DIVERSIFOOD

Embedding crop diversity and networking for local high quality food systems

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D3.5
Development of PPB (Participatory Plant Breeding) methods

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Additional author: INRA, LBI, IPC, ITQB, CSIC, ITAB, RSP, RAS, PSR, Arche Noah

Dissemination level:

☒ **PU:** Public (must be available on the website)

☐ **CO:** Confidential, only for members of the consortium (including the Commission Services)

☐ **CI:** Classified, as referred to in Commission Decision 2001/844/EC

Abstract

Deliverable D3.5 describes the development of the Participatory Plant Breeding (PPB) experiments by all partners involved in DIVERSIFOOD WP3. The strategy and the organisation followed by the partners are presented according to four components:

- Genetic resources (access, choice and management);
- Techniques: a) Breeding and management of populations (EPB- Evolutionary Plant Breeding, CCP-Composite Cross Populations, dynamic mixtures, mass selection and variations, OPV-Open Pollinated Varieties, natural selection, intercropping); b) Statistical tools - Experimental design on farm trials, Statistical methods (D3.1); c) quality assessment;
- Social dimension (stakeholder meetings, education and training) and
- Legislation.

For each experiment, outcomes and conclusion regarding the efficiency and the limits of the approach followed are highlighted, presented as a SWOT analyses:

- Indicating the empowerment and breeding co-learning (both from farmers and researchers), and new germplasm produced as a strength.
- The time spent in the coordination, communication and facilitation, *i.e.*, the scarcity of human and economic resources is limitative, so as some technical limitations regarding individual plant analyses that are considered as weakness.
- Opportunities were referred as the deployment of diversity in the field and its impact to landscape, "greening activities" throughout pollinators' services, mix crops and organoleptic improvement.
- Threats of funding for the PPB process, both considering the growing of the involved people or the long-term breeding activities in which they are involved, or limitations of breeding population sizes on small-scale organic farms can bring to population size

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Introduction

PPB (Participatory Plant Breeding) being transdisciplinary and based on a multi-actor approach needs for its development the integration of four main components:

- Genetic resources (access, choice and management);
- Techniques:
  - Breeding and management of populations (EPB, CCP, dynamic mixtures, mass selection and variations, OPV, natural selection, intercropping);
  - Statistical tools - Experimental design on farm trials, Statistical methods;
  - Quality assessment;
- Social (stakeholder meetings, education and training) and
- Legislation.

The integration of these components allowed understanding how a certain PPB program works and better communicate its strategy (Figure 1). The Figure 1 indicates PPB strategy by the majority of the partners, and Figure 2 to 8 for partners, indicating the methods used that contributed for the development of PPB.

The PPB strategies under DIVERSIFOOD were able to

- develop and reinforce the collective decision making (e.g. wheat populations and mixtures with on farm evaluation and selection lead by INRA and RSP),
- explore the partnership between farmers and researchers (e.g. providing answers to farmers' question, as the example of "differences between CCP and dynamic populations of wheat" - INRA-ITAB),
- explore the evaluating technical possibilities (e.g. improvement of wheat populations on-farm and breeding of legumes for mixed cropping – LBI / comparison of self vs open-pollination breeding approaches in yield and yield determinants – CSIC / protocols development for evolving populations of wheat and tomato – RAS / improvement of colourful tomatoes for direct marketing in decentralised individual breeding programs - Arche Noah / improvement of rare vegetable types by crossing old varieties with commercial varieties of known target qualities, and maintain or improve taste qualities by involve chefs and other stakeholders, PSR),
- explore a multi-actor approach (e.g. search the gaps, to provide answers to improve yield maintaining quality indexes development and providing the connection among the actors' network - IPC + ITQB; building a research community in order to improve co-evolution mechanisms between humans-tomatoes varieties and natural environments RSP+ITAB).

The germplasm used on the PPB program developed in DIVERSIFOOD ranged from landraces, to commercial varieties and breeding lines. Germplasm had its origin on farmers' landraces, OPV, CCP and others created in previous projects (e.g. SOLIBAM), community seed banks, gene banks or commercial cultivars. These backgrounds allowed to produce mixtures or crosses (Wheat INRA+RSP), dynamic mixtures and CCP (INRA-ITAB), selection and adaptation from a population (Spring wheat population from Dottenfelderhof – LBI, tomato populations selection RSP+ITAB) or divergent selection for open pollinated *versus* selfing in faba bean (CSIC)

The techniques used on

- breeding and management of populations included
  - selection and evaluation by farmers and bakers (INRA-ITAB, LBI, IPC),
  - scorecards for practitioners on farm observation (RSP+ITAB),
  - crossbreeding with several combinations,
  - selection of populations along generations (e.g. positive selection for brix and organoleptic – PSR),

- pedigree selection and back-cross breeding programs (e.g. leaf mould resistance – Arche Noah),
- development of decision tools to potentiate certain components (e.g. tocopherols– ITQB+IPC), Performance Index (e.g. Chlorophyll a Fluorescence the OIJP test – ITQB+IPC),
- screening for disease resistance to mycotoxins in maize OPV (ITQB+IPC),
- sensorial analyses (ITQB+IPC), Molecular diversity assessment and nutritional grain quality evaluation (ITQB+IPC),
- genome wide association studies on maize quality traits (ITQB+IPC),
- Digital Image Analysis (CSIC).

- Statistics included the development of PPBstats R package (INRA+RSP) statistical tools - experimental design on farm trials (see deliverable D3.2 on DIVERSIFOOD website).
  - The statistical methods were developed for regional and satellite farms experimental design with hierarchical Bayesian model to analyse using the R package PPBStats,
  - ANOVAs and Multivariate analysis: Principal Components and Discriminant.
- The social component included
  - annual, workshop and regular meetings to present results and take decisions,
  - focus groups, stakeholders' and multi-actor meetings, personal and phone call interviews,
  - field visits, training of farmers and facilitators,
  - product tasting, and chefs involved in the organoleptic evaluation.

The legislation being worked in order

- to contribute for the recognition of the role of farmers organisations in the creation and maintenance of crops genetic resources (INRA+RSP),
- to contribute for the registration as «Niche Varieties» in Switzerland as soon as professional production is promising (PSR).

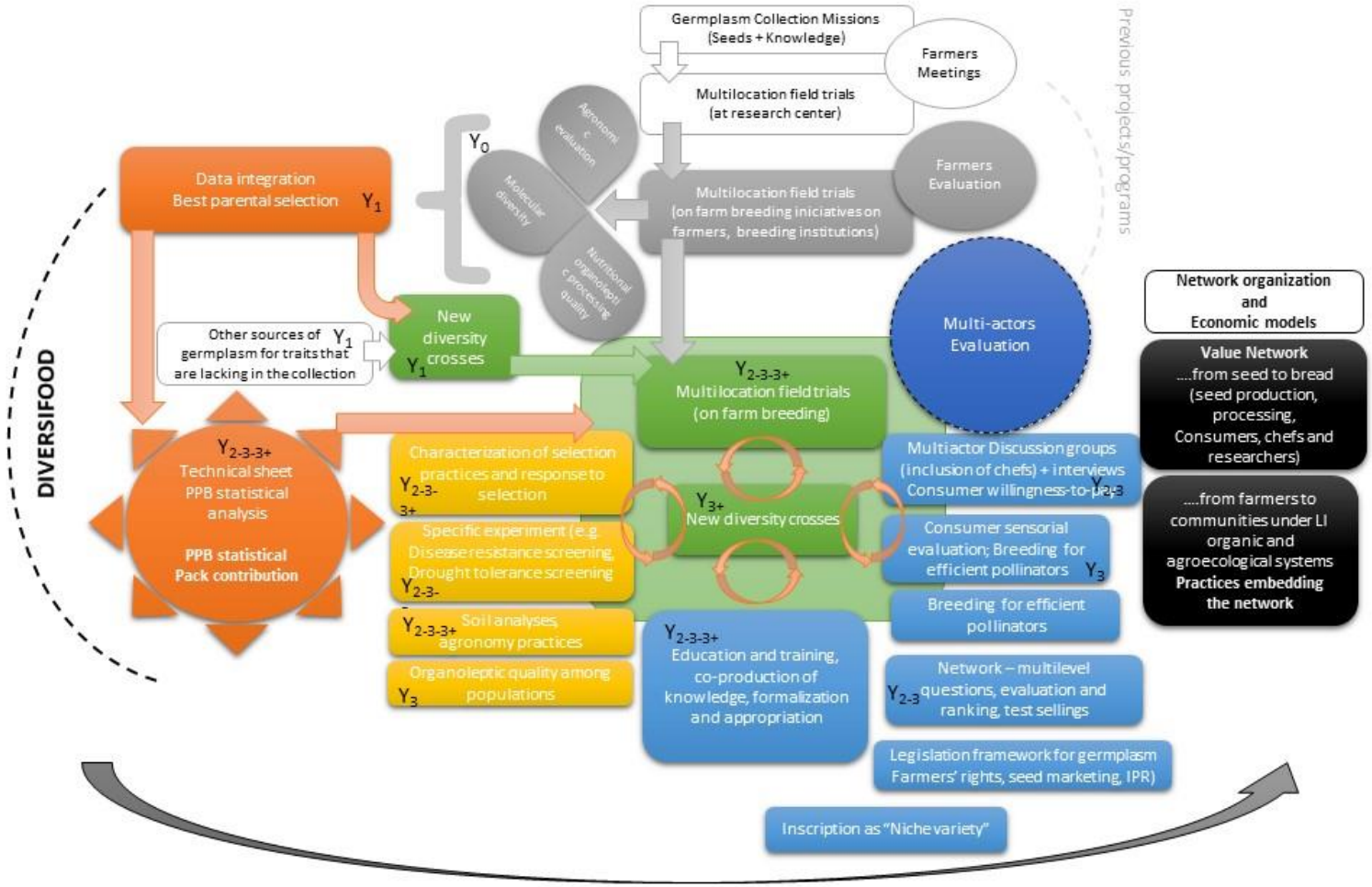

Figure 1 : The integration of components such as: 1) Genetic resources; 2) Techniques 3) Social and 4) Legislation allowed to understand how a certain PPB programs work and better communicate its strategy.

