markers in the bunt resistant varieties Globus and Tommi widely used in European bunt resistance breeding, confirms the phenotypic data indicating that these lines carry Bt5 resistance.
Bt7	2D	607,418,834- 619,576,292	
Bt8	6D		is expected to be located close to Bt10 on chromosome 6D
Bt9	6D	469,248,476 – 469,919,743	remapped to chromosome 6D as by Steffan et al. 2017; Wang et al. 2019
Bt10	6D	1,773,421 – 11,407,937	was remapped to chromosome 6D as by Menzies et al. 2006
Bt12	7D	7,073,045 – 10,835,093	was remapped to chromosome 7D as by Muellner et al., 2020
Bt13	7D	7,073,045 – 10,835,093	is expected to be located close to Bt12 on the same chromosome 7D
Quebon resistance			is concluded to be a combination of Bt5 and an additional gene, most likely Bt2.

Uni Kassel (KU

The GWAS revealed in total 120 SNP markers associated ($r^2 \leq 0.2$) with the response of the wheat to the infection with the common bunt: 43 SNPs for Vr13, 31 for Vr5, 29 for Vr10, 14 for Vr2 and 3 for Vr3 (Figure 22). The physical position of associated markers is given in Figure 23.

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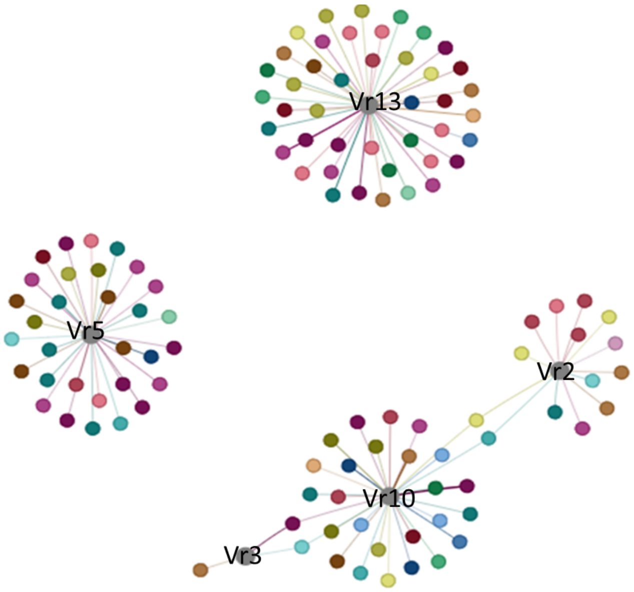

Figure 22 - Strain-marker interactions of 274 genotyped lines of wheat. Each edge represents the connection between two nodes, where each node is a SNP marker (coloured, where each colour represent a chromosome on which a marker is located) or a virulence race (grey).

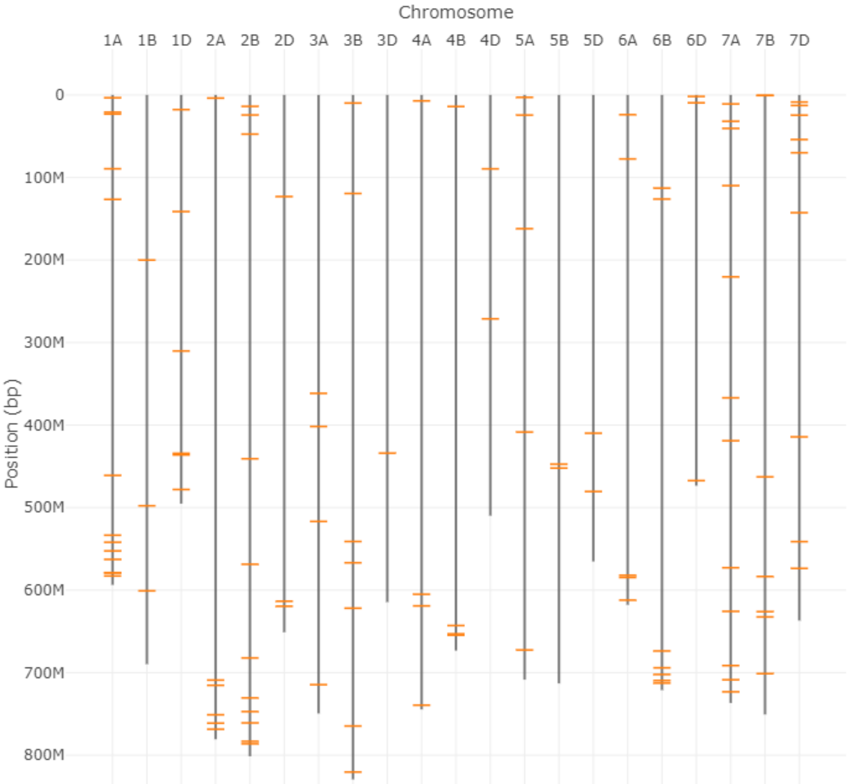

Figure 23 - Physical position of associated markers on wheat chromosomes.

Associated markers are distributed over the whole wheat genome, with the highest number of markers located on chromosomes 1A (14 SNPs), 7A (12) and 2B (11) (Table 28). Some of the identified SNP markers for Vr13 were found in close proximity of the resistance genes Bt12 and Bt9 on

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chromosome 7D and identified SNP markers for Vr10 in the region of resistance gene Bt10 on 6D. Several other promising SNPs have been identified for Vr13 on chromosomes 1A and 7B, as well as for Vr5 on chromosomes 1A and 7A.

Table 28 - Distribution of markers associated to different virulent races over wheat chromosomes

	Vr10	Vr13	Vr2	Vr3	Vr5	Total
1A	3	5		1	5	14
1B	2				1	3
1D	2	2	3			7
2A	1	1			4	6
2B	1	4	1		5	11
2D	1		1	1	1	4
3A	1	3				4
3B	1	3	2	1		7
3D			1			1
4A	2	1			1	4
4B	1	3				4
4D	1	1				2
5A	1	3			1	5
5B	1	1				2
5D		1			1	2
6A	3				2	5
6B	2	1	3		1	7
6D	3					3
7A	2	3	1		6	12
7B	1	5	1		1	8
7D		6	1		2	9

This task of the project is still ongoing and further filtering and annotation of identified SNPs is being done at the moment. Promising SNPs identified for the 5 virulence strains of common bunt, will be used for the design of KASP markers (by end of September) with the goal of being used in the Marker Assisted Selection approach in breeding programs targeting the development of bunt resistant wheat varieties. In order to use synergies, there is a close collaboration with the group of Hermann Bürstmayr from BOKU involved in the marker assisted selection of bunt of the sister project ECOBREED. As soon as the markers are available, they will be published and shared. Latest results were presented at the EUCARPIA –LIVESEED conference in March 2021 and at the international bunt workshop in May 2021.

7.3. New cultivars & breeding material for potential further use

The following wheat cultivars and breeding lines are planned to be released and/or further used for by the partners in LIVESEED subtask 3.4.4 (Table 29)

Table 29 - Summary of exploitation plans for breeding materials from the LIVESEED subtask 3.4.4 on wheat breeding for resistance to common bunt

Partner	Planned cultivar release	Planned year of release	Further use of the material
AGROLOGICA	AGROLOGICA's participation in LIVESEED was focused on bunt research, and bunt resistance will be an important trait incorporated in most wheat material developed from AGROLOGICA.	Farmers, millers, bakers and one plant breeder (AGROLOGICA) has made a membership organisation, Landsorten. Seed will be disseminated within Landsorten to members only without official variety release.	AGROLOGICA participates to Lansorten: a membership organisation together with farmers, millers and bakers. Landsorten will distribute seed of AGROLOGICA cultivars only for experimental trials to the members. Farmers satisfied with the on-farm experiment trials can then use farm-saved seed for further production.
DOTTENFELDERHOF	New bunt resistant cultivars resulting from fully organic breeding program. Breeding varieties which combine a resistance to common bunt with a high baking quality and other important agronomic traits (grain yield, competitive-ness against weeds, other disease resistances)	The lines obtained from crosses with lines in the AGROLOGICA trial will be tested in common bunt trials at DOTTENFELDERHOF in generations F2 - F5. The first lines are therefore tested in 2021-24. Promising lines are further tested in yield trials (in generations F7 and above) and released as varieties not before 2029.	In addition, breeders are provided with specific knowledge about the type of the resistance (Bt genes)

7.4. Multi-actor activities: networks developed and participatory activities

In the subtask 3.4.4 on wheat, each of the partners involved worked on very specific aspects, such as phenotypic evaluation (AGROLOGICA), molecular evaluation (KU) and the potential for further breeding (CULTIVARI and DOTTENFELDERHOF). Nevertheless, the phenotypic evaluation of breeding lines has not only delivered the data for the GWAS, but has indeed also been used in breeding, for performing new crosses, both at AGROLOGICA, CULTIVARI and DOTTENFELDERHOF. This exchange of information allowed to foster and strengthen the collaboration among the organic cereal breeders. Several varieties and mixtures have been selected and are or will be marketed as bunt resistant varieties or organic heterogeneous material based on LIVESEED trials results. The collaboration among the organic wheat breeders has improved during the LIVESEED project. Resistance to common bunt is an important trait for organic wheat, but it is difficult for breeders to test for bunt resistance, since the disease easily spread from the disease nursery to the breeding nursery. Also, it is important to use the right resistance genes or to stack genes, but it is quite complicated to test for specific resistance genes. In LIVESEED, AGROLOGICA has optimised a system to screen wheat germplasm with a collection of different virulence isolates, and thereby to identify which resistance genes a breeding lines contains. AGROLOGICA will offer to test wheat breeding lines in this system, giving breeders and researchers valuable information about their germplasm. AGROLOGICA has the only disease nursery in Europe working with specific virulence races to identify bunt resistance genes in wheat and this activity can be used to further collaborate with the other organic breeders that focus on common bunt resistance.

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8. Tomato

In Subtask 3.4.5, three initiatives aimed at participatory organic breeding of tomato were developed in Spain (UPV) and Italy (RSR/Arcoiris and CREA-OF Monsampolo). Although these initiatives shared many similarities, they also encompassed some differences in approaches, plant populations, breeding strategies, actors, traits evaluated (or ways to evaluate them), parameters, data treatment and participatory approaches (Table 30).

8.1. Methodologies

8.1.1. Activities in Spain (UPV)

In Spain, 270 accessions, mainly corresponding to landraces cultivated or preserved in the COMAV-UPV seedbank, covering a diversity of varietal types and geographical origins from all the country (including Balearic Islands and Canary Islands), were evaluated from 2018 to 2020.
Organic trials were performed in open field in the spring-summer season in 2018, 2019 and 2020 in two locations in the regions of Valencia and Villamartin (Sierra de Cádiz, Andalusia). Small additional pilot trials were performed in Valencia, Cadiz, Catalonia and Murcia aimed at widening the participatory networks for future initiatives (data not shown). Three actor groups were involved in the participatory evaluation: farmers and technicians from the involved cooperatives, scientists from UPV, and citizens/consumers from nearby municipalities.
The following main agronomic traits were evaluated: yield, plant vigour and development traits, earliness, incidence of the main diseases, fruit traits (e.g. setting distribution in the plant, fruits/bench, size, weight, firmness). Fruit quality traits were evaluated depending on the breeding phase; e.g. sugars, acids, ascorbic acid, lycopene, volatiles. Taste was evaluated with un-trained consumers (on-farm open days and local markets) using simple evaluation templates (e.g. aroma, taste, texture, flavour prevalence, overall score), which enabled both feedback/preferences from consumers and diffusion to citizens)

8.1.2. Activities in Italy (RSR/Arcoiris)

The RSR/Arcoiris activities were based on a composite cross population developed during the SOLIBAM FP7 project (2010-2014), which was developed from crossings of four different cultivars. Similar to Spain, organic trials were performed in the spring-summer season in 2018, 2019 and 2020. Trials were conducted in five locations, across the country from north to south.
The participatory evaluation of plants was done on-farm, with the participation of about 400 farmers during the project. Traits evaluated were mainly agronomic, applied and easy to evaluate, adapted to farmers´ approaches, e.g. yield, plant vigour, incidence of diseases, died plants, uniformity, fruit morphology, marketable fruits & waste fruits and overall score (1-4).

8.1.3. Activities in Italy (CREA-OF)

The activities of the CREA unit in Monsanpolo (CREA-OF) were based on a collection of segregating inbreeding lines of a pre-MAGIC population (Multiparent Advanced Generation InterCross). These populations were evaluated in four locations of Italy, also covering the country from North to South, in i) organic experimental trials by CREA in 2018, 2019 and 2020 and ii) in organic participatory trials with farmers in 2019 and 2020. Participatory evaluation of plants was conducted on-farm. Traits

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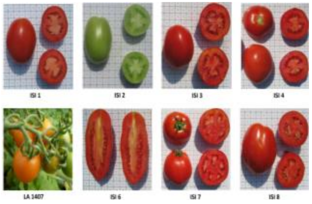
evaluated were mainly agronomic, but covering a larger number of more specific traits than those in 8.1.2, e.g. plant habitus and vigour, ripening homogeneity, disease incidence, fruits per plant, fruit weight, fruit shape, fruit size, fruit colour, fruit firmness, fruit puffiness, overall score, and pH (acidity) and (ºBrix) soluble solids of the fruits.

Table 30 - Summary of the main methodologies used in the tomato participatory activities.