Table 1 : PPB strategy. Methods used by the partners that contributed for the development of PPB

Partner	Strategy	Methods			
		1) Genetic resources	2) Techniques	3) Social	4) Legislation
INRA and RSP	Participatory breeding of wheat populations and mixtures with on farm evaluation and selection and collective decision-making.	Landraces and lines used as such or within mixtures or crosses	Regional and satellite farms experimental design with hierarchical Bayesian model to analyse using the R package PPBStats. Comparison of selection practices of mixtures of populations.	Regular meetings to present results and take decisions. Fields visits. Training of farmers / facilitators.	Contributed to the recognition of the role of farmers' organisations in the creation and maintenance of crops genetic resources.
INRA and ITAB	Looking for answers to farmers' question "differences between CCP and dynamic populations of wheat", in a partnership relationship between farmers and researchers	Populations cultivated at a farmer and used to create a dynamic mixture and a CCP	Involving farmers and bakers in the selection and evaluation (processing) of the populations	Informal relationship (beginning of the process)	
LBI	Participatory breeding to improve wheat populations	Spring wheat population from Dottenfelderhof	Farmers and a bakers were involved in the selection and evaluation	Informal and some meetings to discuss results and follow-up steps	
IPC and ITQB	Multi-actor approach - connect actors in the chain. Improve yield maintaining quality for maize bread. Decision tools for selection of both germplasms.	(1) VASO and SOLIBAM OPV populations and CCP, (2) 4 Portuguese traditional maize landraces and improved populations (Pigarro, Fandango, Caniceira and Bilhó) from different altitudes used in the water deficit field experiment (3) maize inbreed lines	(1) Decision tools for selection of both germplasm and locations to potentiate certain components (e.g. tocopherols); (2) Field evaluation of chlorophyll fluorescence analysis (Fv/Fm - efficiency of Photosystem II- and Performance Index (PI),-OJIP test); Field evaluation of leaf/canopy temperature (Thermal Imaging); Field evaluation of gas exchange parameters: Leaf transpiration, stomatal conductance and net photosynthesis, under saturating light (Infrared gas analyser); Leaf relative water content calculated using leaf discs collected at field trial; Field evaluation of plant growth, flowering time, biomass and yield; (3) Screening for disease resistance to mycotoxins in maize OPV; OPV sensorial analyses tests are being prepared presently (March 2019) (4) Molecular diversity assessment and nutritional grain quality evaluation (5) Genome wide association studies on maize quality traits.	2 focus groups, 2 stakeholders meeting, # interviews	

Partner	Strategy	Methods			
		1) Genetic resources	**2) Techniques**	**3) Social**	**4) Legislation**
CSIC	Comparison of self vs open-pollination breeding approaches in yield and yield determinants	Three populations in two versions open-pollinated *vs* three generations of selfing	Digital Image Analysis. Descriptors. ANOVAs and Multivariate analysis: Principal Components and Discriminant	Informal farmer meetings and collaboration in a multi-actor symposium	
RSP and ITAB	Multi-actors' approach – building a research community in order to improve co-evolution mechanisms between humans-tomatoes varieties and natural environments	Practitioners' tomatoes populations	Scorecards for practitioners on farm observation, PPBstats R package.	Stakeholders meeting twice a year, focus groups phone calls, steering committee phone calls. Building of a research community.	
RAS	Building a research community, developing evolving populations of wheat and tomato	Local varieties of wheat and public gene banks so as RAS community seed bank	Mix the most important varieties of wheat and tomato. Agronomic data were collected	Farmers prepared the trials, collected the data and collaborated in the elaboration of the protocols. In order to train them to carry on with the process RAS organised several meetings and farm visits.	
PSR	Improve rare vegetable types by crossing old varieties with commercial varieties of known target qualities, and maintain or improve taste qualities by involving chefs and other stakeholders	Old varieties and landraces collected and conserved within the PSR network	Crossbreeding with several combinations, reciprocal harvest of seeds, start new population lines out of each harvested lot and select each population over several generations according to breeding targets. Brix content measurements allows positive selection within each generation. Organoleptic selection of the best lines only in hindsight.	Stakeholder meetings to assess the resulting population lines, involvement of chefs for organoleptic evaluation.	Registration as «Niche Varieties» in Switzerland as soon as professional production is promising.
Arche Noah	Participatory research and breeding to improve colourful tomatoes for direct marketing (decentralised individual breeding programs)	Divers: Accessions from the ARCHE NOAH seed archive and other gene banks, commercial varieties, landraces / heirloom varieties, breeding lines	Pedigree selection and back-cross breeding programs. Primary, the site-specific aims indicate the selection techniques. However, one focus across several locations has been laid on leaf mould resistance.	Internal: Annual working group meetings, field days, workshops, product tastings. External: interviews with stakeholders	

1. INRA and RSP (Gaelle van Franck and Isabelle Goldringer from INRA and Pierre Rivière from RSP): Participatory breeding of wheat populations and mixtures with on farm evaluation and selection and collective decision-making

The strategy indicated on the Figure 2 refers to the Participatory breeding of wheat populations and mixtures with on farm evaluation and selection and collective decision-making. It also present the integration of Genetic resources, Techniques, Social aspects and legislation.

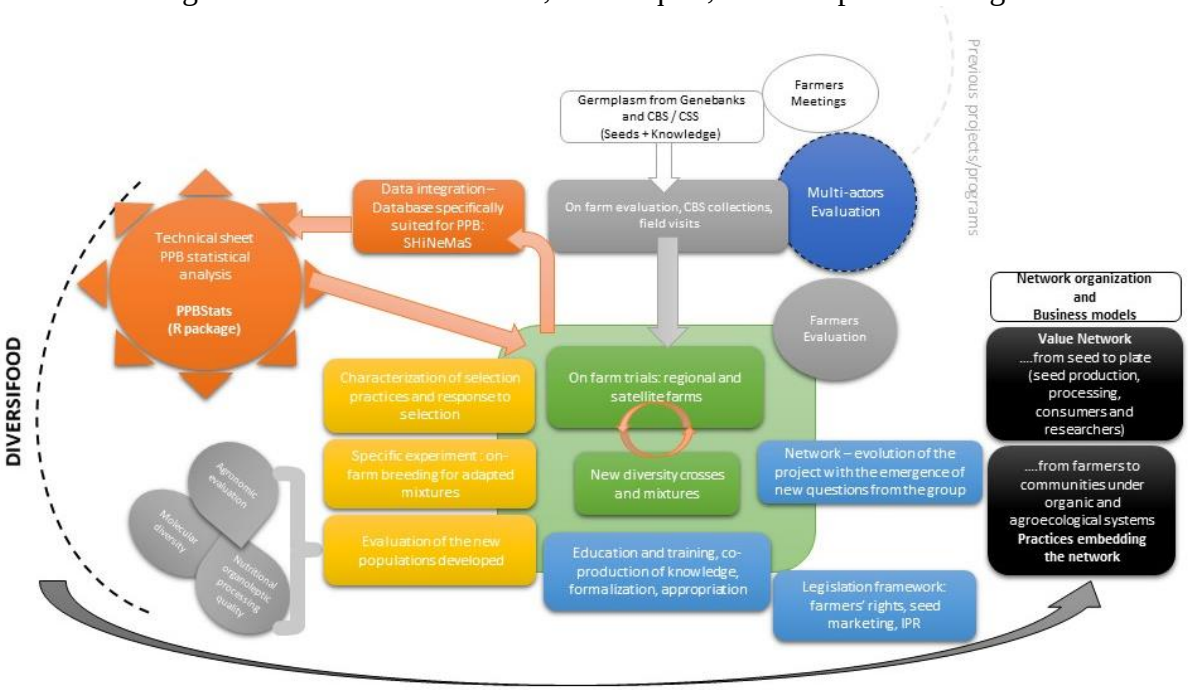

Figure 2 : Participatory breeding of wheat populations and mixtures with on farm evaluation and selection and collective decision-making. The integration of Genetic resources, Techniques, Social aspects and legislation

Genetic resources

Landraces and lines used as such or within mixtures or crosses:

- Crosses developed through earlier PPB projects were used as well as new crosses made within DIVERSIFOOD;
- New mixtures were created by farmers. The components have been chosen to meet farmers' objectives and for their complementarities. These mixtures have been subjected to different selection practices as part of a 3-year experiment;
- New CCPs were developed.

Landraces were obtained from institutional gene banks or from Community Seed Banks or Community Seed Systems most often within the Réseau Semences Paysannes. Each farmer chose the populations to test on his/her farm and selected the ones that behaved best based on several years of observation.

The management is done by farmers and farmers' organisations, and exchanges are organised through "seed exchange days" in some cases or directly between farmers. It is often included in a broader scheme of participatory breeding involving researchers of INRA.

Techniques

a) *Breeding and management of populations;*

The PPB approach has been co-designed between the farmers and facilitators of RSP and the research team of INRA. The process of participatory research is going on since 2006.

b) Statistical tools;

The experimental design allows all interested farmers to participate either through a small trial with 6 to 15 plots (called satellite farm) or through a larger trial with 15 to 50 or more plots (called regional farm). One (for satellite farms) or four (for regional farms) controls are replicated. Analysing all trials over several years is possible using the hierarchical Bayesian models developed by RSP and INRA (Rivière et al 2015; methods M7 in the deliverables D3.1 & D3.2).These design and methods allow to assess the populations and select among them based on their values within a farm or based on their averaged value over all trials and on their sensitivity to environments. The statistical analyses have been done using the R package PPBStats tool developed within DIVERSIFOOD (deliverable D3.2).

c) Quality assessment;

In addition, the farmers may also select within (mass selection) populations. An experiment has been co-designed to evaluate different selection practices for populations mixtures such as selecting plants / spikes within each component before mixing the components, selecting plants / spikes within the mixture, a combination of both and no mass selection at all. The characterisation of farmers' selection practices and the response of populations to those selection practices have been carried out.

Social

Regular meetings among farmers, facilitators and the research team were organised along the project to discuss the results and take decisions. Open field days have been organised by farmers' organisations with researchers' participation. Presentations of PPB approach and of some results of the project were given, a "selection game" was carried out and sometimes discussions between the actors, and different stakeholders outside the project were held. The RSP partner also trained the organisations' facilitators so that they are familiar with research methods and results to be able to disseminate those result both to farmers within the project and to outside people.

Legislation

The knowledge produced within the project contribute to the recognition of the role of farmers organisations in the creation and maintenance of crop genetic diversity. This was used to support the implementation of farmers' rights in the regulation and to broaden the range of seed that is allowed to be marketed.

Outcomes and conclusion regarding the efficiency and the limits of the approach

Deployment of diversity in the field, farmers' empowerment and breeding co-learning (both from farmers and researchers).

The first result is the development of new wheat populations derived from crosses, CCP, mixtures and participatory on farm breeding. These populations proved to be as productive as the current pure line varieties used in organic farming, with longer straws, more competitive against weeds but lodging resistant, with high quality for bread making, and with very diverse and interesting nutritious compositions. In addition, these populations were more stable over time

within each farm for yield and protein content. The collective organisation allowed to include many farmers and over time, both the number of Community Seed Systems and the number of farmers involved in population breeding and / or growing drastically increased. As the populations arrived in the farmer fields and were used in production, this contributed to diversifying agricultural landscapes therefore contributing to more resilience and less vulnerability of these territories.

Limits: The participatory approach requires a strong involvement of all actors and in particular a lot of time spent in the coordination, communication and facilitation. As the number of farmers and trials increased, the time spent both for the facilitation and the recording of data increased too while the people involved are renewed frequently and the funding of the CSS is not durable.

2. INRA and ITAB: Looking for answers to farmers' question "differences between CCP and dynamic populations of wheat", in a partnership relationship between farmers and researchers

The question of which "differences between CCP and dynamic populations of wheat" by farmers is being answered. This answer has been provided with the partnership relationship between farmers and researchers (Figure 3).

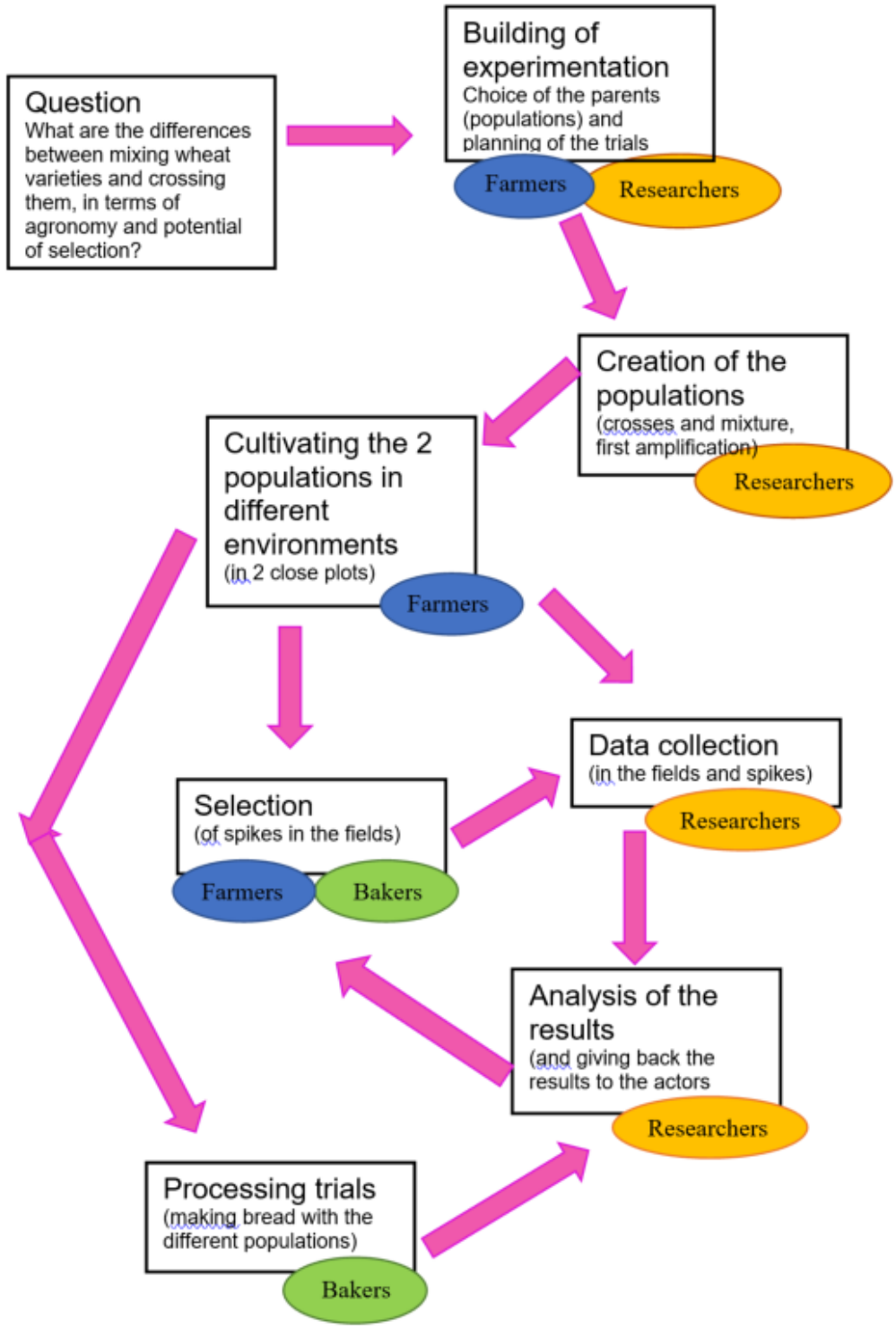

Figure 3 : Looking for answers to farmers' question "differences between CCP and dynamic populations of wheat", in a partnership relationship between farmers and researchers.

Genetic resources

From the farmers' question, a farmer and a researcher chose 6 different wheat populations (landraces cultivated at the farmer for several years) to create 2 experimental populations (a CCP and a dynamic mixture).

Techniques

a) *Breeding and management of populations;*

We have a CCP and a Dynamic mixture to compare, they are cultivated in 2 places at the moment (this could increase in the future), and they are evolving naturally on one hand, and farmer and bakers did a selection on each population (in one place per actor) in 2018. So now we have 10 populations to study (natural selection of the 2 populations in the 2 places = 4 populations; selection of the farmer for each population in place A - 2 populations - and selection of 2 bakers for each populations in place B = 4 populations).

b) Statistical tools;

We did basic descriptive statistics on the data collected and focus on the diversity indexes (CV and NEI index) of each criterion, in order to try to visualise the diversity structuration of each population.

c) Quality assessment;

It will start in 2019: a baker will make bread with CCP and Dynamic mixture of harvest 2016, 2017 and 2018 if place B. We will ask him what the differences will be felt during the process.