	SPAIN-UPV	ITALY-RSR/Arcoiris	ITALY-CREA-OF
Materials	270 landraces from all the Regions of Spain and different varietal types 	Composite Cross Popul. (CCP, SOLIBAM Project): Cuore bue, Coeur boeuf, Muchamiel, 	Pre-MAGIC lines = 8 parents combined, highly segregating offsprings 
Actors	Farmers, scientists, techn. consumers & society	Farmers, technicians, breeders	Farmers, technicians, scientists
Traits	**Agronomic:** e.g. yield, earliness, vigour, diseases, fruit traits, **Analytics:** sugars, acids, volatiles + **Taste panels** organoleptic profiling	**Agronomic** e.g. yield, plant vigour, diseases incidence, missed plants, uniformity, overall score, market. fruits, waste fruits	**Agronomic** *Plant habitus* & vigour, ripening homog., healty, n° fruits, fruit weight, shape, size, color, firmness, overall score **Analytics** °Brix, pH
Actors/trait	**Agronomic** Farmers + Scientists **Analytics:** Scientists **Taste panels:** Consumers	**Agronomic** Farmers + Researchers **Visual evaluation:** Farmers	**Agronomic** Farmers + Scientists + Technicians **Analytics:** Scientists
Locations	**Two main (>1000 pl.)**: - Valencia (Med. coast) - Cadiz mount, Med. 160m + Some pilots (100-200 pl) 	**Five locations** - Rubano (North) - Sestola (Centre-North) - Molise (Centre-South) -Pollino (2 farms, South) 	**Four locations** - Padova (North) -Fermo & CREA-OF (Center) - Metaponto (South) 

8.2. Main results

8.2.1. Activities in Spain (UPV)

The breeding strategy in Spain involved the evaluation of the whole collection in the years 2018 and 2019, distributed across the two main locations (Valencia and Cadiz). Control varieties (in common in the main trials and also shared with the partners Italy) were included in the trials. Based on a two-step selection procedure (i.e. preliminary on-farm selection with farmers, complemented with simple fruit analytics and taste panels with consumers), 15-20 accessions per location were pre-selected each year

(Figure 24). The preselected varieties (and some controls) were re-evaluated in the trials in 2020, evaluating the same agronomic and composition traits of 2018 and 2019, and additional evaluation with HPLC analytics (sugars, acids, bioactive), GC/MS (volatiles) and trained taste panels (due to the covid-19 situation no on-farm testing with consumers was possible) (Figure 24).

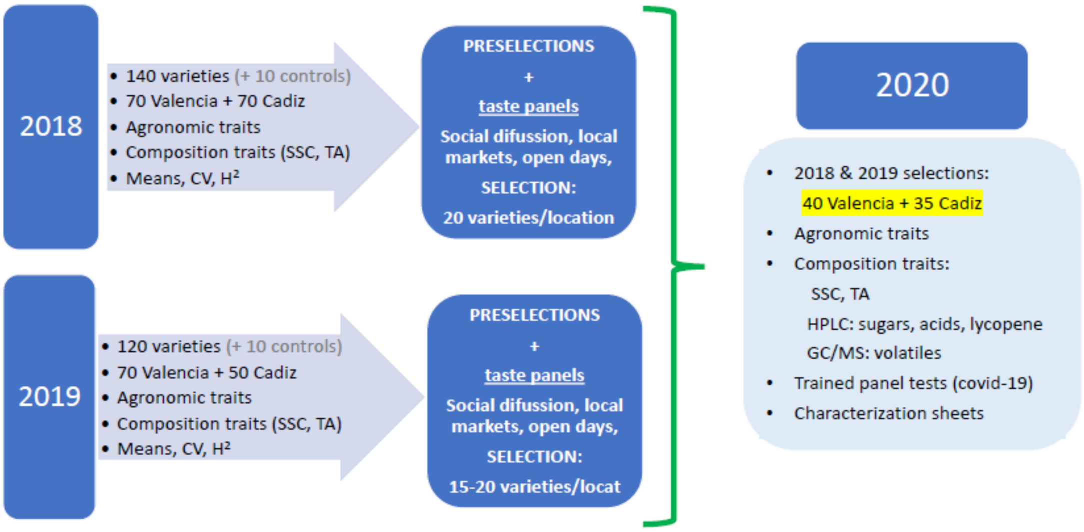


Figure 24 - Breeding workflow followed in Spain

<u>Phase 1: pre-selections in 2018 and 2019</u>

Our findings from 2018 and 2019 on-farm trials confirmed a considerable variation in many agronomic and fruit quality traits and enabled the identification of many cultivars with a satisfactory adaptation to organic farming and farmers´ preferences. In addition, participatory selection considered varietal groups by fruit sizes and typologies separately (Table 31). Thus, selection was based not only on agronomic criteria, but also encompassing as much varietal diversity (e.g. fruit quality and taste) as possible. Moreover, within each group of fruit sizes a considerable variation was found, which enabled the selection of 20 accessions in Valencia and Cadiz in 2018 and 20 and 15 accessions in Valencia and Cadiz, respectively, in 2019 (Figure 25, Table 31).

In this regard, yield was considerably higher in the big-sized and mid-sized fruit varieties than in small-fruited group. Many varieties showed yields higher than 4 kg/m^2 and, therefore, suitable for selection in terms of productivity (Table 31). Within the group of small-fruited varieties, many of them had yields higher 2 kg/m^2 and several of them reached 3-4 kg/m^2, giving the opportunity to select many accessions on the basis of productivity within this group. This trait, together with earliness, fruit morphology and plant management (data not shown) were used to perform preliminary selections.

In addition, the incidence of the main pests and diseases affecting tomatocrop was relatively low or negligible in both locations and years (Table 31), which could be due to the efficiency of crop rotation in the organic farms involved. Nevertheless, some accessions appeared to be affected by these diseases, which was used for additional on-farm selection. Finally, a wide variation was also found for taste/flavor traits, i.e. soluble solid content (SSC) and acidity (TA) (Table 31). These differences enabled the performance of a final round of preselection, prior to the panel tests and diffusion activities with consumers. Small-fruited accessions showed higher levels of soluble solids and acidity, although considerable levels were also found in many accessions within the groups of big-sized and mid-sized fruits (Table 31). The variation was large enough to perform selection within each group. Thus, many accessions within mid- and big-sized groups achieved levels higher than 6% in SSC and higher than 1% in TA and many small-fruited varieties even reached SSC higher than 7% (Table 31).

Table 31 - Summary of agronomic participatory evaluations and analyses in 2018 and 2019 in Valencia and Cadiz, and selected accessions after the evaluations with consumers (taste panels). Total mean, varietal means slots (minimum and maximum means), and the number (or %) of accessions showing a specific threshold are provided for each trait on within each year and location.

	2018				2019			
	VALENCIA		CADIZ		VALENCIA		CADIZ	
TRAITS [1]	**Mean (min-Max)**	**Nº access.**	**Mean (min-Max)**	**Nº access.**	**Mean (min-Max)**	**Nº access.**	**Mean (min-Max)**	**Nº access.**
>50 g group								
Yield (kg/m2)	3.15 (1.52-7.34)	18 (>4 kg/m^2)	3.53 (1.82-7.18)	22 (>4 kg/m^2)	3.43 (1.32-7.12)	19 (>4 kg/m^2)	3.64 (2.15-6,90)	19 (>4 kg/m^2)
Fruit weight (g)	79 (54-470)	14 (>100 g)	85 (61-512)	16(>100 g)	64 (51-380)	12 (>100 g)	68 (56-400)	11(>100 g)
L/W fruit (mm)	52/84		49/78		49/81		51/76	
TSWVincidence	Low	<10% access.	Nil	0% access.	Very low	<5% access.	Very low	<5% access.
ToMV incidence	Nil	0% access.	Nil	0% access.	Nil	0% access.	Very low	<5% access.
Tuta incidence	Low	<10% access.	Very low	<5% access.	Low-Medium	10-20% access.	Nil	0% access.
SSC (%)	5.8 (4.6-7.3)	12 (>6%)	5.6 (4.4-7.1)	15 (>6%)	5.5 (4.3-7.2)	11 (>6%)	5.6 (4.5-7.5)	14 (>6%)
TA (%)	0.78 (0.50-1.15)	7 (>1%)	0.72 (0.48-1.22)	8 (>1%)	0.75 (0.52-1.21)	9 (>1%)	0.69 (0.45-1.18)	7 (>1%)
Selected acc.	**8**		**10**		**7**		**5**	
25-50 g group								
Yield (kg/m2)	3.54 (1.10-7.65)	12 (>4 kg/m^2)	3.78 (2.21-8.05)	16 (>4 kg/m^2)	3.70 (1.20-7.85)	12 (>4 kg/m^2)	2.90 (2.21-6.75)	11 (>4 kg/m^2)
Fruit weight (g)	35 (29-48)	5 (>40 g)	34 (26-47)	9 (>40 g)	38 (25-50)	5 (>40 g)	34 (26-46)	6 (>40 g)
L/W fruit (mm)	38/56		37/58		35/52		34/55	
TSWV incidence	Low	<10% access.	Nil	0% access.	Low	<10% access.	Very low	<5% access.
ToMV incidence	Nil	0% access.	Nil	0% access.	Nil	0% access.	Nil	0% access.
Tuta incidence	Low	<10% access.	Nil	0% access.	Medium	20-30% access.	Very low	<5% access.
SSC (%)	6.1 (4.9-7.4)	10 (>6.5%)	6.0 (4.7-7.2)	9 (>6.5%)	6.0 (4.8-7.6)	11 (>6.5%)	5.9 (4.6-7.0)	8 (>6.5%)
TA (%)	0.82 (0.55-1.31)	6 (>1%)	0.80 (0.58-1.35)	8 (>1%)	0.78 (0.51-1.28)	6 (>1%)	0.80 (0.60-1.32)	7 (>1%)
Selected acc.	**7**		**6**		**7**		**5**	
< 25 g group								
Yield (kg/m2)	2.67 (1.15-3.56)	12 (>2 kg/m^2)	2.87 (1.82-4.21)	13 (>2 kg/m^2)	2.54 (1.10-3.16)	11 (>2 kg/m^2)	2.65 (1.80-4.1)	12 (>2 kg/m^2)
Fruit weight (g)	18 (3-25)	8 (>10 g)	16 (4-24)	10 (>10 g)	17 (4-24)	7 (>10 g)	14 (3-19)	9 (>10 g)
L/W fruit (mm)	35/39		34/40		32/41		25/34	
TSWV incidence	Nil	0% access.	Nil	0% access.	Nil	0% access.	Nil	0% access.
ToMV incidence	Nil	0% access.	Nil	0% access.	Nil	0% access.	Nil	0% access.
Tuta incidence	Nil	>10% access.	Very low	>5% access.	Nil	0% access.	Nil	0% access.
SSC (%)	6.8 (5.9-8.1)	10 (>7%)	6.9 (5.8-7.8)	12 (>7%)	6.5 (5.7-8.2)	9 (>7%)	7.1 (5.7-8.1)	11 (>7%)
TA (%)	0.91 (0.70-1.35)	7 (>1%)	0.89 (0.72-1.45)	8 (>1%)	0.85 (0.65-1.35)	7 (>1%)	0.91 (0.76-1.45)	9 (>1%)
Selected acc.	**5**		**4**		**6**		**5**	

[1] L/W: fruit length/width ratio, TSWV and ToMV incidence: incidence of Tomato Spotted Wilt Virus and Tomato Mosaic Virus (% of affected accessions, at least 2 plants within each accession), SSC: soluble solids content (%) and TA: acidity (%) measured with hand digital refractometer.

Broad sense heritability was moderate to high (0.44-0.76) for yield and fruit weight.
Particularly yield also showed higher coefficients of variation (CV), suggesting a remarkable response to selection for these traits (Table 32). The length/width ratio of the fruits showed the highest heritability among the studied traits and a moderate CV, indicating that this trait, as usually, concurs with fruit shape as a highly heritable trait, with a low impact of the environment or the year effect. Finally, moderate heritability values (0.48-0.62) were found in soluble solids (SSC) and acidity (TA), suggesting that selection of the best accessions for these traits could be moderately efficient, but a bit limited in the case of SSC due to their low CV (Table 32).

Table 32 – Heritability (broad sense, H^2) and coefficients of variation (CV) for different quantitative traits evaluated in 2018 and 2019 trials.

	H^2				CV			
Trait	**VAL 18**	**CAD 18**	**VAL 19**	**CAD 19**	**VAL 18**	**CAD 18**	**VAL 19**	**CAD 19**
Yield	0.74	0.71	0.76	0.69	85.1	73.2	86.6	71.2
Fruit weight	0.53	0.44	0.60	0.49	41.1	38.3	44.3	39.7
L/D fruit	0.81	0.85	0.92	0.88	35.6	36.6	37.8	36.7
SSC	0.51	0.56	0.55	0.48	19.5	22.1	21.9	18.2
TA	0.62	0.63	0.58	0.55	32.1	33.2	31.1	29.8

In the second step, within each year (2018 and 2019) and each location, preliminary on-farm selections were evaluated in the taste panels with consumers. Like in the brassica organoleptic evaluations, evaluation templates were also prepared for tomato, which included parameters such as aroma, juiciness, acidity, sweetness, firmness during mastication/ chewing, flavour persistence and overall evaluation. These activities involved more than 250 consumer and over 1000 evaluations (each consumer evaluated 4-5 varieties) in tasting sessions on-farm and in local markets. These evaluations were used to perform final selections within each year and location, to be re-evaluated in the final trials in 2020. Such activities also enabled social diffusion, reaching more than 1000 citizens, to explain the importance to preserve agrobiodiversity, local organic production, and to strengthen the link between farmers and consumers, as well as the main principles and objectives of LIVESEED (Figure 25).