Social

The trials are conducted in collaboration between the researchers and farmers directly because very few farmers are involved at the moment.

Outcomes and conclusion regarding the efficiency and the limits of the approach

At the moment, the experimentation is conducted with very few people and the populations don't seem really different in terms of agronomy. The limit of this approach is that not a lot of people are involved at the moment, but it would need other types of actor (facilitator) to increase participation.

3. LBI: Participatory breeding to improve wheat populations.

So far, the work focused mostly on evaluating technical possibilities in terms of improvement of wheat populations on-farm and breeding of legumes for mixed cropping.
All activities where conducted on-farm. In the case of wheat, some farmers actively participated to improve the existing populations. However, no clear progress was observed.
For the legumes, the work focused on what sort of selection methods can be used for selection for mixed cropping in populations of faba bean and lupin, e.g. a preparatory phase before doing actual PPB.

Genetic resources

Work was conducted with wheat CCPs from Dottenfelderhof, Faba Bean from CSIC and lupin from own material.

Techniques

a) Breeding and management of populations;
Improvement of wheat populations for mono-cropping using mass selection; improvement of faba bean and lupin for mixed cropping using mass selection.

b) Statistical tools;
For wheat populations ANOVA was used in consecutive seasons to assess possible progress. For faba bean and lupin no statistical tools were used, as it was not relevant at this stage yet;

c) Quality assessment
None.

Social

With the wheat, evaluation of the results was conducted with the farmers. Farmers gave suggestions for simpler selection methods (like changing sowing rates and selection for seed size). Another option could be changing sowing depths.
For the selection in the legumes, farmers were less involved, at this stage, some principles of selection for mixed cropping were investigated.

Outcomes and conclusion regarding the efficiency and the limits of the approach

Working with farmers leads to new ideas on selection, which can be easily done on-farm and are cost-effective. Some not planned suggestions were the result of unplanned deviations from farmers' activities leading to new ideas.
For possibilities in regard to selection for mixed cropping, more research is needed to better develop the principles of on-farm selection for mixed cropping. So far, results suggest that it can be a promising approach.

4. IPC and ITQB: Multi-actor approach - connect actors in the chain. Improve yield maintaining quality for maize bread. Decision tools for selection both germplasms.

The Figure 4 shows a diagram that presents the research operationalisation of the Portuguese maize PPB program, with agronomic, molecular and quality data integration on the development of innovative, more resilient diverse maize populations with increased "breadability".

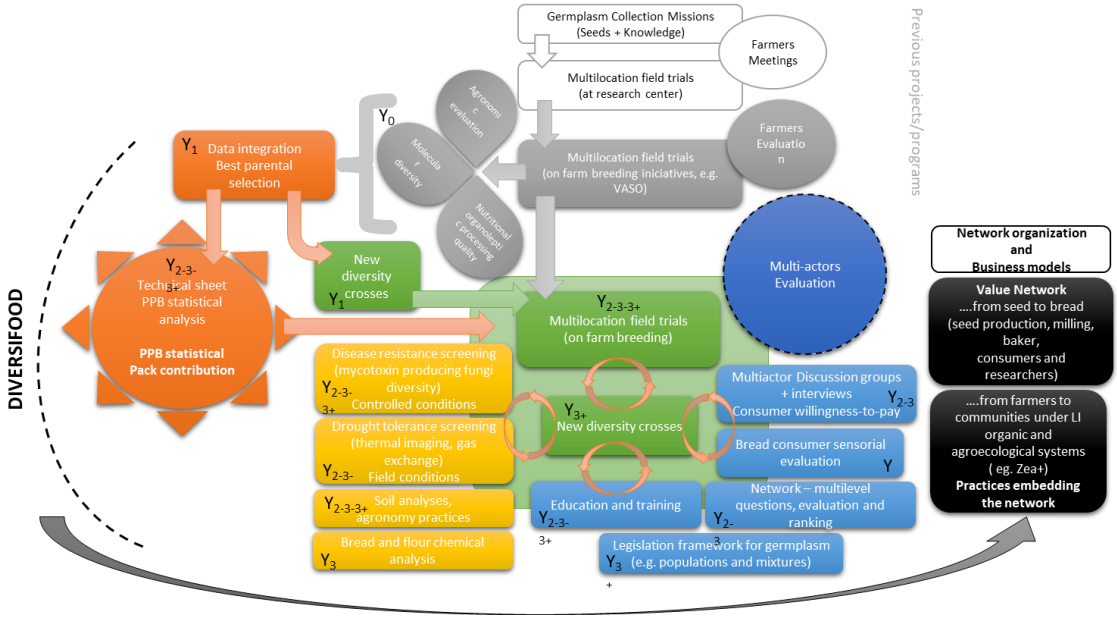

Figure 4 : Multi-actor approach - connect actors in the chain. Improve yield maintaining quality for maize bread. The integration of components such as: 1) Genetic resources; 2) Techniques 3) Social and 4) Legislation

The IPC strategy is to connect the actors of the maize bread chain, improving the production traits, maintaining diverse and rare qualities of the maize bread. To achieve these goals, we worked on the following components.

Genetic resources

Populations derived from VASO (Sousa Valley in Portugal, where PPB started in 1984 with local landraces and CCP, VASO also integrates collecting missions) and SOLIBAM project (GA n°245058) were used, but also some new accessions from collected missions were obtained.

Techniques

a) *Breeding and management of populations; b) Statistical tools;*

1) Development of decision tools to support an efficient and effective management and breeding of underused genetic resources to allow increasing the agronomic performance of the populations but also tocopherol levels, both selecting the germplasm and the locations that could potentiate the response to selection (Alves et al., 2017).

ITQB was responsible for creating decision tools for selection of both germplasm and most promising locations to establish new PPB activities based on the integration of molecular, agronomic and quality assessment of VASO and SOLIBAM maize open pollinated, and of a collection of inbred lines partially derived from traditional maize landraces. The aim was to develop molecular tools to assist on the implementation of a participatory breeding program focused on maize quality improvement, as a way to also promote the on-farm conservation and

management of the Portuguese maize landraces. To achieve this, we (Objective 1) conducted genome wide association studies to identify allelic variants associated with the organoleptic, nutritional and technological quality traits, and (Objective 2) characterised and selected the accessions genetically more dissimilar with superior quality to be used as parental lines in controlled crosses. The quality traits assessed in both open-pollinated populations and inbred lines were compounds related to nutritional value of maize flour (for example, protein and fiber content), flour technological and rheological properties (for example, flour viscosity parameters), potential bioactive compounds (for example, carotenoids and tocopherols content) and aroma-related compounds (volatile aldehydes). Overall, the integration of these different aspects had, as main objective, to improve production traits while maintaining diverse and unique qualities of these genetic resources for bread production ("breadability").

- Objective 1) Phenotypic data (quality traits) was measured on maize flour from 132 inbred lines obtained from 2 years of field trials. The genotypic information retrieved from the Maize50K Illumina BeadChip array, enquiring more than 50,000 Single Nucleotide Polymorphisms (SNPs) across the maize genome.
- Objective 2) phenotypic data (quality traits) was measured on maize flour from 32 potential parental maize open-pollinated populations, and on maize flour from 134 maize inbred lines, and genotypic data was obtained genotyping the open-pollinated populations with 20 microsatellite markers, and the inbred lines with 57 microsatellite markers.

The identification of allelic variants associated with several quality traits (Objective 1) was carried out using a genome-wide association mapping approach. Association mapping consists essentially in finding significant molecular marker-trait associations in genetically diverse populations varying for the trait of interest.

To characterise and select genetically more dissimilar lines with superior quality for future crosses under DIVERSIFOOD project (Objective 2), phenotypic information (quality traits) was used to rank the lines and the genotypic information was used to calculate the genetic distances among the different lines. The information on the lines' rank combined with the genetic distance information was then used to select, from the most promising lines, the ones that are genetically most distant.

2) To optimise under growth chamber conditions and field application of a rapid screening approach to maize OPV drought responses, the Performance Index (PI) (using the OJIP test) characterised for being an expedite, low cost and efficient field phenotyping approach for drought-resistant maize (Bicho et al, 2018).

Field evaluation of different parameters on four maize landraces cultivated at different altitudes and grown under two water regimes: well-watered conditions and water deficit. Experimental design: completely randomised with two blocks (well-watered and water deficit) and three repetitions. Each of the 24 plots had an area of 9,675 m2 and an average of 48 plants. All plants were kept under well-watered conditions (more or less at field capacity) until the 6th expanded leaf (T0 - 38 days after sowing). After that, plants belonging to the water deficit treatment were not watered anymore. The soil water content decreased to 20% one month later. The control plants were kept well-watered to maintain a good water status (Leaf relative water content around 90-95%). The following measurements were taken in the field every week during 5-6 weeks: chlorophyll fluorescence (Fv/Fm - efficiency of photosystem II- and Performance Index (PI)); leaf/canopy temperature (Thermal Imaging); gas exchange parameters: leaf transpiration, stomatal conductance and net photosynthesis, under saturating light (Infrared gas analyser); leaf relative water content calculated using leaf discs collected at field trial; field evaluation of plant growth (height, number of leaves) and flowering time. Seed production, root and aerial biomass were measured at the end of the season. Univariate analysis of variance (GLM) to test mean differences between water regimes and landraces and multivariate analysis (PCA) to access the overall landraces behaviour were performed.

c) quality assessment;

3) Fungi producing mycotoxins were isolated from different Portuguese geographical origins and varieties. The screening of resistance in 15 maize OPV to 4 selected fungi isolates is on-going. Sterilised maize grains are being inoculated with spore suspensions from the four isolates and the susceptibility/resistance is being assessed for each OVP, using a scale of incidence level of infection. The mycotoxins accumulated in the grains will be also determined soon.

4) OPV sensorial analyses tests with a consumer panel was organised in Lousada from 24 to 25 of February 2019, data are being analysed.

Social

Stakeholder meetings include breeders, farmers, millers, bakers and local politicians. On year (Y0) SWOT analyses took place at COPAGRI (Lousada Farmers Cooperative). Field days, focus groups and interviews to the stakeholders (Y1-Y3+) were established to better understand and work with stakeholders as referred in Dinis, I., Mendes-Moreira, P., & Padel, S. (2017). Developing marketing strategies for food diversity: A Case-Study In Northern Portugal. In International scientific conference RURAL DEVELOPMENT 2017 (pp. 985-991). The Sense-Bus was organised in Lousada from 24 to 25 of February 2019.

Outcomes and conclusion regarding the efficiency and the limits of the approach

Two scientific publications were made as a result of the ITQB-IPC activities: a publication on the development of decision tools to support an efficient and effective management and breeding of underused genetic resources (Alves M.L., Brites C., Paulo M., Carbas B., Belo M., Mendes-Moreira P.M., Brites C., Bronze M.R., Gunjaca J., Satovic Z., & Vaz Patto M.C. (2017) Frontiers in Plant Science, 8: 2203. DOI: 10.3389/fpls.2017.02203). And a publication on the effectiveness of participatory breeding methodologies on agronomic improvement of two historical maize open-pollinated populations, while maintaining high molecular diversity (Alves M.L., Belo M., Carbas B., Brites C., Paulo M., Mendes-Moreira P., Brites C., Bronze M.R., Satovic Z., & Vaz Patto M.C. (2018) Evolutionary Applications, 11(2): 254–270. DOI: 10.1111/eva.12549).

Following a genome wide association approach, we were able to identify putative allelic variants associated genomic regions controlling both for a decrease and increase in the average value of 27 quality-related traits in maize wholemeal flour. As scientific outcomes of this part of the work one publication is currently under review and two more publications are being prepared.

The expeditious and non-destructive physiological methods used to phenotype water deficit responses on maize landraces under field conditions proved to be efficient to clear differentiate plants under contrasting water regimes. Plant responses to water deficit were only visible (leaf curling recorded with RGB) under severe stress (20% SWC - T3) imposition. However, the more sensible leaf gas exchange measurements detected these responses earlier during the stress imposition (40% SWC - T2). Leaf temperature was higher in plants under water deficit due to stomatal closure. Differences were also clear between water regimes on the plant growth, clearly reduced in some of the genotypes under water deficit, with male flowering completed well before the starting of female flowering. The maize architecture posed some limitations on thermal imaging measurements at field conditions. Thermal imaging allowed to clearly distinguishing plants under water deficit with higher leaf temperature when compared to the well-watered plants, but it was not possible to distinguish between the different maize landraces.

A research paper is being prepared from these results and two Posters were already presented:

Bicho MC, Alves ML, Leitão ST, Ferreira E, Mendes-Moreira P, Costa JM, Vaz Patto MC (2018) Multiple-criteria approach to select drought-resistant maize populations. DIVERSIFOOD Final Congress: Cultivating diversity and food quality. 10-12 December. Rennes, France.

Leitão ST, Ferreira E, Alves ML, Araújo SS, Santos D, Mendes-Moreira P, Costa JM, Vaz Patto MC (2018) Field phenotyping of maize landraces under imposed water stress conditions: First steps on the establishment of a physiological breeding approach. Integrative Plant Biology Conference. 7-9 November. Poznan, Poland.

5. CISC: Comparison of self and open-pollination breeding approaches in yield and yield determinants.

In the Figure 5, is presented the research operationalisation of participatory agronomic and pollinator-related traits integration on the development of bi-crop adapted open-pollinated bee-friendly faba bean populations.

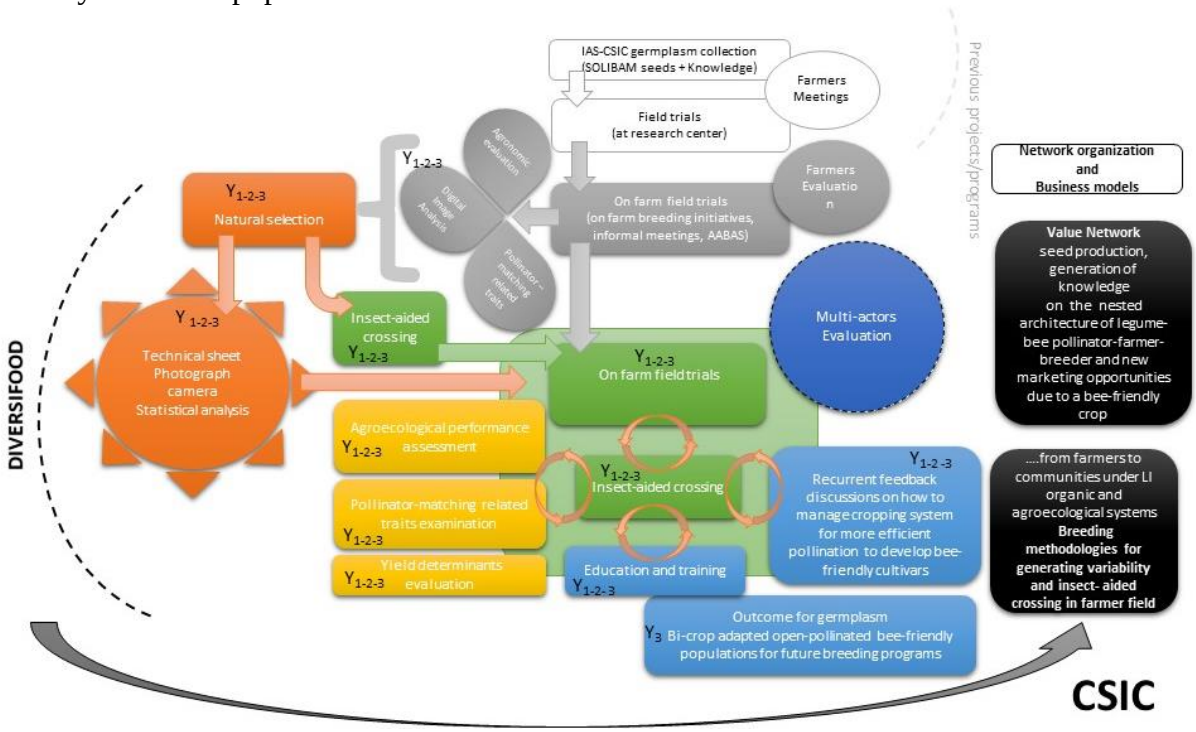

Figure 5 : Research operationalisation of participatory agronomic and pollinator-related traits integration on the development of bi-crop adapted open-pollinated bee-friendly faba bean populations.

CSIC strategy is to explore breeding approaches (self and open-pollination) for developing a bi-crop adapted and resilient faba bean crop (Figure 5).

Genetic resources

Three populations in two heterozygosity/homogeneity versions derived from SOLIBAM. The initial synthesis of each gene-pool involved different components selected from the world germplasm collection of the Institute of Sustainable Agriculture (CSIC). Under the frame of SOLIBAM, gene-pools were subjected to two pollination environment strategies: open-pollination *vs* pollinator exclusion. Thus, populations genepools highly homozygous, derived from selfing *vs.* highly heterozygous and heterogeneous, derived from open-pollination were used. In addition, and in the frame of WP2, we created a bi-crop system, faba bean-spelt, to compare two cropping management systems (alternate stips intercropping vs. monoculture).

Techniques

a) *Breeding and management of populations;*

Research was conducted in a farmer field. Natural-unconscious selection was applied (plants harvested as bulk for next year's sowing). Field preparation and cultivation practices were conducted by the farmer applying local farmers' agronomic techniques. The gene-pools were hand planted in a randomised block experiment design, with two replications. (3 genepools x 2 genetic structures + local control (nationally popular cultivar) x 2 cropping systems (faba bean-

monoculture + faba bean/spelt -1:1) x 2 replications in one location. The following trait super-categories were considered: plant architecture and seed production patterns. In addition to usually employed agronomic traits involved in coping with abiotic and biotic stresses, plant-pollinator matching traits were recorded. Leaves and flowers were collected and scanned. Digital Image Analysis was performed by using the Image Tool program to measure the pollinator related traits. No incidence of any disease and pest was detected.

b) Statistical tools; ANOVAs and multivariate approaches- principal component analysis (PCA) and discriminant function analysis (DFA) - were performed to explore differences in agronomic and plant–pollinator matching traits among breeding approaches, cropping systems and genepools. Data from each year were analysed separately because year-to-year heterogeneity.