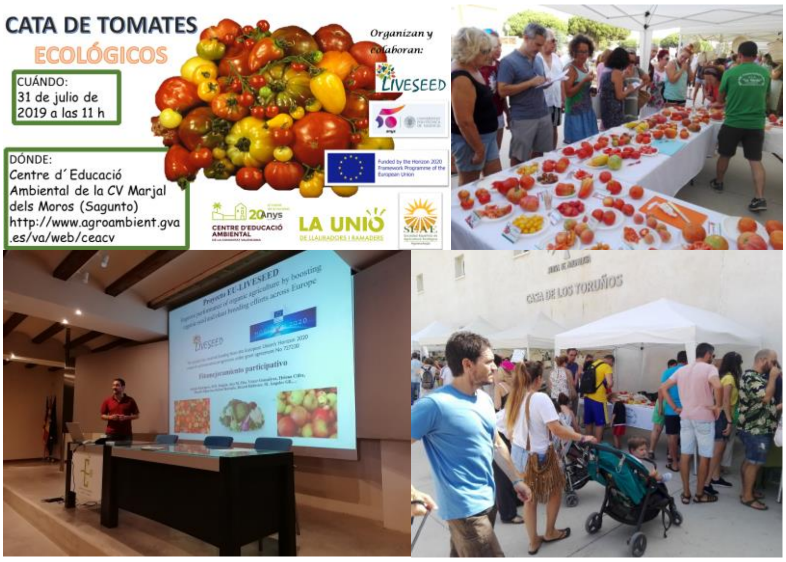
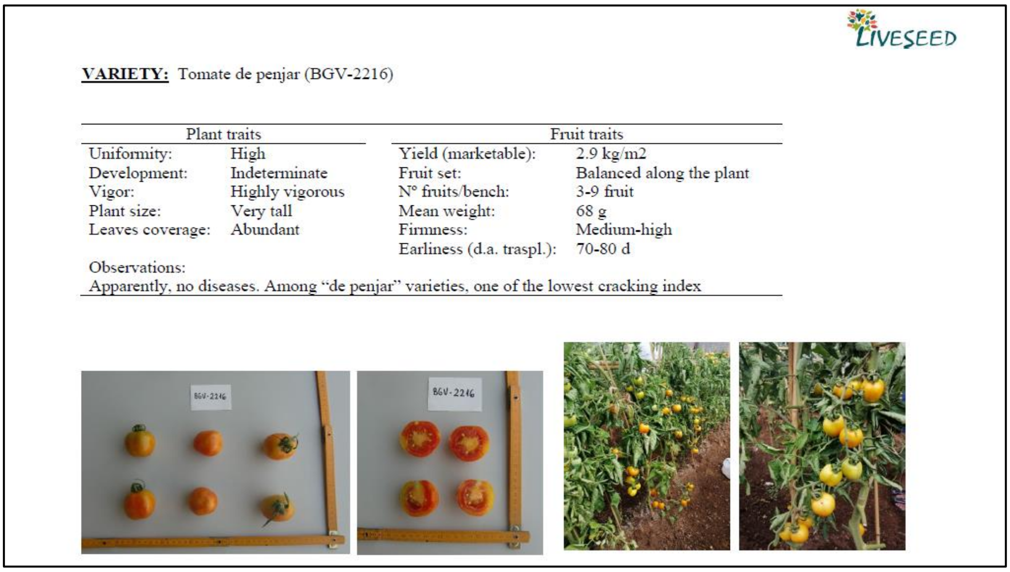


Figure 25 – Example of advertisements, social activities, diffusion, and taste panels with consumers in years 2018 and 2019 in open days and local markets in both locations (Valencia and Cadiz)

Phase 2: Final evaluations 2020

In the last year 2020, the varieties selected in 2018 and 2019 were re-evaluated to confirm their agronomic performance under organic and their SSC and TA levels, in order to estimate the efficiency of our pre-selections, to discard those varieties which finally did not confirm their previous evaluation, as well as to add more details and technical observations from farmers. Illustrative characterization sheets (done for the 2020 final accessions) are also being prepared, which will be available for organic farmers and networks created (Figure 26).



VARIETY: Tomate de penjar (BGV-2216)

Plant traits		Fruit traits	
Uniformity:	High	Yield (marketable):	2.9 kg/m2
Development:	Indeterminate	Fruit set:	Balanced along the plant
Vigor:	Highly vigorous	N° fruits/bench:	3-9 fruit
Plant size:	Very tall	Mean weight:	68 g
Leaves coverage:	Abundant	Firmness:	Medium-high
		Earliness (d.a. traspl.):	70-80 d

Observations:
Apparently, no diseases. Among "de penjar" varieties, one of the lowest cracking index

Figure 26 - Example of characterization sheet ("Tomate de Penjar" - UPV seedbank BGV-2216)

In addition, the activities in 2020 involved more detailed analytics of i) sugars (fructose, glucose and sucrose) and organic acids (malic and citric) by HPLC and ii) composition of the volatile fraction (key factor for aroma and flavour during mastication) by GC/MS (Table 33, Figure 27). These analytics were complemented by taste panel evaluations. Due to the covid-19 situation, this year taste panels were performed with trained panellists of our COMAV-UPV fruit quality unit. This also enabled to tune and to correlate more efficiently fruit composition with organoleptic quality.

We found a considerable range of variation in most quality composition traits and many varieties reached high values, confirming the efficiency of our selections from 2018 and 2019. Thus, regarding the measurements based on the refractometer, the soluble solids content (SSC) means ranged between 3.56% (worst variety) and 6.68% (best variety), and the quartile (25%) of the best accessions reached a mean value of 5.56% (Table 33). Similarly, mean levels in total acidity (TA) were comprised between 0.63% and 1.15% and the quartile of the best accessions achieved a mean value of 0.95%.

The HPLC analyses showed that fructose was the main sugar (variety means ranged from 14 g/L to 28 g/L), followed by glucose (range means of 9.5 to 23 g/L). As found in SSC, a remarkable variation was found for these sugars and total sugars, and the quartile (25%) of the best accessions reached mean values of 21.4 g/L, 17.4 g/L and 39 g/L for fructose, glucose and total sugars, respectively (Table 18). Finally, citric acid and, at a lesser extent, malic acid were the main organic acids in the composition of the evaluated varieties (Table 33), and a remarkable variation was also found, with mean values of the varieties in the best quartile reaching contents of 5.30 g/L and 1.28 g/L, respectively (Table 33). The levels of oxalic acid were very low and only a few accessions reached levels of 0.05 g/L. Furthermore, we also found positive and high correlation between SSC and total sugars (0.87) and moderate to high corelation between TA and total acids (0.60) (data not shown), which indicates that

refractometric measurements are a fast, suitable and efficient measurements of the
real content in sugars and acids, in particular in the former, which is of interest for farmers and breeders.

Table 33 - Summary of 2020 analytics in Spain (n=40 varieties, 5 data each)

Trait	Mean ± SE	Range (min-max)	Mean25% Best	Mean 25% worst
SSC (%)	4.58±0.05	3.56-6.68	5.56	3.88
TA (%)	0.81±0.01	0.63-1.15	0.95	0.70
SS/TA	5.7±0.1	4.3-8.9	6.7	4.9
Fructose (g/L)	18.1±0.2	13.8-27.9	21.4	15.0
Glucose (g/L)	13.7±0.2	9.5-23.2	17.4	10.6
Total sugars (g/L)	31.7±0.4	24.1-51.2	39.0	26.1
Citric acid (g/L)	3.90±0.08	2.60-8.86	5.30	2.98
Malic acid (g/L)	0.72±0.03	0.13-1.66	1.28	0.17
Oxalic acid (g/L)	0.032±0.001	0.010-0.056	0.046	0.018
Total acids (g/L)	4.65±0.08	3.45-9.34	6.19	3.69
Ascorbic acid (mg/L)	138±6	8-288	231	37
DH ascorbic acid (mg/L)	104±5	28-400	193	46
Lycopene (mg/kg)	40.0±1.8	0.3-81.6	69	11
B-carotene (mg/kg)	0.476±0.072	0-5.438	1.391	0.031

In the same trend, our 2020 analytics found a considerable variation for bioactive compounds, enabling the identification of many varieties with high levels in ascorbic and dehydro-ascorbic acids (i.e. vitamin C), reaching mean values of 231 mg/L and 193 mg/L in the best quartiles of varieties, respectively (Table 33). Also, a remarkable variation among varieties was found in Lycopene and b-carotene levels, with ranges of 0.3-81.6 mg/kg and 0-5.4 mg/kg, respectively for each carotenoid (Table 33). All these results indicate that there are ample opportunities to select varieties on the basis of their levels in vitamin C and the main carotenoids as a high added value for our varieties adapted to organic.

Finally, we have found a considerable variation in terms of volatile composition of the varieties studied in 2020 (Figure 27). In this regard, in future steps, the taste and flavour compounds (i.e. sugars, acids, volatiles) will be compared to the 2020 organoleptic profiling (and those from 2018 and 2019) in order to identify which compounds can be considered key factors for consumers´ preferences and specific quality profiles.

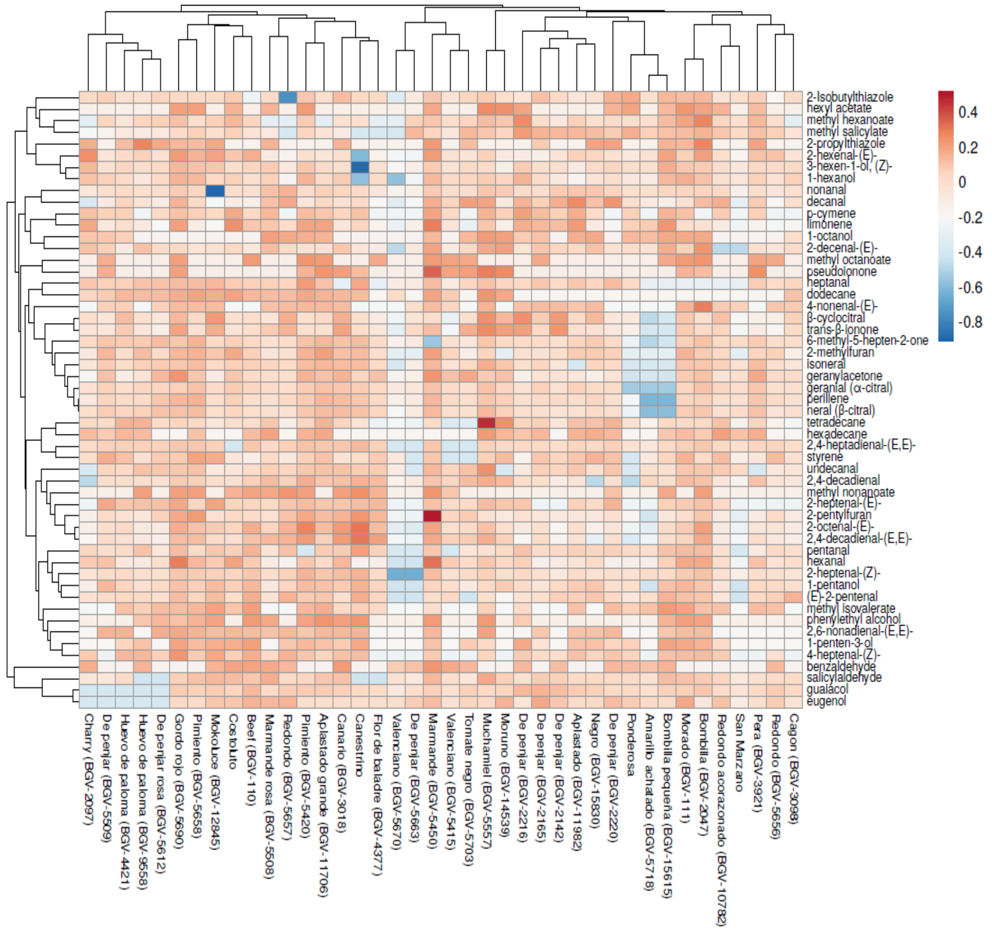


Figure 27 - Volatile profile (GC/MS) heatmap of the 40 varieties (preliminary identification 52 volatiles, expected > 150 volatiles).

8.2.2. Activities in ITALY (RSR/Arcoiris)

In Italy, RSR and Arcoiris conducted a 3-years participatory-evolutionary breeding programme on four organic farms and one experimental farm/research station managed organically (see sections 3.5 and 8.1 (Figure 28). Starting with the Ox-Heart (Cuore di bue) tomato CCP population developed during the FP7 project SOLIBAM, two cycles of participatory selections were carried out in 2018 and 2019. The selection was carried out on 400 plants per location, grown under organic conditions and according to the management prevalent on each farm.

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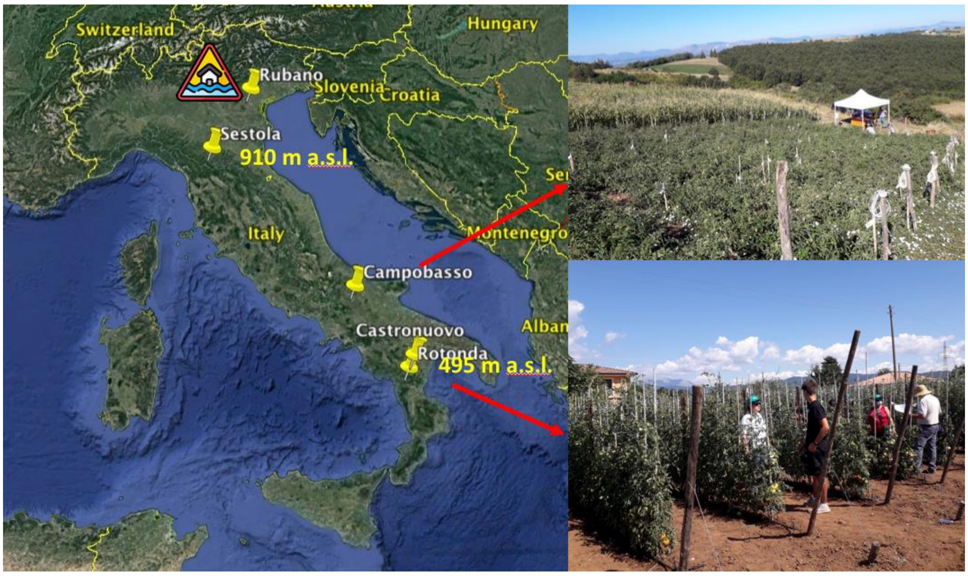

Figure 28 - The five locations hosting participatory selection and comparative trials. The farms were chosen to encompass a wide range of geographical conditions and management practices.