Social

Informal meetings and training seasons with the farmers resulted in co-learning, knowledge exchange and information transfer concerning the DIVERSIFOOD concepts regarding breeding strategies and role of bee-pollinators and cropping systems.

Knowledge exchange via collaboration and participation with NGOs in a multi-actor-symposium: The AABAS symposium (Arte, Agriculturas, Biodiversidad, Alimentación y Salud -Art, Agriculture, Biodiversity, Food and Health). In this event the ideas of DIVERSIFOOD were presented by MJ Suso, presentation of "Construyendo una nueva cultura de la alimentación. La diversidad agrícola, la multifuncionalidad y la conectividad entre todos los actores"("Building a new food culture. Agricultural diversity, multi functionality and connectivity between all actors"). The multi-actor symposium was hosted by the C3A (Centro de Creación Contemporánea de Andalucía- Center for Contemporary Creation of Andalusia) and was able to join several stakeholders to share knowledge on the critical importance of cultivar diversity and the use of ecosystem services in ensuring food and nutritional security.

In addition, a Biodiversity Patch was created right on the entrance to the C3A. The Biodiversity Patch was structured into three parts for the description of the different levels of diversity, inter-crop and intra-crop and weeds, and the ecosystem services focused mostly in the pollination. Faba beans and lupines from CSIC and spelt and weeds from the farmer were sown with the help of the community. Some stakeholders carried out agricultural practices, seed sowing, weeding, flowering and pollinators observation at the Biodiversity Patch. The facility was a living and complex piece, to reflect on the ways of obtaining food and the biodiversity associated with agricultural ecosystems.

Outcomes and conclusion regarding the efficiency and the limits of the approach

Outcomes include: 1) Bi-crop adapted open-pollinated faba bean populations available for future breeding programs and 2) Exploration of efficient breeding methodologies for generating intra-population variability and for insect-aided cross-pollination

Faba bean is an entomogamous partially allogamous crop with a mixed mating system. Regarding breeding approaches, two basic philosophies have been held by faba bean breeders to deal with the partial allogamy of *V. faba*: (a) open-pollinated or synthetic populations and (b) inbred line cultivars. Open-pollinated cultivars make use of yield, yield stability and resistance to biotic and abiotic stresses mediated by heterosis. Open-pollination, although seldom used by faba bean breeders, is a method of crossing well adapted to farmer management. It is possible to recommend the crossing to the local pollinators by designing a crop that encourages their density

and visits. For breeders thinking in terms of pollinators needs is a new challenge. A few of the existing-breeding strategies have been considered from the perspective of the role of bee-pollinators and within an inter-play crop-pollinator framework. However, there are several advantages of this crossing method. It is ease to conduct with little effort many crosses can be made. Any natural selection that occurs happens under conditions of the target pollination environment supporting the development of varieties particularly adapted to social and ecological local conditions. Thus, opening new possibilities on the development of win–win options where both seed production and pollinators co-benefit and at the same time creating new opportunities for farmers to get additional incomes because their contribution to the development of the "greening economy" by maintaining wild bee populations.

6. RSP and ITAB: Multi-actors approach – building a research community in order to improve co-evolution mechanisms between humans-tomatoes varieties and natural environments

The Multi-actor approach is represented in the Figure 6, in which there is the building of a research community in order to improve co-evolution mechanisms between humans-tomatoes varieties and natural environments.

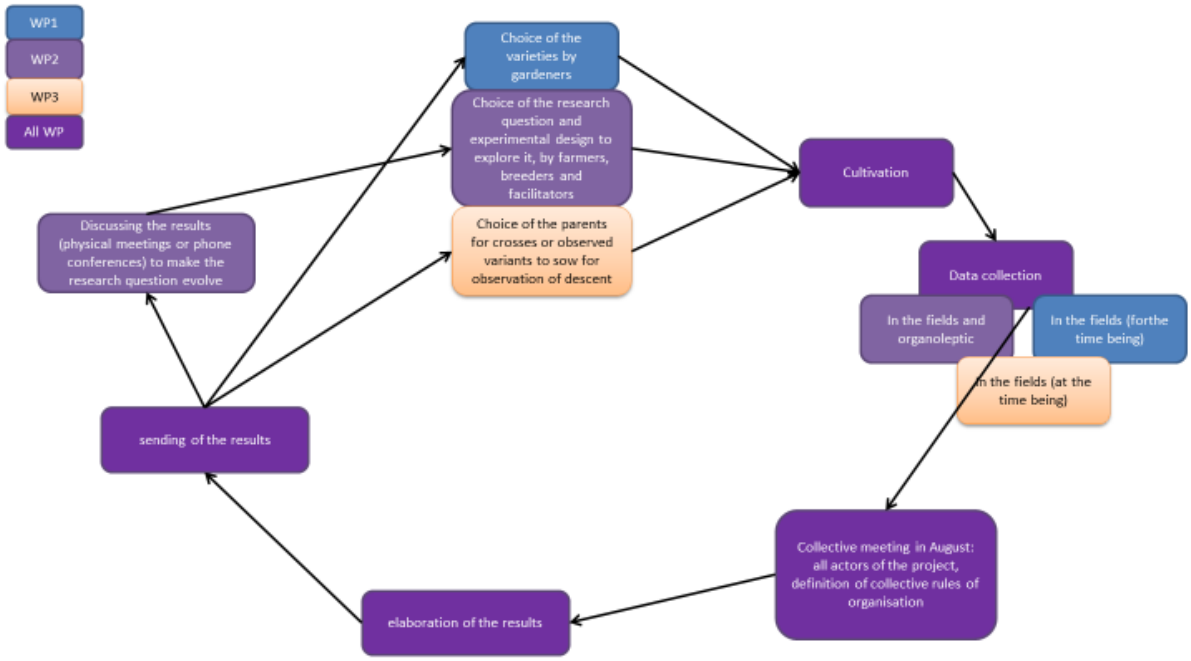

Figure 6 : Multi-actor approach – building a research community in order to improve co-evolution mechanisms between humans-tomatoes varieties and natural environments.

Genetic resources

Populations belonging to gardeners and seed producers' members of RSP were used.

Techniques

a) Breeding and management of populations;

In 2016, RSP and ITAB organized a training on how to make tomato crosses. Some people (gardeners and producers) realized some crosses during the summer and collected seeds to observe the cross descent in 2017. Beside this, the majority of populations used for tomatoes PPB trials were managed by mass selection. However, it is important to notice that tomatoes PPB French group members prefer to talk about "co-evolution" rather than "breeding" or "selection". The concept of co-evolution between humans, plants and natural environments reflects better the relations between the members of the group and their tomatoes varieties than the word "selection" which focuses only on human will without taking into account the plants behaviours and environment characteristics' effects on "breeders' will".

b) Statistical tools;

In 2015 and 2016, multi-local trials of several varieties of Coeur de bœuf tomatoes were carried out. In 2017, multi-local trials were carried out in order to assess the influence of different technical practices on a series of tomatoes varieties characteristics (yield, taste, diseases, etc.). The trials carried out in 2018 consisted in the evaluation of several strains of several tomatoes'

varieties in 2018. The idea was to observe yield, taste and phenotypic differences among strains of the same variety. Scorecards were collectively elaborated together with researchers and practitioners in order for practitioners to carry out on farm observations and data collection. Collected data were analysed using PPBstats R package through descriptive (boxplots, barplots) and analytic stats (ANOVA).

c) quality assessment;

Some qualities (taste, yield, diseases) of each trials' varieties and strains were observed by practitioners and some other (phenologic, morphologic) qualities were assessed by students trainees (in 2016 and 2018).

Social

Steering committee phone calls (at least 4 per year), stakeholders meeting (2 per year) and e-mails were carried out in order to organise the trials, discuss previous trials results, elaborate internal rules for the group, get stakeholders to know each other and share ideas and points of view. Beside trials and tomatoes observations, the aim of the tomatoes PPB French group was to build a research community gathering practitioners and researchers.

Outcomes and conclusion regarding the efficiency and the limits of the approach

Regarding the participatory approach, the limiting factor is the time needed to communicate sufficiently in order for everyone's needs to be taken into account.

Regarding the building of a research community, the initial participants start to know each other quite well and the group is being opened to other collective structures working on the same subject (collective structures more than individuals). However, there is a challenge in managing the opening of the group while continuing building the research community (increasing diversity means increasing ideas numbers: how to go in the same direction with more and more different purposes and contexts?). This is management of people diversity, with people that are used to manage biological diversity.