In 2018, in each location, the 20 best plants were selected after a participatory evaluation by local farmers (value of cultivation based on agronomic quality), whilst in parallel one fruit per plant was collected to maintain the overall genetic diversity of the original population.
In 2019, two separate 400 plant plots were established on each farm: one deriving from the complete population (natural selection); the other from a balanced population (farmer selection) created by mixing equal number of plants of the offspring from the 20 best plants selected the previous year.
The participatory evaluation was conducted on the "farmer selection" population, and seed collected from the best 20 plants, as well as from all of the 400 plants of the "natural selection" population. Due to flooding in the Veneto region, the Rubano farm suffered complete crop failure and, therefore, the selection process was interrupted here, and the number of locations was reduced to four.
As a consequence, by the end of 2019 we had generated:

- 4 random populations (2 seasons of natural selection)
- 4 farmer selection populations (2 seasons of farmer selection)

In 2020 comparative trials were conducted in all four locations, in order to assess whether specific adaptation with measurable effect had occurred during the two previous growing cycles and rounds of selection. This was done by setting up a randomised block design with two replications including (Table 34):

- 4 natural selection SOLIBAM Cuore di Bue populations
- 4 farmer selection SOLIBAM Cuore di Bue populations
- 4 local varieties chosen by farmers as their local check (one per location)
- 1 open pollinated modern variety chosen by farmers as control
- 1 Cuore di Bue type F1-hybrid chosen by RSR in cooperation with the breeder (ISI sementi)

Table 34 - Random populations, farmers' selections and controls included in the final comparative trials.

Entry Name	Category
MOLISE RANDOM	SOLIBAM tomato Random Population
ROTONDA RANDOM	SOLIBAM tomato Random Population
CASTRONUOVO RANDOM	SOLIBAM tomato Random Population
SESTOLA RANDOM	SOLIBAM tomato Random Population

MOLISE FARMER SELECTION	SOLIBAM tomato Farmers’ Selection Population
ROTONDA FARMER SELECTION	SOLIBAM tomato Farmers’ Selection Population
CASTRONUOVO FARMER SELECTION	SOLIBAM tomato Farmers’ Selection Population
SESTOLA FARMER SELECTION	SOLIBAM tomato Farmers’ Selection Population
KERO F1	Local check Molise – F1 Hybrid
POMODORO DI ROTONDA	Local check Rotonda – Local variety
ROSA DI CASTRONUOVO	Local check Castronuovo – Local variety
COSTOLUTO DI PARMA	Local check Sestola – Conservation variety
BELMONTE – SEMENTI BIOLOGICI	Modern Control – Open Pollinated
DEKO F1 – ISI SEMENTI	Modern Control – F1 Hybrid

The 14 entries were planted in plots of 20 plants in a Randomised Complete Block Design with two replications. All plots were assessed in participatory evaluations during field days held in the four farms between August and September 2020. Participating farmers, researchers and citizens assessed for: disease resistance, plant vigour, yield potential, homogeneity and a general score. In parallel, hosting farmers and local technicians harvested all the ripe fruit of all the plants in each plot recording number and weight of marketable and non-marketable berries.

Collected data were submitted to ANCOVA using missing plants as covariates (Genstat software). PCA was performed using GGE-Biplot R package to explore G x E interactions. Preliminary results were presented at the LIVESEED-Eucarpia Conference in March 2021. A shift between Farmers’ Selections and Random Populations was visible for potential yield at the first harvest (marketable + non marketable berries) (Figure 29), suggesting farmers were selecting for earlier maturing genotypes during the two previous growing cycles.

The material resulting from the trials will be destined to different uses: SOLIBAM Ox-heart random populations multiplied in 2018 and 2019 are already available from the RSR's Community Seed Bank to farmers and growers (via MTA). The first accessions of the SOLIBAM Cuor di Bue Random Populations were distributed in April 2021. SOLIBAM Cuor di Bue farmers' selections will be used for further breeding and evaluation by Arcoiris and RSR, with a view of registering the most promising ones as Organic Heterogeneous Material starting from January 2022. As the 2020 comparative trials were purely agronomic and for participatory visual evaluation, further characterisation work on morphological traits (fruit shape, size, etc.) and level of intra-population variability is needed for full scale commercial use.

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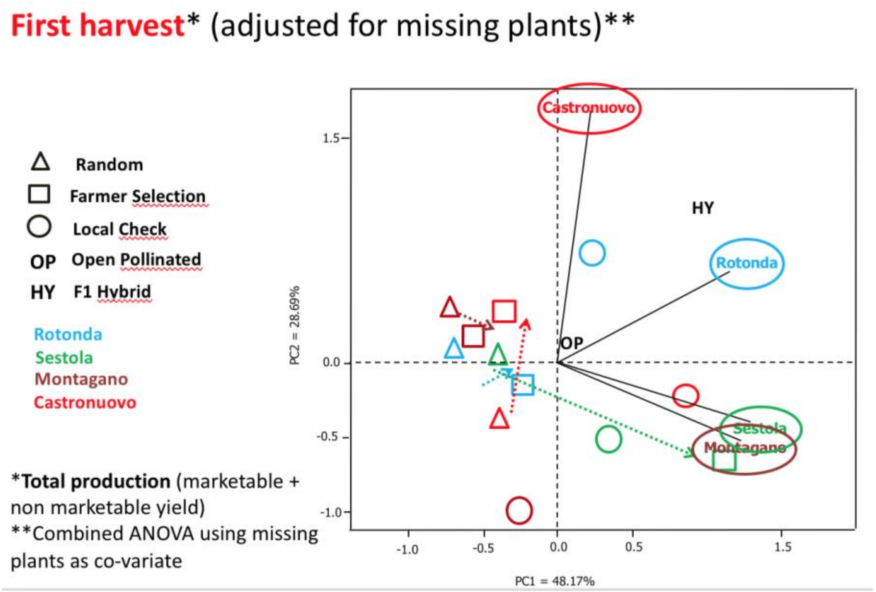

Figure 29 - GGE Biplot (all entries and all locations) for potential yield at first harvest (marketable + nonmarketable) adjusted for missing plants. The Biplot shows a yield increase in the farmers' selections within the original population, compared to the unselected population (random).

8.2.3. Activities in ITALY (CREA-OF)

Activity at CREA-OF in 2017-2019

In 2017, 400 G3 plants (4 plants for each single cross obtained) of a tomato MAGIC population were cultivated, developed by ISI Sementi Seed Company (Italy) (scheme detailed in Figure 30) were cultivated at the Research Centre for Vegetable and Ornamental Crops (CREA) on the Monsampolo organic vegetable long-term field experiment—MOVE LTE (AP, 42◦53' N, 13◦48' E). The MOVE LTE reproduces a typical cropping system of an organic vegetable farm (four-year crop rotation with four cash crops and three different agro-ecological service crops), including the most important crops in the area. Agronomic management follows an agro-ecological approach based on both conservation tillage and crop diversification strategies.

From the 400 MAGIC population phenotypically evaluated a total of 30 plants were selected based on the following main agronomic traits: plant habitus, time of flowering, ripening time, leaf and fruit shape, firmness, shelf-life, colour, ◦Brix and pH. The selection was made considering the initial single-crosses, thus each of the 30 plants selected represent different crosses obtained at the G3 generation cycle. Seeds of all the 400 plants, enclosed the 30 selected ones, were collected, and used in the 2018 trials.

In the 2018 summer season, 400 G4 individual plants from the MAGIC, one offspring plant from each of the 30 selected G3 and 25 tomato local varieties from Italy and Spain (replicated twice) were cultivated (12 x 40 rows and lines) for a total of 480 plants. A partially replicated experimental design was adopted. Based on the phenotypical evaluation, 22 G4 plants were selected, and the seeds (G5) were collected for the next trials.

In the 2019 summer season the G5 progenies of the 22 selected G4 plants (20 plants from each selected G4 plant) were cultivated and phenotypically evaluated (Figure 31).

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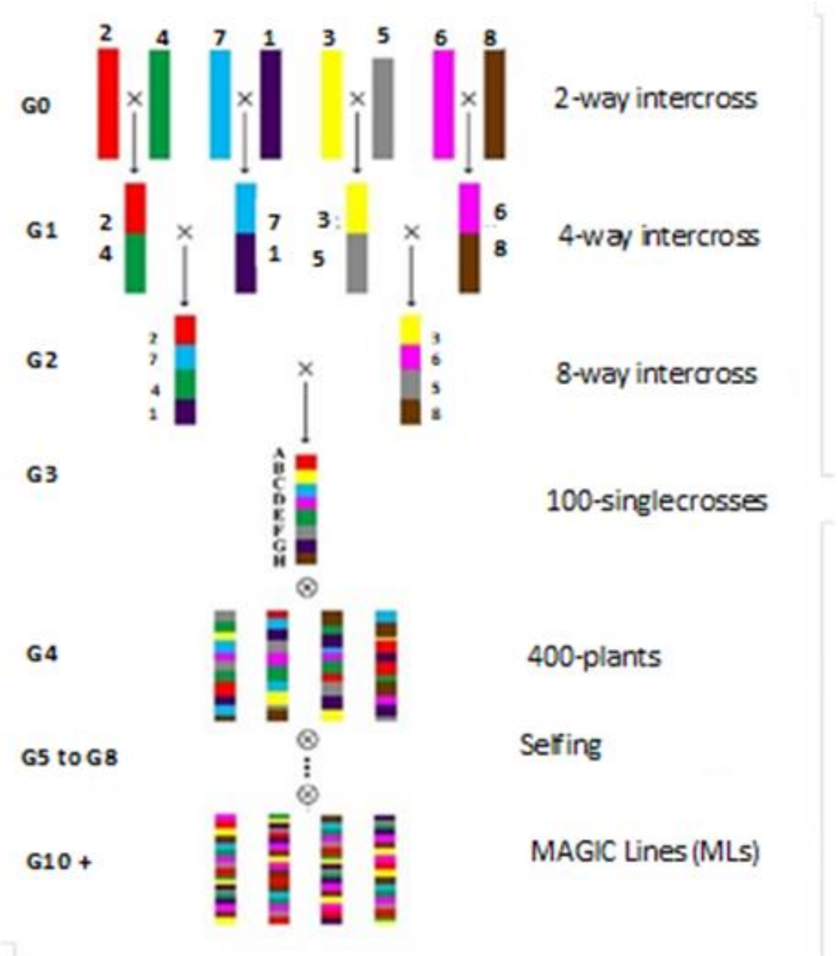

Figure 30 - Generation scheme of crossing and selfing, followed by the CREA-OF with the tomato MAGIC population used in their experiments.

Figure 31 - Plants selected at CREA-OF

The 22 selected tomato plants (G5), together with the 8 parental lines, were analysed with molecular markers to detect resistance genes as FOL = *F. oxysporum* f.sp. *Lycopersici* (race 2), FORL = *F. oxysporum* f.sp. *radicis-lycopersici*, Mi = Meloidogyne spp., TSWV = Tomato spotted wilt virus; Pto = *Pseudomonas syringae* pv. *tomato*, and fruit shape Ovate gene (pear shape) (Figure 31). The molecular analysis confirmed the phenotypic selection (Table 35).

Table 35 - Presence of resistance and fruit shape genes in the 22 selected plants and 8 parental lines of the MAGIC population.

		Resistance	**Fruit shape**
Selected	1	Meloidogyne; FORL; TSWV	
	2	Meloidogyne; TSWV	
	3	Meloidogyne; FORL; TSWV	
	4	Meloidogyne; P. syringae; TSWV	
	5	Meloidogyne; FOL; FORL; TSWV	Pear shape
	6	Meloidogyne; FOL; FORL; TSWV	Pear shape
	7	Meloidogyne; FOL; FORL; TSWV	Pear shape
	8	Meloidogyne; FORL; P. syringae; TSWV	Pear shape
	9	Meloidogyne; FOL; P. syringae; TSWV	
	10	Meloidogyne; FOL; FORL; TSWV	Pear shape
	11	Meloidogyne; P. syringae; TSWV	Pear shape
	12	Meloidogyne; FOL; FORL; P. syringae; TSWV	
	13	Meloidogyne; FOL; FORL; P. syringae; TSWV	Pear shape
	14	Meloidogyne; FOL; TSWV	Pear shape
	15	Meloidogyne; FOL; FORL; P. syringae; TSWV	Pear shape
	16	Meloidogyne; FOL; FORL; P. syringae; TSWV	Pear shape
	17	Meloidogyne; P. syringae; TSWV	Pear shape
	18	Meloidogyne; TSWV	
	19	Meloidogyne; TSWV	
	20	Meloidogyne; FOL; FORL; TSWV	Pear shape
	21	Meloidogyne; FOL; FORL; P. syringae; TSWV	
	22	Meloidogyne; FOL; FORL; TSWV	Pear shape
Parents	23	Meloidogyne; FOL; FORL; P. syringae; TSWV	
	24	Meloidogyne; FORL; TSWV	
	25	TSWV	Pear shape
	26	Meloidogyne; TSWV	
	27	Meloidogyne; FOL; FORL; TSWV	
	28	Meloidogyne; FOL; FORL; P. syringae; TSWV	Pear shape
	29	Meloidogyne; TSWV	Pear shape
	30	Meloidogyne; FORL; TSWV	Pear shape

PPB at farms in 2018-2019

In 2018 the same material cultivated at CREA-OF, in particular 400 (SG4) individual plants from the MAGIC, 30 selected plants (SG4) and 25 tomato local varieties from Italy and Spain (replicated twice) were grown at three certified organic farms. In each trial, the single plant evaluation was visually performed by farmers, researchers, and technicians, who individually indicated a score ranging from 1 (bad value) to 4 (very good value) (Figure 32).

Figure 32 - PPB at organic farms in North, Central and South Italy

The participatory evaluation in the three organic farms allowed to select different genotypes particularly characterized by plant habitus and fruit colour. The parameters with a higher variability among the three locations were plant vigour, health and production rate (Table 36). In particular, the plants selected in the North showed a higher vigour, healthier plants, higher production rate and mainly red fruits, while the ones selected in South Italy showed fruits with higher solid soluble solids content (Brix). Among all the material evaluated by PPB, 30 plants were selected in each organic farm.

Table 36 - Mean values in 2018 in participatory evaluations in three organic farms (range from one to four except for fruit firmness, which was expressed as kg/cm2 and solid soluble as ◦Brix) corresponding to different plant parameters (mean ± standard deviation).