7. RAS: Building a research community, developing evolving populations of wheat and tomato.

Wheat-YEAR 1

1. To select the farm in which trials will be developed
2. To choose the wheat varieties to be tested. The priority is to choose those used by farmer partners and that work well. They could choose some varieties characterised by RAS at previous projects (better if they choose another) and others that have not been characterised before.
3. Preparation of a protocol for the trial
4. Implementation and monitoring of the trial
5. Data collection

Wheat-YEAR 2

6. Implementation and monitoring of the trial. The seeds used came from the harvest of the first year.
7. Data collection

Tomato- YEAR 1

1. To select the farm in which trials will be developed.
2. To choose the tomato populations to be tested. The priority is to choose those used by the farmer partners and that work well. They could choose some varieties characterised by RAS at previous projects (better if they choose another) and others that have not been characterised before.
3. Preparation of a protocol for the trial
4. Implementation and monitoring of the trial
5. Data collection

Tomato-YEAR 2

6. Implementation and monitoring of the trial. The seeds used came from the harvest of the first year.
7. Data collection

Genetic resources

WHEAT

Farms:
Location 1: El Viso de Los Romeros (Yunquera-Málaga)
Location 2: Los Portales (Castilblanco de los Arroyos)

Number	Type	Name	Reason for chosen it	Donor
Variety 11	Durum	Mixture of 6 durum wheats	Local variety and enough seeds available	Organic farmer
Variety 12	Bread wheat	Mixture of 4 bread wheats	Local variety and enough seeds available	Organic farmer

TOMATO

LOCATION 1: El Viso de Los Romeros (Yunquera-Málaga)

Number variety	Name Variety	Type of variety	Reason of chosen it	Donor
9	Mixture 1: Tomate corazón de buey de Jerez de la Frontera Tomate Corazón de toro de los Huertos de Zambullo de Baena Tomate corazón de toro de Huetor de Santillan Tomate Corazón de Aroche	Mixture of local varieties	Local bull's heart type from complementary regions	Public gene banks RAS community seed bank

LOCATION 2: Huerta La Alegría (Marchena, Sevilla)

Number variety	Name Variety	Type of variety	Reason of chosen it	Donor
10	Mixture 2: Tomate corazón de buey de Fuente Obejuna Tomate rojo corazón de toro de Benaoján Tomate morado corazón de toro de San Nicolas del Puerto Tomate Corazón de Toro del carnicero de Alozaina	Mixture of local varieties	Local bull's heart type from complementary regions	Public gene banks RAS community seed bank

Techniques

a) *Breeding and management of populations;*

Wheat

RAS has mixed most interesting wheat varieties tested in WP3 and WP2. RAS has tested a mix of 4 varieties of bread wheat and 6 varieties of durum wheat. The trials have been conducted for 2 years to see the evolution of the mixture (sowing in autumn 2015 and 2016, with crop seed-mix of the previous year). Only agronomic data have been collected.

Tomato

RAS has mixed seeds of different types of bull's heart tested in WP3 (Summer 2016 and 2017). Two different mixtures will be tested (4 varieties in each mix in each location), with crop seed-mix of the previous year. Only agronomic data have been collected.

Social

- Wheat: The trial has been carried out in an organic farm in Castilblanco de los Arroyos (Sevilla) and Yunquera (Málaga)
- Tomato: The trial has been carried out in an organic farm in Marchena (Sevilla) and Yunquera (Málaga)

b) Statistical tools;
Farmers have prepared the trials, collected the data and collaborate in the elaboration of the protocols. In order to train them to carry on with the process RAS has done several meetings and farm visits.

Outcomes and conclusion regarding the efficiency and the limits of the approach

- Populations' evaluation records.
- Protocol for the implementation and development of a trial of tomato and wheat mixtures.

First steps of evolutive populations of wheat and tomato. RAS needs more time to continue with the trials, generate more data and assess the populations and their development.

8. PSR: Improve rare vegetable types by crossing old varieties with commercial varieties of known target qualities and maintain or improve taste qualities by involve chefs and other stakeholders

In Figure 7 is presented the participatory organoleptic quality integration on crossbreeding old and new varieties for the agronomic improvement of rare vegetable types (e.g. of carrots, onions).

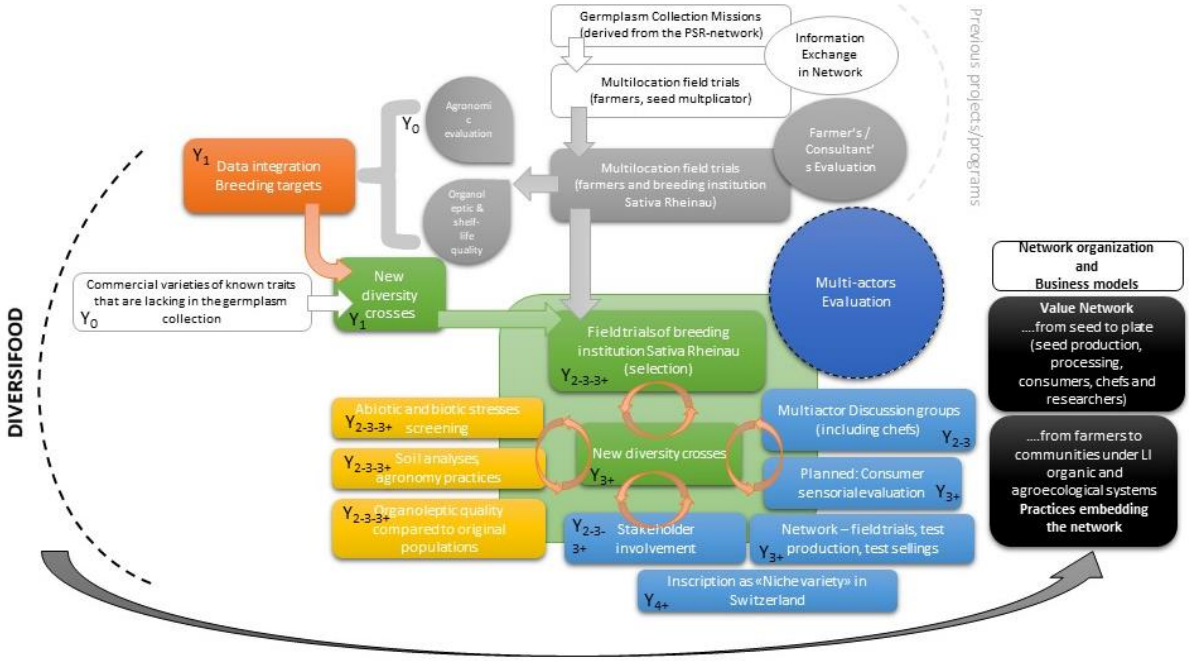

Figure 7 : Participatory organoleptic quality integration on crossbreeding old and new varieties for the agronomic improvement of rare vegetable types (e.g. of carrots, onions).

Genetic resources

PSR collects rare varieties and secures them in the network of PSR by multiplication of seeds with private gardeners, partner institutions, as well as breeding institutions like Sativa Rheinau. By collecting and conserving these germplasms within the PSR-network, PSR is able to receive many impressions from practice. Promising vegetable varieties for professional organic production are tested step by step, first at PSR station, second at Sativa Rheinau and at FiBL, third at farms. Agronomic evaluation is as important as are organoleptic tests and observation of shelf life and other qualities during processing. From these tests a stakeholder consortium decides which varieties stand a chance for professional production, and which varieties need some breeding efforts.

Depending on the characteristics to improve, breeding targets are defined. In some cases, a strong selection within a population is adequate in order to improve the most impeding shortcomings. In other cases, smart crosses between the old varieties and commercial varieties of known target traits are mandatory for a sufficient improvement. (For example, the purple and white carrot 'Gniff' was in a rather depressed plant condition, resulting probably from inbreeding in generations before. Crossings with other purple varieties were mandatory in order to save the carrot type of 'Gniff'.)

Techniques

a) *Breeding and management of populations;*

Flowering of original germplasms together with modern varieties and harvesting of filial seeds on both parents (A x B, B x A). Then starting new population lines out of each harvested lot of Y1.

b) Statistical tools

Positive selection within each population line according to the breeding targets.

Each line features enough seed bearers in each generation in order to omit inbreeding, as these lines shall work each as healthy populations that may be developed as fast as possible to rather homogeneous varieties again.

Selection of single plants generally done by "breeders eye", i.e. visual examination. Complementary selection by Brix content of each plant, i.e. with onions and carrots (Y1 - Y3+).

c) Quality assessment

However, organoleptic qualities cannot provide criteria for plant by plant selection, as features of processed products need to be evaluated as well. Organoleptic quality therefore is pursued in hindsight that is by evaluating the harvest of a certain line/generation, and deciding about further pursuing this line (Y2 - Y3+).

Social

Stakeholder meetings include breeders, farmers, consultants, merchants, and for tasting meetings most important, also chefs. In a first meeting (with Y0 – Y2) participants tried to evaluate taste qualities of the original varieties and to provide breeding targets (note: a target may very well be to keep the qualities just as is).

In later meetings (with Y3+) evaluation states which lines are on track, and which lines will not be pursued any more. Also stakeholders will now get an idea of what to expect from the resulting breeding varieties. First test productions with the current populations might be arranged.

Legislation

When all trials and test productions are positive, and the commercialisation of a new variety is promising, PSR will register such as «Niche variety» in Switzerland. A «Niche Variety» may be traded only with a certain total amount of seeds per year. From PSR's own perspective the new variety needs to be in accordance with the ProSpecieRara plant criteria guidelines in order to allow the label with its commercialisation.

Outcomes and conclusion regarding the efficiency and the limits of the approach

- As commercial success will be limited to the level of a «niche variety», a rather low technical input is adequate.
- As organoleptic quality shall be one of the pillars for the unique selling proposition, and the varieties shall be interesting for consumers and the gastronomy alike, efforts in culinary improvement are important. Even more resources and time for organoleptic improvement is advisable.
- However, the limits of organoleptic selection are given by the possible evaluation of a single plant/fruit.

- Brix content can be measured with a small quantity of juice from a single onion or carrot, that can still be used as seed bearer. Positive selection on Brix content is therefore easy and promising for fast improvement.
- Taste evaluations and selection should be done by several people. It should treat the raw product as well as the processed product. A single onion or carrot cannot deliver enough substance for such evaluations and still keep a viable seed bearer. Taste selection therefore can only be done in hindsight, in order to dismiss inferior lines. Taste selection therefore slows down the development of a new breed.

9. Arche Noah: Participatory research and breeding to improve colourful tomatoes for direct marketing (decentralised individual breeding programs).

The strategy is to support the own initiative of individual organic farmers and create a collaborative framework for open exchange of experiences, knowledge and seeds. In the centre are the decentralised breeding programs aiming to improve colourful tomatoes for direct marketing. Research issues (like resistance against leaf mould or late blight) evolve from the farmer's realities. Then the researchers in the network try to develop answers for the emerging questions. ARCHE NOAH is responsible for building a collaborative framework to enable these dynamic processes and for supporting farmers by their decentralised on-farm breeding activities (Figure 8).

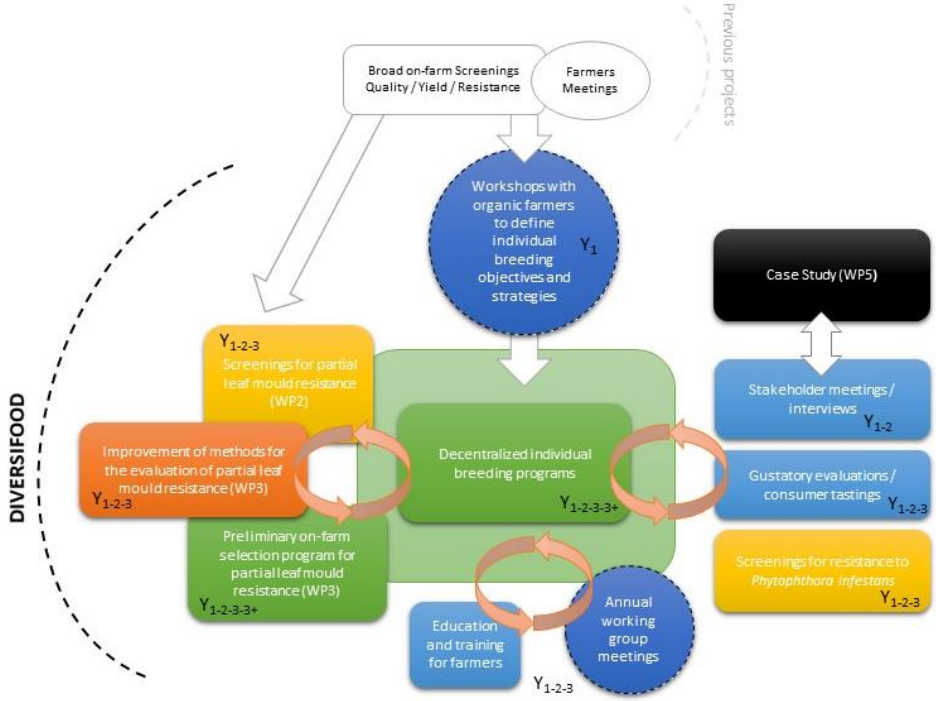

Figure 8 : shows a diagram that presents the operationalisation of participatory research and breeding activities for the improvement of colourful tomatoes for direct marketing in the framework of the 'Bauernparadeiser' ('farmer's tomatoes') working group in Austria.

Genetic resources

The genetic resources used in the breeding programs are very diverse. You find accessions of the ARCHE NOAH seed archive and other gene banks, as well as common commercial varieties or on-farm cultivated landraces. Further, breeding lines from tomato breeders and universities have been used.

Techniques

a) *Breeding and management of populations;*

The individual objectives and programs ask for different techniques. However, a focus in this project has been laid on the development of efficient on-farm methods to determine levels of partial leaf mould resistance.

Regarding breeding, we work with classic pedigree selection and back-cross breeding programs. The objective is always to develop OP varieties (homozygous lines).

Social

For the internal exchange we have organised annual working group meetings, field days and product tastings. Special issues (e.g. inoculation method for the selection of virus resistance, gustatory evaluations) have been presented and discussed in workshops.
For the external exchange we have conducted interviews with different stakeholders.

Outcomes and conclusion regarding the efficiency and the limits of the approach

During the project the participatory tomato breeding initiative 'Bauernparadeiser' evolved very well. It became possible to shift awareness from variety screenings to breeding activities. Meanwhile 14 organic farms are working on about 35 individual breeding programs. Starting from an agronomic moderate performance level of heirloom varieties in most cases, there are already improvements visible for the farmers. Furthermore, consumers (especially in the context of CSA farms) value the engagement for breeding and seed sovereignty.
However, there are also challenges regarding technical aspects, especially limitations of breeding population sizes on small-scale organic farms. And also regarding strategic aspects: it's difficult to ensure a continuous funding to support these long-term breeding activities, because most projects won't fund routine actions.

Conclusion

The development of PPB methods developed in DIVERSIFOOD can be presented as a SWOT analyses.

Strength

- Empowerment and breeding co-learning (both from farmers and researchers). The work with farmers leads to new ideas on selection, which can be easily done on-farm and are cost-effective.
- Favours the development of expeditious and non-destructive physiological methods used for phenotyping under field conditions (e.g. water deficit responses on maize proved to be efficient to clear differentiate plants under contrasting water regimes).
- Provide the opportunity to establish a protocol for the implementation and development of trials.
- New wheat populations derived from crosses, CCP, mixtures and participatory on farm breeding proved to be as productive as the current pure line varieties used in organic farming, with longer straws, more competitive against weeds but lodging resistant, with high quality for bread making, and with very diverse and interesting nutritious compositions. For some experiments more time is needed.
- Shift awareness from variety screenings to breeding activities (e.g. 'Bauernparadeiser').
- Consumers (especially in the context of CSA farms) value the engagement for breeding and seed sovereignty.
- Provide the local adaptation within either individual or collective breeding programs.
- The collective organisation allowed to include many farmers and over time, both the number of Community Seed Systems and the number of farmers involved in population breeding and / or growing drastically increased.
- As commercial success will be limited to the level of a «niche variety», a rather low technical input is adequate.

Weakness

- The participatory approach requires a strong involvement of all actors and in particular a lot of time spent in the coordination, communication and facilitation, i.e., the scarcity of human resources is limitative.
- Challenges in enlarging a group of work,
- For selection for mixed cropping, more research is needed to understand the co-evolution of crops.
- Limits of organoleptic selection are given by the evaluation of a single plant/fruit.
- The limited product, considering the single plant/fruit and several people to taste it, for taste evaluation and selection of raw and processed material slows down the development of a new breed

Opportunities

- Deployment of diversity in the field for more resilient crops.
- The diversity of populations on farmers' fields contribute to diversify the agricultural landscapes therefore contributing to more resilience and less vulnerability of these territories.
- Open-pollinated cultivars of faba bean make use of yield, yield stability and resistance to biotic and abiotic stresses mediated by heterosis. Thus, opening new possibilities to pollinators co-benefit and at the same time creating new opportunities for farmers to get

additional incomes because their contribution to the development of the "greening economy" by maintaining wild bee populations.

- Development of the principles of on-farm selection for mixed cropping. So far, results suggest that it can be a promising approach
- As organoleptic quality is one of the pillars for differentiation as a selling proposition. The varieties shall be interesting for consumers and the gastronomy alike, efforts in culinary improvement are important. More resources and time for organoleptic improvement is advisable.
- Positive selection on Brix content as promising for fast improvement (e.g. brix measuring of small quantity of juice from a single onion or carrot that can still be used as seed bearer).

Threats

- As the number of farmers and trials increased, the time spent both for the facilitation and the recording of data increased too while the people involved are renewed frequently and the funding of the CSS is not durable.
- It’s difficult to ensure a continuous funding to support the long-term breeding activities, because most projects won’t fund routine actions.
- There are also challenges regarding technical aspects, especially limitations of breeding population sizes on small-scale organic farms.