Organic Farm	Plant vigor	Plant health	Abundance fruiting	Fruit size	Homogeneity ripe fruit	Fruit firmness	Puffiness appearance	Solid soluble
North	4.13±0.57	3,53±0.68	4,20±0.85	2,97±0.81	3,37±0.89	6,20±0.93	2,83±0.75	5,22±0.75
Center	3.10±0.71	2,80±0.85	3,50±0.63	2,80±0.61	3,20±0.85	6,17±1.61	2,97±0.89	5,47±0.99
South	3.03±0.85	2,83±0.79	3,30±0.75	3,10±0.85	3,73±0.74	6,04±1.77	3,23±0.77	5,71±0.86

In 2019, at the three organic farms were cultivated and evaluated 32 plants for each individual plant selected in 2018. We consider the 32 plants as belonging to the same family, and therefore, 20 families were cultivated at the organic farms in North and Central Italy, while 24 families were cultivated at the farm in South Italy. The participatory evaluation allowed to select families and individual plants within the family in the two organic farms of Central and South Italy. The trial in the North of Italy failed due to environmental adverse conditions, thus seeds were not collected. Seeds of the selected plants were collected for further evaluation. Specifically, 9 families from 2018 and 10 individual plants planted in 2019 were selected in the Central Italy farm, while 10 families from 2018 and 16 individual plants planted in 2019 were selected on farm in South Italy. Seeds of the best families were collected by mass selection.

In 2020, 8 and 10 families selected in 2019 (32 G6 plants per family), were cultivated at the organic farm in the Central and South Italy, respectively. Each farmer collected seeds:

- *Central Italy*: the farmer selected the best plants among the 10 families cultivated and created a heterogeneous material having specific agronomic and qualitative characteristics and determinate plant habitus. The farm production is based on short chain and the consumers really appreciated the developed material.

- *South Italy*: the farmer performed mass selection of some families selected based on uniformity, homogeneity, health, productivity, and other characteristics important for the local market. He collected seeds.

Activities in 2020-2021 at CREA-OF

In 2020, the material selected at the two organic farms in 2019 were cultivated also at CREA-OF due to COVID-19 emergency. A total of 13 families selected in 2019, 4 from CREA and 9 from PPB activity at the organic farms (i.e. 4 from Central Italy and 5 from South Italy organic farms), were cultivated and phenotypically evaluated based on the following traits: habitus, number of plants corresponding to the typology selected, homogeneity of ripening, vigour, health, abundance and uniformity of fruiting, fruit shape, fruit size, fruit colour at ripening, total yield, fruit consistency, and ºBrix (Table 37). Of these 13 families, 10 families (G7) were chosen mainly based on the level of uniformity and correspondence to the typology selected during the project. Seeds of the 10 selected families, the 8 parental lines and 400 RILs (S11) developed from the MAGIC population in collaboration with ISI Seed Company at Fidenza (Italy), were sown in plastic pots filled with sterilised peat-based soil mixture and the young leaves were collected for DNA extraction. The genotyping of the families, RILs and parental lines, by Single Primer Enrichment Technology (SPET) is ongoing at IGATech. The genotyping will allow to identify parental-specific SNP markers and multi-site loci for the segregation analysis. The 10 selected families (10 plants per family) and 400 RILs (2 plants per RILs) will be phenotypically evaluated in open field at CREA-OF during the 2021 summer season.

Table 37 - Phenotypic data of fruit traits in the 13 families evaluated

Accessions ID	FNo	FW (g)	Yield (Kg)	FFirm (Kg/cm^2)	FPuff	SSC (°Brix)	FCol	N° of good characteristics	Health
MO 1-12	25.7	75.5	1.9	2.6	2	4.7	R	2	3
MO 7-29	31.0	65.8	2.0	4.1	1	5.5	R	8	4
MO 9-39	114.7	30.8	3.5	2.7	2	4.4	A	3	4
MO 9-39A	81.7	35.5	2.9	3.1	3	3.6	A	0	3
ME 8-4	26.3	51.7	1.3	2.1	3	4.7	A	1	2
ME 10-14	36.3	24.5	0.8	3.4	1	4.4	R	1	3
ME 11-38	18.0	30.3	0.6	2.2	1	5.7	R	2	2
ME 12-10	58.7	50.2	2.9	4.2	3	4.0	R	4	2
ME 10-37 5B	150	26.3	4.1	3.2	3	6.2	R	5	4
FE 4-32	35.7	36.8	1.4	3.8	2	4.9	R	0	2
FE 11-14-2	38.3	61.5	2.4	3.7	2	4.9	R	2	3
FE 3-34-2	31.0	67.8	2.1	1.5	2	5.4	R	0	3
FE 8-23-12-	48.7	79.0	3.8	4.1	2	5.7	R	6	4

FNo: mean number of fruits per plant, FW: mean fruit weight, Yield: mean yield per plant, FFirm: fruit firmness, FPuff: fruit puffiness (1 min, 5 max), SSC: soluble solids content, FCol: fruit colour at ripening, Health: health ranking of fruits produced (1 min-poor, 5 max-excellent)

The accessions MO 7-29 selected in Monsampolo del Tronto (AP) and FE 8-23-12 selected in Fermo (FM) could be considered ''varieties'', well adapted to organic production and low input crop management.

8.3. New cultivars & breeding material for potential further use

The following tomato cultivars and breeding lines are planned to be released and/or further used for by the partners in LIVESEED subtask 3.4.5 (Table 38)

Table 38 - Summary of exploitation plans for breeding materials from the LIVESEED subtask 3.4.5 on tomato

Partner	Planned cultivar release	Planned year of release	Further use of the material
UPV	The first comprehensive and public list of dozens of Spanish heirloom cultivars and landraces identified for their good adaptation to organic farming under Mediterranean conditions. Also, characterization sheets for each cultivar will be available, including brief description of yield, fruit traits and quality (taste, flavour, composition)	2021: diffusion of the list of selected cultivars	There is a remarkable amount of small/medium organic farmers and organic consumers interested in the recovery of the morphology and "taste of the past" cultivars (i.e. ancient heirlooms). The COMAV-UPV seedbank, founded in the 80s will provide the seed service for the selected cultivars. UPV will continue the research on this material and further use the broad range of accessions conserved in COMAV-UPV seed-bank.
RSR	Well-performing, open pollinated, Ox-Heart tomato cultivars for organic production adapted to different pedo-climatic conditions in Italy. The SOLIBAM Ox-Heart tomato has a 10-year history of organic breeding: parental material was evaluated under organic conditions. All selection and multiplication stages following the initial crosses were carried out in organic farms. Ox-Heart tomato are a high value tomato for the Italian fresh market.	2021: Preparation for notification of OHM for 2022 seed multiplication 2022 - onwards: SOLIBAM Ox-heart OHM seed marketing through Arcoiris' catalogue	Monitor evolution of the population and keep selecting (positive and negative selection)
CREA-OF	The tomato MAGIC population developed and evaluated is a powerful breeding material to create varieties adapted to different environmental condition and organic management techniques. The 10 families selected are under evaluation other than for important agronomic traits also for the uniformity and stability characteristics.	2021: evaluation of the families and RILs developed during the project 2022: list of varieties, HMs and RILs adapted to organic management cultivation will be released. Seeds will be available for organic farmers.	The developed varieties adapted to organic production and low input crop management, represent a stable, long-lasting collection and an important genetic resource both for scientists and farmers communities

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8.4. Multi-actor activities: networks developed and participatory activities

In each project, the networks were developed differently. In Spain the network consisted of a coordinated and well-balanced mixture of university researchers, farmers, cooperatives´ technicians and consumers. In the Italian initiatives, the networks consisted of researchers and farmers mainly. In each of the networks, there was a strong focus on finding suitable locally adapted varieties with a good balance in yield, agronomic performance, taste and nutritional quality.

Each of the three sub-projects organised comprehensive on-farm trials, with a considerable number of farmers and agricultural technicians to perform efficient participatory selection focused on agronomic, technical and scientific perspective. In Spain, also consumers were involved in the subsequent selection process. The way farmers were involved in the activities differed per project. This allowed the development of specific protocols for involving other actors in participatory breeding, depending on the goals set, resources and available knowledge, agronomic and socioeconomic (local) preferences, and the type of actors involved. Finally, the activities performed have enabled the development of new networks and reinforced already existing ones, as well as made different actors aware of their role and relevance in the breeding process and the agri-food chain.

Also, in 2021, a common workshop with the Horizon2020 sister project BRESOV (LIVESEED-BRESOV tomato joint meeting, February 22nd 2021) aimed at organic tomato breeding was organized. This meeting enabled us to exchange the latest results between the involved partners and to widen approaches to face future challenges in organic breeding of this vegetable.

9. Integrated summary of the main issues of the five crops, discussion, conclusions and future initiatives

9.1. White lupin

The research work on white lupin produced the following achievements:

- Development of genetically broad-based pre-breeding material for European breeding of the crop, comprehensive composite cross population and selection of advanced lines for cultivar registration;
- Development of screening tools for anthracnose resistance in the field and under controlled conditions, and of NIRS for quality traits,
- Evaluation of many inbred lines for key traits and target environments;
- The generation of experimental data that could allow for the genome-enabled prediction of important traits that may be expensive to be evaluated phenotypically, and produced genotyping data that could be used for future predictions relative to lines that did not undergo evaluation;
- Stimulation of the future development of at least 10 evolutionary populations adapted to specific climatic regions and growing conditions, using the composite cross population distributed to several partners;
- New collaborations and joint research initiatives among several partner institutions within the project and even outside the project.

Future research initiatives, including project partners that contributed to the evaluation of inbred lines and/or received the composite cross population in order to develop adapted populations, could aim

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to fully exploit the different components of the novel genetic base (evaluated inbred lines; genotyped but non-evaluated inbred lines; CCP) and the generated scientific knowledge, by:

- assessing the agronomic value of each evolutionary population in its target conditions with respect to: (i) the initial composite cross population that generated it (thereby measuring the adaptive gain achieved through evolutionary adaptation); (ii) the best-performing inbred line as determined by earlier testing in the target conditions or as predicted from evaluation data of other environments that are not too different from the target environment. The latter aspect has high scientific value: to understand under which conditions which of the two types of organic varieties for inbred crops (pure lines, or evolutionary populations) has higher potential for organic farming
- assessing the evolution of alkaloid content in CCP populations, in which bitter-seed mutants with selective advantage may increase their frequency within the originally sweet-seeded population and lead, in time, to crop alkaloid content to exceed acceptable levels for feed or food use. This issue is relevant for the design of procedures for exploitation of lupin populations, which should be based on the same mutant for low alkaloids.
- assessing the value of QTLs and genomic predictions for specific resistance (anthracnose) and quality traits (protein content, low alkaloid level) and/or growing conditions (drought, calcareous soil, winter or spring sowing), by evaluating the predictivity of superior genotypes on the ground of available genotyping data for inbred lines that were not evaluated, along with top-performing lines as emerged from the earlier evaluation for the specific traits/environments. This work may also allow the assessment of the cost-efficiency of genome-enabled selection relative to phenotypic selection.
- include some additional key traits for the evaluation of material and the development of genomic predictions. Tolerance to low winter temperatures (assessed in controlled conditions) is one such trait, given its importance for autumn-sown crops in southern and western Europe (where autumn sowing is expected to raise crop yields and the crop ability to control weeds). Adaptation to intercropping with cereals might be another key trait for organic farming.
- involve farmers into participatory selection activities, by including their agronomic preferences into the evaluation initiatives relative to evolutionary populations and inbred lines

Through the above-mentioned activities, elite inbred lines could be registered as varieties for formal seed systems, and composite cross populations could be notified and grown and spread through formal or informal seed systems in different European target regions.

9.2. *Brassica* vegetables

The main achievements of the Brassica sub-task were the following:

- Building a network of breeders, researchers and producers interested in testing and developing organic (cell-fusion free) *Brassica* cultivars; jointly developing evaluation protocols for a range of *Brassica* crops (Kohlrabi, broccoli, cauliflower and head cabbages)
- Evaluating, often for the first time, organic *Brassica* cultivars/breeding lines in Southern Europe that had been developed in Northern Europe; identifying material with commercial potential and, if not available, collecting data on the deficits of the current material.
- Assembling positive lists of cell-fusion free *Brassica* cultivars for both central and southern Europe

To this end, more than 35 trials were conducted throughout Europe. It was shown that organic OP cultivars with commercial potential are already available in kohlrabi as well as in red, white, pointed and Savoy cabbage. In head cabbage, the cultivars' list is long enough to make a choice according to the growing period and consumers' demands. However, since these types of cabbages are usually stored over the whole winter in Northern Europe, the need for organic cultivation in Southern Europe is not high. Regarding kohlrabi, which is being exported to Northern Europe in winter, two organic open-pollinated cultivars were identified as promising for cultivation in winter/early spring. On the other side, organic breeding material of broccoli and cauliflower were shown to be insufficient for commercial cultivation in Southern Europe. This is probably due to the strong genotype x environment interaction in both crops on the one hand, and on the other hand due to the overall challenges related to breeding open-pollinated broccoli and cauliflower cultivars. Thus, it seems essential to select breeding material in the target environments in an early stage of the breeding process. Some OP varieties appeared to show more potential than others.

Future work within the organic *Brassica* network should include:

- Establishing a durable trial network in Southern Europe (with connections to breeding initiatives in northern Europe) in order to allow variety development for Southern European conditions, including:
  a) the definition of trait profiles for the different regions/cultivation times, but also for different market chains;
  b) field visits and an active exchange about breeding and trial activities;
  c) integration of specific collaborations between partners, e.g. in particular laboratory analyses for nutritional quality and taste evaluations
- Integrating the entire value chain in order to promote organic (cell-fusion free) *Brassica* cultivars through dissemination and communication activities towards organic consumers, traders and growers' associations. The organic value chain needs to move closer together and contribute to financing organic breeding activities in order to scale them up.

9.3. Apple

The research work on apple produced the following achievements:

- Launch of the European Participatory Organic Fruit Breeding Network, the EURO FRUITS NETWORK (EUropean RObust FRUITS) and Balkan network on the conservation and sustainable use of plant genetic resources to foster networking and collaborations in apple and other fruit breeding across Europe;
- Protocol for on-farm testing of candidate apple lines as tool for collaborative on-farm testing of new candidate lines of fruit crops;
- Apple lines from collective organic on-farm testing ready for variety registration.

The next step towards market introduction of the two selected lines that resulted from the on-farm testing includes (i) networking with the retail sector and (ii) set-up an extended trial cultivation (ca. 4000 trees) with the possibility of test sales. The registration of the variety can take place at the same time as the propagation of the trees. A stakeholder group has been formed to launch the market introduction of these new varieties. Members of the group are producers as well as companies involved in the distribution and sale of organic food. The bioverita association (organisation that certifies with private label varieties from organic breeding) is also a member.

Future work within the organic apple network should include:

- Development of a long-term strategy to save, spread and exchange knowledge, data as well as genetic material.
- Active support of trust building as essential element to allow the established networks to function smoothly.
- Translation of the protocol for on-farm testing of candidate apple lines as important tool to other fruit crops to allow fast and efficient evaluation of the cultivation and market potential of new candidate lines for different fruit species. This allows a smoother testing of new organic variety candidates and therefore important to support the work of organic fruit breeders.

9.4. Wheat

The research work on wheat produced the following achievements:

- Increased knowledge on common bunt resistance genes in wheat;
- Identification of marker-trait associations in GWAS that can be further exploited to develop markers of marker-assisted selection;
- New varieties and populations with improved bunt resistance have been developed and released based on LIVESEED research;
- Optimisation by AGROLOGICA of a system to screen wheat germplasm with a collection of different virulence isolates, and thereby to identify which resistance genes breeding lines contain. This can be offered as a service to plant breeders working with common bunt, as well as geneticists and plant pathology researchers;
- A new Community Seed Bank, LANDSORTEN, has been created in Denmark with organic farmers, as well as with farmer members from the UK, Belgium, Sweden, Norway, Germany and Finland, as a direct result of LIVESEED.

Before LIVESEED, resistance breeding by many organic breeders (e.g. AGROLOGICA, CULTIVARI and DOTTENFELDERHOF) was based on crossing well adapted varieties with bunt resistant germplasm in order to develop well adapted resistant varieties. We have learned that this approach can be improved. If breeders don’t know which resistance gene they use, or how many resistance genes a given variety has, then the resistance may not be durable in the field. The winter wheat variety Butaro with resistance gene Bt7 and Tilliko with BtZ are very resistant in the nursery, but after a few years in the field, these varieties lose their resistance as virulence tends to develop against many of the resistance genes used in organic breeding. It is therefore crucial for organic breeders to be able to test the bunt resistance to different virulence races or have access to genetic markers.

Future work within the organic wheat breeding network should include:

- Application of MAS for common bunt resistance selection;
- Strengthening of the collaboration between researchers and breeders in order to develop genetic tools of practical interest to the breeders;
- Expanding the network of organic cereal breeders that test their breeding material for resistance to common bunt with the screen system optimised in LIVESEED.
- Developing Organic Heterogeneous Material (CCPs) with bunt resistance. This poses a new challenge with respect to bunt. It is commonly accepted that CCPs may have more durable resistance to diseases. The CCPs can be developed from resistant parents, but will contain also some susceptible lines. It is unknown how susceptible lines can be accepted within a population before bunt will become a problem. New research should investigate this.

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9.5. Tomato

From the activities in tomato, several achievements and lessons learned can be drawn:

- The three approaches used in LIVESEED can be useful to develop tomato populations adapted to a range of socioeconomic demands and farmers´ and consumers´ preferences.
- The use of collections of landraces and ecotypes conserved by farmers and seedbanks offers the opportunity of selecting those cultivars with the best adaptation to organic farming conditions and farmers´ preferences. As landraces evolved (and were bred) decades ago under low-input farming systems, they were expected to show good performance under organic farming. Many of the varieties used in the trials in Spain showed a satisfactory behaviour in the participatory organic trials and, after considering many agronomic and fruit quality traits, a considerable collection of tens of varieties were selected and are available for organic farmers.
- On the other side, the development of composite cross populations (CCPs) in Italy based on crossing landraces and following a dynamic participatory mass selection have proved useful to develop new materials adapted to local conditions and farmers´ preferences, with specific fruit typology (oxheart in this case), while preserving a heterogeneous genetic pool at the same time.
- Finally, even experimental populations like MAGIC lines can be useful not only for scientists and genome wide association studies, but also for organic breeding as they are based on crossing several parent lines. They offer a range of agronomic traits and a huge segregating gene pool, offering the opportunity of breeding new populations adapted to many growing conditions, including organic farming.
- The tomato cultivation is quite demanding in terms of costs of production and time-consuming and, therefore:
    - (i) a high commitment is necessary between farmers and breeders to achieve success in participatory breeding initiatives;
    - (ii) the scale of the trial may change depending on the farmer, as there are small and medium-scale farmers who cannot perform big trials, being limited to trials of 100-400 plants per trial;
    - (iii) close interaction with farmers is essential, i.e. not limited to a mere evaluation based on 1-2 visits to the farms, but based on intense and continuous trusted networks.
- Consumers may also play an essential role in participatory initiatives, and their activity must be integrated in a rational way to increase the efficiency of PPB in tomato.

To this end, many participatory trials, lab analyses, and activities with consumers were performed during the tomato task to evaluate hundreds of landraces, CCPs and experimental MAGIC populations, involving thousands of plants. On the whole, the three approaches were proved to contribute to a more diverse and, therefore, resilient farming systems, by promoting i) intervarietal diversity (the one based on collections of heirlooms and partly the one based on MAGIC populations) and ii) intravarietal diversity (the one based on CCPs and partly the one based on MAGIC populations) by providing heterogeneous materials. As a result, these approaches may contribute to stable, long-lasting collections and important genetic resources for both scientists and organic farmers communities. In addition, organic consumers are demanding actors who are interested in agri-food products coming from sustainable farming systems, but also expect that such food is of high quality and taste (i.e. traditional varieties with the taste-of-the-past). In this regard, consumers can be attracted to PPB by offering them the participation in taste panels, contributing with their opinions to the improvement of the efficiency of the process of selection. Thus, these activities, together with breeders, scientist and farmers, make consumers feel to be part of the process and also contribute to strengthen the links between all these actors, particularly the connection between farmer and consumer, essential to durable participatory networks. Finally, our activities have enabled the development of a range of participatory networks "breeders/scientists-farmers-(consumers)". Some of them already existed but now reinforced, like those from the activities of RSR & Arcoiris in Italy, others newly developed, like those in Spain in the two main trials and other pilot (minor) trials, which have been widened also to other related crops like peppers or brassica.

Future work within the organic tomato network should include:

- The release of tomato heirlooms and heterogeneous materials selected in the frame of LIVESEED, making them available to farmers and other associations interested on them.
- The testing of new heirlooms and CCPs in future local activities.
- The reinforcement of the tomato networks created during LIVESEED through i) local ongoing activities and also ii) internationally, with the exchange of plant materials and knowledge (with visits and workshops) between the three initiatives and their networks, and potentially other initiatives.
- The validation of the utility of DNA polymorphisms derived from CREA in tomato organic breeding initiatives.
- The further investigation of the relationship between fruit composition (sugars, acids and volatiles) and consumers´ preferences. Firstly, based on our results. Then widened to new studies with other cultivars and experimental populations.

9.6. General conclusion

The five case studies show different approaches to build networks and to close breeding gaps. As was described in Deliverable 3.4, many different technical and social aspects together make up the breeding process. How breeding gaps are dealt with is shaped by various factors such as crop type (annual, perennial), available cultivars, available knowledge on various fields (agronomy, genetics, breeding, etc), the type of actors involved (farmers, breeders, researchers, consumers, etc) and the available markets. From these five case studies, it can be concluded that there is no single approach to build networks and to close gaps, but that the approach is a contextual development shaped by many different factors. Hence, no single advice can be suggested to breeders who want to initiate breeding networks or close breeding gaps for other crops. Instead, they can best compare the five case studies in this deliverable and develop an approach that fits their context best. An advice to policy makers is also to develop an enabling environment to stimulate the diversity in breeding approaches in order to close breeding gaps and to initiate different types of networks. Stimulating multi-actor approaches can be an important and powerful tool to stimulate diversity in approaches for building networks and closing breeding gaps in an efficient way, and hence at the same time to stimulate the development of more crop diversity.

However, considering the four IFOAM principles (care, fairness, health and ecology), we need to remind ourselves continuously that not every problem can and/or needs to be solved through breeding. Taking a systems-based perspective (Lammerts van Bueren et al. 2018), we need to ask ourselves: can this problem be solved through other means, or a combination of different solutions, or is breeding really the best solution? For example, in the case of wheat, it is important to realise that proper screening of the seed on infections with bunt remains one of the most important measures for farmers to do, even when working with bunt-resistant cultivars. Another issue is how to prevent a rat rice between disease and breeder introducing new resistance genes with the development of new types of bunt. In the case of tomato, it is also important to realise that the nutritional quality is not only improved through breeding, but is also highly influenced by the crop management, in particular the manuring level. In that respect, the value chain also has a role to play in determining what type of farming system can be best meet the IFOAM principles. In the case of apple, despite the advances made in improving the organic management of apple orchards, a critical reflection is needed in what is really the best way to reduce the pest and disease incidences in apple orchards. As was recognised by Tamm et al. in 2004, innovative solutions are needed, in particular given the conventional market demands that influence the shape of organic markets today more and more. Hence, the use of molecular tools such as in the case of white lupin may provide more in-depth knowledge of the genetic background of the crop. But also in white lupin, the market players and policy makers both have a role to play to make the cultivation more economically viable for organic farmers (and conventional farmers alike). This not only applies specifically to white lupin but to all leguminous crops. In the case of the brassica, some trials were set up in which traders were involved. The results suggested that various value chain players see the importance of using cultivars bred according to the organic principles. Follow up projects are needed to involve value chain players more in this theme and to make substantive progress in this respect.

10. References

Alkemade, J. A., Messmer, M. M., Arncken, C., Leska, A., Annicchiarico, P., Nazzicari, N., Książkiewicz, M., Voegele, R. T., Finckh, M. R., & Hohmann, P. (2021). A high-throughput phenotyping tool to identify field-relevant anthracnose resistance in white lupin. Plant Disease, 105. https://doi.org/10.1094/pdis-07-20-1531-RE

Alkemade, J. A., Messmer, M. M., Voegele, R. T., Finckh, M. R., Hohman, P. Genetic diversity of Colletotrichum lupini and its virulence on white and Andean lupin. Scientific Reports (under review)

Allen AM, Winfield MO, Burridge AJ, Downie RC, Benbow HR, Barker GL, Wilkinson PA, Coghill J, Waterfall C, Davassi A (2017) Characterization of a wheat breeders' Array suitable for high-throughput SNP genotyping of global accessions of hexaploid bread wheat (Triticum aestivum). Plant Biotechnol J 15(3):390–401

Borgen A, Backes G, Müller K-J, Gallehr A, Scherrer B, Ytting N, Spieß H M (2019) Identifying resistance genes in wheat against common bunt (*Tilletia caries*) by use of virulence pattern of the pathogen. In: Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs (Ed), 69. Jahrestagung 2018, 19-21 November, Raumberg-Gumpenstein, pp 13-15. BOKU-University of Natural Resources and Life Sciences, Vienna, Austria. ISBN-13: 978-3-900932-63-3

Christensen, D.K. & Borgen, A. 2021, 'Starke-II NIL based common bunt resistance gene mapping', XXI International Workshop on Bunt and Smut Diseases.

Hoffmann, J.A. 1982, 'BUNT OF WHEAT', Plant Disease, vol. 66, no. 11, pp. 979-86.

J. G. Menzies, R. E. Knox, Z. Popovic, and J. D. Procunier (2006). Common bunt resistance gene Bt10 locatedon wheat chromosome 6D. Canadian Journal of Plant Science, https://doi.org/10.4141/P06-106

Ksiazkiewicz, M., Nazzicari, N., Yang, H.a., Nelson, M.N., Renshaw, D., Rychel, S., Ferrari, B., Carelli, M., Tomaszewska, M., Stawinski, S., Naganowska, B., Wolko, B. & Annicchiarico, P. 2017, 'A high-density consensus linkage map of white lupin highlights synteny with narrow-leafed lupin and provides markers tagging key agronomic traits', Scientific Reports, vol. 7.

Lammerts van Bueren, E. T., Struik, P. C., van Eekeren, N., & Nuijten, E. 2018, Towards resilience through systems-based plant breeding. A review. Agronomy for Sustainable Development, 38(*5*), [42]. https://doi.org/10.1007/s13593-018-0522-6

Laroche A, Demeke T, Gaudet DA, Puchalski B, Frick M, McKenzie R. 2000, Development of a PCR marker for rapid identification of the Bt-10 gene for common bunt resistance in wheat. Genome 43:217-223. DOI: 10.1139/g99 -113

Mascher, F., A.Borgen, V. Dumalasova, K.-J Müller, D. Hole, F. Dell'Avo, Ž. Liatukas, A. E. Müllner,T. Henrikson, A. Pregitzer, E. M. Al-Maroof, A. Morgounov 2016: Multilocal resistance assessmentagainst common bunt of wheat (Triticum aestivum). 67. Tagung der Vereinigung der Pflanzenzüchter undSaatgutkaufleute Österreichs, 21.-23. November 2016, HBLFA Raumberg-Gumpenstein, Irdning,Österreich. p37-38.

Menzies, J.G., Knox, R.E., Popovic, Z., Procunier, J.D. (2006). Common bunt resistance gene Bt10 located on wheat chromosome 6D. Can J Plant Sci 86:1409–1412

(16) (PDF) Mapping of common bunt resistance gene Bt9 in wheat. Available from: https://www.researchgate.net/publication/314036278_Mapping_of_common_bunt_resistance_gene_Bt9_in_wheat [accessed Jun 14 2021].

Muellner, A.E., Buerstmayr, M., Eshonkulov, B., Hole, D., Michel, S., Hagenguth, J.F., Pachler, B., Pernold, R. & Buerstmayr, H. 2021, 'Comparative mapping and validation of multiple disease resistance QTL for simultaneously controlling common and dwarf bunt in bread wheat', Theoretical and Applied Genetics, vol. 134, no. 2, pp. 489-503.

Steffan, P.M., Torp, A.M., Borgen, A., Backes, G. & Rasmussen, S.K. 2017, 'Mapping of common bunt resistance gene Bt9 in wheat', Theoretical and Applied Genetics, vol. 130, no. 5, pp. 1031-40.

Tamm, L., Häseli, A., Fuchs, J.G., Weibel, F.P., Wyss, E. 2004, Organic fruit production in humid climates of Europe: Bottlenecks and new approaches in disease and pest control. Acta Horticulturae, 638, 333-339.

Van Loqueren G., Baret P.V. (2008) Why are ecological, low-input, multi-resistant wheat cultivars slow to develop commercially? A Belgian agricultural 'lock-in'case study. Ecological Economics 66(2), 436-44

Wang S, Wong D, Forrest K, Allen A, Chao S, Huang BE, Maccaferri M, Salvi S, Milner SG, Cattivelli L, Mastrangelo AM, Whan A, Stephen S, Barker G, Wieseke R, Plieske J, International Wheat Genome Sequencing Consortium, Lillemo M, Mather D, Appels R, Dolferus R, Brown-Guedira G, Korol A, Akhunova AR, Feuillet C, Salse J, Morgante M, Pozniak C, Luo M, Dvorak J, Morell M, Dubcovsky J, Ganal M, Tuberosa R, Lawley C, Mikoulitch I, Cavanagh C, Edwards KJ, Hayden M, Akhunov E (2014) Characterization of polyploid wheat genomic diversity using a high-density 90,000 single nucleotide polymorphism arrayPlant. Biotechnol J. 12(6):787-796

Wang, R., Gordon, T., Hole, D., Zhao, W., Isham, K., Bonman, J.M., Goates, B. & Chen, J. 2019, 'Identification and assessment of two major QTLs for dwarf bunt resistance in winter wheat line 'IDO835'', Theoretical and Applied Genetics, vol. 132, no. 10, pp. 2755-66.

Annex 1. Sampling templates for kohlrabi trials

KOHLRABI TEMPLATES		BEFORE PLANTING AND AT TRASPLANTING			
Cultivar	Origin	No. seeds seeded	No. seedlings germinated	Remarks on seeds	No. plants planted
Name, code, etc.	Company, seedbank, etc				
……					
……					
……					

FOLIAGE				
Leaf blade length (1 very short to 5 very long + cms)	Leaf blade width (1 very narrow to 5 very broad +cms)	Leaf attitude (1 horizontal to 5 erected	Petiole thickness (1 thick to 5 fine + mms)	Colour (white green, green, dark green

GENERAL ASPECTS OF HARVEST					
Beginning of harvest (date)	Nº plants at commercial size	50% plants harvest (date)	End harvest (date)	Nº harvests (and dates)	Pictures (at 50% harvest)

BULB TRAITS				
Bulb shape (1 flat to 5 high round)	Bulb high (cm)	Bulb diameter (cm)	Woodiness (1 high to 5 absent)	Colour (white green, green, dark green)

OTHER HARVEST DATA					
Nº bulbs >8cm	Weight of bulbs >8 cm (g)	No. bulbs marketable	Weight bulbs marketable (g)	Ø marketable bulbs (g)	Nº non commercial bulbs

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OTHER HARVEST DATA		OBSERVATIONS
Weight non marketable bulbs (g)	Why no marketable?	

Annex 2. Sampling templates for broccoli trials

BROCCOLI TEMPLATES		AT SOWING / PLANTING			
Cultivar	Origin	No. seeds seeded	No. seedlings germinated	Remarks on seeds	No. plants planted
Name, code, etc.	Company, seedbank, etc				
……					
……					
……					

VEGETATIVE DEVELOPMENT					
Vigour (scale 1 to 5 high vigour)	Foliage homogen. (1 to 5 scale)	Heads homogen. (1 to 5 scale)	Colour (light green to dark/blue green)	Leaf shape (1 spread to 5 erected)	Leaf surgace (1 smooth to 5 curly)

VEGETATIVE DEVELOPMENT	GENERAL ASPECTS OF HARVEST				
Disease/pest description and scoring (1 very infested to 5 nothing)	Beginning harvest (date)	50% harvest (date)	End harvest (date)	Nº Harvest	Pictures at 50% harvest

GENERAL ASPECTS OF HARVEST-HEADS					
Colour (light, middle, dark green, blue or grey shades)	Shape (1 flat to 5 round; 3 ideal)	Regularity (1 irregular =ping-p balls to very regular)	Ramification (1 low to 5 high)	Compactness (1 large to 5 short)	Buds (1 very small to 5 very big; ideal 3)

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GENERAL ASPECTS OF HARVEST-HEADS						
Nº heads harvested	Weight heads harvested (g)	Nº Heads (1st choice)	Weight heads 1st choice (g)	Nº Heads (2nd choice)	Weight heads 2nd choice (g)	Nº non market. heads

OTHER HARVEST DATA		OBSERVATIONS
Weight non marketable heads (g)	Why no marketable?	

Annex 3. Sampling templates for cauliflower trials

CAULIFLOWER TEMPLATE		AT SOWING / PLANTING				
Cultivar	Origin	No. seeds seeded	No. seedlings germinated	Remarks on seeds	No. plants planted	No. plants evaluated
Name, code, etc.	Company, seedbank, etc					
……						
……						
……						

GENERAL ASPECTS OF HARVEST					
Beginning harvest (date)	50% harvest (date)	End harvest (date)	Nº Harvest	Utility value (1 useless to5 good performance)	Pictures at 50% harvest

GENERAL ASPECTS OF HARVEST-HEADS					
Colour (1 white to 3 yellow; shade of purple 1-3)	Shape (1 concave, 3 flat, 5 spheric, 7 eliptic)	Regularity (1 irregular =ping-p balls to very regular = ballon)	Ramification (1 low to 5 high)	Compactness (1 large to 5 short)	Buds (1 very small to 5 very big; ideal 3)

	GENERAL ASPECTS OF HARVEST-HEADS						
Nº heads harvested	Weight heads harvested (g)	Ø heads harvested (g)	Nº Heads (1st choice)	Weight heads 1st choice (g)	Nº Heads (2nd choice)	Weight heads 2st choice (g)	Nº non market. heads

OTHER HARVEST DATA		OBSERVATIONS
Weight non marketable heads (g)	Why no marketable?	

Annex 4. Sampling templates for white and savoy cabbages trials

WHITE & SAVOY CABBAGE TEMPLATE		AT SOWING / PLANTING			
Cultivar	Origin	No. seeds seeded	No. seedlings germinated	Remarks on seeds	No. plants planted
Name, code, etc.	Company, seedbank, etc				
……					
……					
……					

VEGETATIVE DEVELOPMENT			
Plant vigour (scale 1 to 5 high vigour)	Average Ø of plants (cm)	Average height of plants (cm)	Outer leaf colour (1 yellow green, 2 green, 3 grey gree, 4 blue green, 5 violet)

GENERAL ASPECTS OF HARVEST					
Harvest maturity 70% (date)	Shelf life on field (1 weak to 5 strong)	Head bursting resistance (1 weak to 5 strong)	Utility value (1 useless to 5 very good performance)	Bursting resistance stem at harvest	Pictures at 50% harvest

HARVEST-HEADS OUTSIDE			HARVEST-HEADS INSIDE in longitudinal section			
Shape (1 triangle, 2 ovate, 3 obovate, 4 eliptic, 5 spheric, 6 cylindric, 7 transverse)	Colour (1 light to 5 dark)	Waxiness (1 absent to 5 very strong)	Relative length interior stem (1 short to 5 long)	Head density (1 very loose to 5 very dense)	Inter. struct. (1 fine to 5 coarse)	Colour intensity (1 light to 5 dark)

GENERAL ASPECTS OF HARVEST-HEADS				
Nº heads harvested	Nº marketable heads	Weight heads marketable (g)	Head-forming overlap (1 curled outwards, 2 erect, 3 curled inwards, 4 slight overlap, 5 strong)	Uniformity of marketable heads (1 very heterogeneous to 9 very uniform

OTHER HARVEST DATA		OBSERVATIONS
Weight non marketable heads (g)	Why no marketable? (bursted, rotten, small, too big, …)	

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.

Annex 5. Infection rate (%) of wheat lines with Bt1 postulated resistance gene infected with 7 different virulence isolates of *Tilletia caries* in seasons 2018/19

- *Infection rate (%) of wheat lines with **Bt1** postulated resistance gene infected with 7 different virulence isolates of Tilletia caries in seasons 2018/19*

	Vr4	VrG	VrR	Vr1	Vr2	Vr10	Vr13
Pi-554-108	6.9	0.0	0.0	21.7	20.7	0.0	0.0
PG3540	0.0	0.0	0.0	52.9	26.7	0.0	0.0
Starke NIL Bt1	2.4	0.0	0.0	36.5	8.2	0.0	0.0
Butaro	0.0	0.0	0.0	37.1	70.6	0.0	0.0
Courier	0.0	0.0	0.0	48.8	37.5	2.2	0.0
Ring 61: HSI-664-11	0.0	0.0	0.0	29.8	10.9	2.2	0.0
ButQue 1	0.0	0.0	0.0	17.1	51.7	0.0	0.0
ButQue 2	0.0	0.0	0.0	10.0	13.5	0.0	0.0
ButQue 3	0.0	0.0	0.0	19.4	48.9	0.0	0.0
ButQue 4	0.0	0.0	0.0	30.0	43.6	0.0	0.0
ButQue 5	0.0	0.0	0.0	12.8	16.7	0.0	0.0
ArPG	0.0	0.0	0.0	40.0	20.0	0.0	0.0
PerBut 1	0.0	0.0	0.0	38.3	10.0	0.0	0.0
PerBut 2	0.0	0.0	0.0	55.9	58.3	0.0	0.0
PerBut 3	0.0	0.0	0.0	51.4	68.8	0.0	0.0
PerBut 4	0.0	0.0	0.0	38.5	14.6	0.0	0.0
PerKo 1	0.0	0.0	0.0	47.5	17.1	0.0	0.0
PerKo 2	0.0	0.0	0.0	23.7	11.8	0.0	0.0
PerXe 1	0.0	0.0	0.0	68.2	22.6	0.0	0.0
PerXe 2	0.0	0.0	0.0	62.5	47.8	0.0	0.0
PerXe 3	0.0	0.0	0.0	48.8	55.3	2.2	0.0
CorPG 1	0.0	0.0	0.0	26.0	18.4	0.0	0.0
CorPG 2	0.0	0.0	0.0	47.5	17.1	0.0	0.0
CorPG 3	0.0	0.0	0.0	57.7	18.0	0.0	0.0
CorPG 4	0.0	0.0	0.0	47.3	26.3	0.0	0.0
FoPG	0.0	0.0	2.0	12.5	7.4	0.0	0.0
DZW1107p	0.0	0.0	0.0	25.0	22.6	2.4	0.0
AB-5	0.0	0.0	0.0	16.0	22.0	0.0	0.0
CCP 16	0.0	0.0	0.0	22.0	7.8	0.0	0.0
CCP-20	0.0	0.0	0.0	15.1	5.3	2.2	0.0
CCP-29	2.2	0.0	0.0	10.0	13.5	0.0	0.0
CCP-34	0.0	0.0	0.0	9.1	25.6	0.0	0.0
Butaro	0.0	0.0	0.0	38.7	70.0	4.3	2.3
HSi 439-2-10	0.0	0.0	0.0	17.5	55.3	0.0	0.0
HSi 447-10	0.0	0.0	0.0	7.0	16.7	0.0	0.0
HSi 672-10	0.0	0.0	0.0	14.7	10.3	4.8	0.0
HSi 528-11	0.0	0.0	0.0	11.1	2.4	0.0	0.0
HSi 664-11	0.0	0.0	0.0	9.1	8.2	2.2	2.6

HSi 612-11	0.0	0.0	0.0	11.4	14.7	0.0	0.0
HS 191-04	0.0	0.0	0.0	31.9	30.8	0.0	0.0
WIF109Bt.01	0.0	0.0	0.0	44.0	42.2	0.0	
WIF9Bt.741	0.0	0.0	0.0	32.3	61.9	0.0	
WAR109Bt.01	0.0	0.0	0.0	23.8	12.0	2.4	
SePen 6A	0.0	0.0	15.2	37.5	25.0	47.1	4.2
DZW1107c	0.0	0.0	2.2	26.3	7.7	43.6	0.0
DZW1107h	0.0	0.0	0.0	21.4	41.3	10.8	0.0
DZW1107i	0.0	0.0	0.0	25.9	19.0	6.5	0.0
DZW1107k	0.0	0.0	0.0	28.6	22.0	17.2	2.6
DZW1107m	0.0	0.0	0.0	19.4	20.0	23.3	2.9
DZW1108a	0.0	0.0	0.0	6.3	20.0	18.0	0.0
DZW1108b	0.0	0.0	0.0	14.8	46.2	25.5	0.0
DZW1019a1	0.0	0.0	0.0	6.3	5.7	20.4	0.0
DZW1019e	0.0	0.0	0.0	8.9	0.0	10.2	0.0
DZW1019h1	0.0	0.0	0.0	4.3	3.8	8.0	0.0
DZW1019h2	0.0	0.0	0.0	6.3	22.0	12.2	0.0
DZW1019p1	0.0	0.0	0.0	6.3	8.7	20.0	0.0
DZW1019g	0.0	0.0	0.0	5.7	5.4	5.4	0.0
HSi 367-10	0.0	0.0	0.0	39.7	16.7	21.6	

- *Infection rate (%) of wheat lines with **Bt2** postulated resistance gene infected with 7 different virulence isolates of Tilletia caries in seasons 2018/19*

	Vr4	VrG	VrR	Vr1	Vr2	Vr10	Vr13
Skotte	0.0	0.0	69.5	69.5	76.5	0.0	0.0
Inthaler	0.0	0.0	7.0	3.8	14.0	0.0	0.0
Solstice	0.0	0.0	69.8	65.5	70.7	0.0	0.0
Complet	0.0	0.0	26.9	33.3	24.1	0.0	0.0
Hereward	0.0	0.0	37.3	41.5	32.6	0.0	0.0
Ochre	0.0	0.0	25.5	12.0	20.8	0.0	0.0
SegQue Veksel	0.0	2.8	47.1	35.5	20.0	0.0	0.0
ButQue 1	0.0	0.0	39.6	78.0	59.3	0.0	0.0
ButQue 2	0.0	0.0	15.7	10.0	7.0	0.0	0.0
ArPG 1	0.0	0.0	13.0	27.3	11.5	0.0	0.0
ArPG 2	0.0	0.0	35.0	41.2	2.4	0.0	0.0
ArPG 3	0.0	0.0	20.8	44.9	6.3	0.0	0.0
FoPG	0.0	0.0	59.6	38.0	16.1	0.0	0.0
P.LA.P 87.1	0.0	0.0	40.7	62.5	24.0	0.0	
Pi-554-103	6.1	4.3	47.8	50.0	39.5	4.3	0.0
Pi-554-097	0.0	11.8	64.7	65.2	52.5	4.8	0.0
Bussard	0.0	6.3	55.8	31.3	66.1	0.0	0.0
Skagen	0.0	4.8	21.6	20.3	11.5	0.0	0.0
Paroli	0.0	4.8	59.2	63.3	71.7	0.0	0.0
Format	0.0	2.2	61.3	63.6	81.3	0.0	0.0
Dream	0.0	8.9	100.0	72.7	86.8	0.0	0.0

Fold	19.6	7.7	20.5	22.2	0.0	18.2	0.0
Helge	23.7	14.3	4.8	12.3	3.8	11.3	0.0
Rubrik	3.8	27.0	34.1	46.2	24.1	0.0	0.0
Ring 39 :DS-6395-6	4.3	14.3	19.4	40.8	4.3	0.0	0.0
Ring 37 :DS-6583-2	0.0	6.3	13.0	17.0	0.0	2.0	0.0
HypBut 20	0.0	2.4	12.1	46.7	25.8	0.0	0.0

- *Infection rate (%) of wheat lines with **Bt5** postulated resistance gene infected with 7 different virulence isolates of Tilletia caries in seasons 2018/19*

	Vr4	VrG	VrR	Vr1	Vr2	Vr10	Vr13
Pi-554-104	0.0	8.2	0.0	0.0	0.0	0.0	0.0
Promesse	0.0	17.1	0.0	0.0	0.0	0.0	0.0
Globus	4.8	73.1	0.0	0.0	3.2	16.9	0.0
Starke NIL Bt5	0.0	17.4	0.0	0.0	2.2	0.0	0.0
Tommi	0.0	81.8	0.0	0.0	0.0	12.1	0.0
Bill	0.0	18.0	0.0	0.0	0.0	0.0	0.0
SegQue-1	0.0	27.1	4.8	0.0	0.0	0.0	2.3
SegQue 2	0.0	47.7	0.0	2.4	6.3	0.0	0.0
SegQue 3	0.0	8.6	2.2	5.4	0.0	0.0	0.0
SegQue 4	0.0	38.6	0.0	0.0	0.0	0.0	0.0
SegQue 5	0.0	25.0	0.0	0.0	0.0	10.4	0.0
QueGlo 13	0.0	24.1	2.4	0.0	0.0	0.0	0.0
QueGlo 2	2.0	7.0	0.0	0.0	2.4	3.9	0.0
PerWin	0.0	10.3	0.0	0.0	0.0	0.0	0.0
DZW0510e30	0.0	6.3	0.0	0.0	0.0	0.0	0.0
DZW0405s0	0.0	68.4	0.0	0.0	0.0	0.0	0.0
DZW0605e	0.0	33.3	0.0	0.0	0.0	11.8	0.0
BiFo 5	0.0	13.5	0.0	0.0	0.0	0.0	0.0
CCP 15	0.0	10.0	0.0	0.0	0.0	0.0	0.0
CCP 42	0.0	27.3	0.0	0.0	0.0	0.0	0.0
CCP-27	0.0	37.9	0.0	0.0	0.0	0.0	0.0
GloCor 20	0.0	36.4	0.0	0.0	0.0	0.0	0.0
GloTat 11B	0.0	30.3	0.0	0.0	0.0	0.0	6.1
GloTat 3A	0.0	40.0	0.0	0.0	0.0	0.0	0.0
GloTat 3B	0.0	52.0	0.0	0.0	0.0	0.0	0.0
GloTat 3C	0.0	22.0	0.0	0.0	0.0	2.3	0.0
LaHyp10	0.0	22.2	0.0	0.0	0.0	0.0	0.0
PerHyp 17	0.0	40.9	0.0	0.0	0.0	0.0	0.0
QueGlo 13	0.0	26.1	0.0	0.0	0.0	0.0	0.0
QueGlo 9	0.0	25.0	0.0	0.0	7.7	0.0	0.0
SePen 17	0.0	16.7	0.0	0.0	0.0	6.3	0.0
SePen 6B	0.0	45.5	0.0	0.0	0.0	0.0	0.0
SePen 9	0.0	16.0	0.0	0.0	6.3	0.0	0.0
SkaLa 18A	0.0	11.4	0.0	0.0	2.4	0.0	0.0
TomBut 20	0.0	68.4	0.0	0.0	0.0	4.3	3.4
TomHyp 16	0.0	22.5	0.0	0.0	0.0	0.0	0.0

HSi 1010-12	0.0	30.4	0.0	0.0	0.0	2.2	0.0
HSi 676-11	0.0	20.0	0.0	0.0	0.0	2.2	0.0
HSi 186-13	0.0	6.3	0.0	0.0	0.0	0.0	
CP 88.4	0.0	68.2	1.7	0.0	0.0	0.0	
CP 91.6	0.0	83.3	0.0	0.0	0.0	0.0	
CP 88.7	0.0	89.7	0.0	0.0	0.0	2.4	
CP 71.1	0.0	61.2	0.0	0.0	0.0	5.0	
CP 87.5	0.0	30.0	0.0	2.8	0.0	9.1	
CP 86.2	0.0	50.0	0.0	0.0	0.0	2.8	
CP 75.2	0.0	47.1	0.0	0.0	0.0	4.5	
CP 95.4	0.0	77.1	0.0	0.0	0.0	6.3	
CP 78.3	0.0	63.6	0.0	0.0	0.0	0.0	
CP 73.2	0.0	25.0	0.0	0.0	0.0	0.0	
CP 77.2	0.0	61.9	0.0	0.0	0.0	0.0	
CP 81.5	0.0	72.2	0.0	0.0	0.0	3.8	
CP 95.2	0.0	44.8	0.0	3.8	0.0	3.2	
CP 74.1	0.0	81.3	0.0	0.0	0.0	5.4	
Hohenheimer Bt5	0.0	20.0	0.0	0.0	0.0	0.0	
SePen 20	0.0	7.4	0.0	0.0	4.3	2.4	0.0

- *Infection rate (%) of wheat lines with **Bt7** postulated resistance gene infected with 7 different virulence isolates of Tilletia caries in seasons 2018/19*

	Vr4	**VrG**	**VrR**	**Vr1**	**Vr2**	**Vr10**	**Vr13**
SegQue Veksel	0.0	4.8	0.0	21.2	0.0	0.0	0.0
Pi-554-100	0.0	21.2	0.0	21.3	0.0	24.3	0.0
Tambor	0.0	0.0	0.0	13.0	39.1	6.3	0.0
Korrund	0.0	23.9	0.0	35.3	44.6	33.3	2.2
Xenos	2.4	44.7	4.3	61.5	85.1	56.9	0.0
Segor	0.0	27.3	0.0	51.3	57.4	57.8	8.3
Ring 59: HSI-672-10	0.0	6.3	0.0	8.2	0.0	5.7	0.0
Ring 60: HSI-367-10	0.0	28.1	0.0	70.7	38.1	18.8	0.0
Jacoby	7.7	9.1	0.0	75.0	25.0	7.0	0.0
SegQue	0.0	14.3	4.8	23.3	21.1	3.0	0.0
ButQue	0.0	7.0	0.0	24.5	50.8	7.0	0.0
PerXe	0.0	51.4	0.0	63.3	79.2	41.7	0.0
DZW1107e	0.0	8.2	0.0	32.4	17.9	42.9	0.0
DZW1107f	0.0	9.7	0.0	25.6	32.3	2.8	0.0
DZW1108c	0.0	9.3	0.0	40.4	27.7	73.3	0.0
DZW1109a	0.0	17.1	0.0	55.0	37.5	17.9	0.0
DZW1109b	0.0	6.4	0.0	35.1	18.5	38.8	0.0
DZW1019d	0.0	8.2	0.0	24.5	19.5	42.9	3.8
SePen 3	0.0	31.8	0.0	22.7	7.7	50.0	0.0
SkaKo-20	0.0	5.4	0.0	22.5	25.9	6.3	0.0

HSi 585-10	0.0	7.0	0.0	4.8	35.6	8.3	0.0
HSi 637-10	0.0	3.2	0.0	46.4	4.5	28.1	0.0
HSi 430-10	2.2	8.3	0.0	13.6	0.0	4.3	0.0
HSi 681-10	0.0	28.6	0.0	34.2	6.3	28.9	0.0
HSi 379-11	0.0	6.3	0.0	32.1	31.4	4.3	0.0
HSi 1004-12/2	0.0	6.3	0.0	26.2	55.6	42.1	4.2
HSi 1005-12	0.0	16.7	0.0	46.9	0.0	58.3	0.0

- *Infection rate (%) of wheat lines with **Bt10** postulated resistance gene infected with 7 different virulence isolates of Tilletia caries in seasons 2018/19*

	Vr4	VrG	VrR	Vr1	Vr2	Vr10	Vr13
Weston	0.0	0.0	0.0	0.0	4.3	41.8	0.0
Starke NIL Bt10	0.0	0.0	0.0	0.0	0.0	16.2	0.0
SegQue Veksel	0.0	0.0	0.0	0.0	0.0	12.2	0.0
PerWin 1	0.0	0.0	0.0	0.0	0.0	37.8	0.0
PerWin 2	0.0	0.0	0.0	0.0	0.0	6.4	0.0
CCP 13	0.0	0.0	0.0	0.0	0.0	25.7	0.0

- *Infection rate (%) of wheat lines with **Bt13**postulated resistance gene infected with 7 different virulence isolates of Tilletia caries in seasons 2018/19*

	Vr4	VrG	VrR	Vr1	Vr2	Vr10	Vr13
Thule III	0.0	0.0	2.4	4.3	0.0	0.0	5.2
SegThul 1	0.0	0.0	0.0	0.0	0.0	0.0	7.1
SegThul 2	0.0	0.0	0.0	0.0	0.0	0.0	7.7
SegThul 3	0.0	0.0	0.0	0.0	0.0	0.0	35.7
SegThul 4	0.0	0.0	0.0	0.0	0.0	0.0	12.1
SegThul 5	0.0	0.0	0.0	0.0	2.4	0.0	7.1
SegThul 6	0.0	0.0	0.0	32.0	39.1	28.1	43.5
SegThul 7	0.0	0.0	0.0	2.4	0.0	0.0	16.3
SegThul 8	0.0	0.0	0.0	27.0	23.2	51.4	46.9
SegThul 9	0.0	0.0	0.0	0.0	0.0	0.0	5.0
SegThul 10	0.0	0.0	0.0	0.0	0.0	0.0	23.5
SegThul 11	0.0	0.0	0.0	0.0	0.0	0.0	41.3
SegThul 12	0.0	0.0	0.0	4.3	0.0	0.0	26.9
SegThul 13	0.0	0.0	0.0	0.0	0.0	0.0	35.7
SegThul 14	0.0	0.0	0.0	0.0	0.0	0.0	14.1
SegThul 15	0.0	0.0	0.0	0.0	0.0	0.0	3.4
SegThul 16	0.0	0.0	0.0	0.0	0.0	0.0	7.9
SegThul 17	0.0	0.0	0.0	0.0	0.0	0.0	28.9
SegThul 18	0.0	0.0	0.0	12.1	0.0	2.2	15.6
SegThul 19	0.0	0.0	0.0	0.0	0.0	0.0	24.1
SegThul 20	0.0	0.0	0.0	0.0	0.0	0.0	17.5
SegThul 21	0.0	0.0	0.0	2.4	0.0	0.0	41.7
SegThul 22	0.0	0.0	0.0	0.0	0.0	0.0	9.5
SegThul 23	0.0	0.0	0.0	0.0	0.0	0.0	15.6

- *Infection rate (%) of wheat lines with* ***BtZ*** *postulated resistance gene infected with 7 different virulence isolates of Tilletia caries in seasons 2018/19*

	Vr4	VrG	VrR	Vr1	Vr2	Vr10	Vr13
DZW1105b	0.0	0.0	0.0	0.0	0.0	44.4	0.0
DZW1110	0.0	0.0	0.0	2.2	0.0	79.5	0.0
DZW1126e	0.0	0.0	0.0	0.0	0.0	35.0	0.0
DZW1126f	0.0	0.0	0.0	0.0	0.0	39.4	0.0
DZW1126g	0.0	0.0	0.0	0.0	0.0	63.0	0.0
DZW1126h	0.0	0.0	0.0	0.0	0.0	25.7	0.0
DZW1127a	0.0	0.0	0.0	0.0	0.0	37.8	0.0
DZW1127b	0.0	0.0	0.0	0.0	0.0	50.9	0.0
DZW1004a	0.0	0.0	0.0	0.0	0.0	100.0	0.0
DZW1007ka	0.0	0.0	0.0	0.0	0.0	37.7	0.0
DZW1007b2	0.0	0.0	0.0	0.0	0.0	48.0	0.0
DZW1007f	0.0	0.0	0.0	0.0	0.0	62.3	0.0
DZW1007h	0.0	0.0	0.0	0.0	0.0	36.2	0.0
DZW0402g	0.0	0.0	0.0	0.0	0.0	42.9	0.0
DZW0716b	0.0	0.0	0.0	0.0	0.0	55.3	0.0
DZW0716p	0.0	0.0	0.0	0.0	0.0	27.9	0.0
HSi 68-07	0.0	0.0	0.0	0.0	0.0	10.3	0.0

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.