



DIVERSIFOOD

Embedding crop diversity and networking for local high quality food systems

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D3.3

Assessment of the potential of the diverse methods to create relevant diversity for plant breeding

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Abstract

To carry out on-farm PPB, farmers use and develop various types of varieties depending on their breeding objectives and on the crop mating system. Usually they aim at developing varieties with a certain level of diversity, i.e. population-varieties, which are suited to their specific environmental conditions, farming practices and marketing objectives. In D3.3, we first describe the different types of varieties and populations-varieties, the methods used for creating them and we highlight their advantages and their limits. Then, examples of population-varieties developed within DIVERSIFOOD T3.1 are described with their breeding objective and an assessment of their potential to reach their goal.

Table of content

1. Introduction.....	4
2. Types of varieties, populations and methods for creating genetic diversity for PPB	4
2.1. Pure lines and F1 hybrids	4
2.2. Landraces and old varieties	4
2.3. Mixtures of landraces or old varieties (or pure line varieties bred for organic farming)	5
2.4. Bi-parental crosses	5
2.5. Composite Cross Populations (CCP) / Mixtures of crosses	6
2.6. Open-pollinated population.....	6
3. Analysing examples of new populations created within DIVERSIFOOD T3.1.....	7
3.1. Development of different composite crosses populations of white lupin (<i>Lupinus albus</i> L.) (FiBL).....	7
3.2. Identification of maize germplasm with superior quality traits for new hybrid or synthetic populations' development (ITQB-NOVA).....	8
3.3. Tomato participatory breeding (RSP-ITAB).....	12
3.4. Evolutionary – Participatory Breeding generates wheat populations adapted for organic agriculture in Italy (Rete Semi Rurali, RSR)	15
3.5. Comparison of phenotypic diversity of 2 populations (CCP and Dynamic populations) (ITAB-INRA).....	18
3.6. Evaluation of new faba bean gene-pool (CSIC).....	20
3.7. Effect of the type of variety used in binary crosses of bread wheat (INRA-RSP).....	21

1. INTRODUCTION

Crop diversity is required to support more resilient and sustainable agricultural practices and diversified food systems. Developing PPB approaches together with farmers is a good way to increase diversity in the fields and plates. To carry out on-farm PPB, farmers use and develop various types of varieties depending on their breeding objectives and on the crop mating system. Usually they aim at developing varieties with a certain level of diversity, i.e. population-varieties, which are suited to their specific environmental conditions, farming practices and marketing objectives. In the following, we describe different kinds of those populations-varieties, we highlight their advantages and their limits, and we illustrate with examples from DIVERSIFOOD T3.1. The breeding and management practices used for the development of the populations are described and their potential to create relevant diversity for PPB is analysed.

2. TYPES OF VARIETIES, POPULATIONS AND METHODS FOR CREATING GENETIC DIVERSITY FOR PPB

2.1. *Pure lines and F1 hybrids*

Pure line varieties are composed of highly homozygous plants that are almost all genetically identical. It is the main variety type currently marketed for self-pollinated crops. They can be reproduced unchanged on-farm by farmers, but their potential for evolution and for response to selection is very low. They can be used as parents for crosses or as components of mixtures (see below). They also can be developed using classical selection methods such as pedigree or single seed descent.

For out-crossing crops, F1 hybrids are often marketed. They are obtained by crossing two pure lines and therefore all plants are genetically identical and highly heterozygous. Due to segregation of genes in the progeny, F1 cannot be maintained unchanged by farmers. They can be used as parents to create open-pollinated varieties (see below). They also can be developed but it requires particular systems to produce enough seed amount for marketing.

Interest: They may bring an interesting source of diversity if they have been bred under organic farming practices respecting natural barriers and plant mating systems as it is mentioned in IFOAM principles.

Limits: Most PPB programmes do not aim at developing these types of varieties since they have a very narrow genetic basis and they don't allow for adaptability and resilience of the crop under low-input, organic and/or sustainable farming systems.

2.2. *Landraces and old varieties*

Landraces are genetically heterogeneous populations locally adapted through on-farm management. Old varieties have usually been developed through mass selection within landraces or after crosses by breeders before the 50's. They maintain some internal diversity. Since both are reproduced under their natural mating system, self-pollinated landraces are mostly composed of related inbred individuals, while out-crossing landraces consist in heterozygous plants. They can be reproduced on-farm with a specific attention to limit genetic drift by growing populations of sufficiently large size. They can also be used as parents for crosses or as components of mixtures.

Interest: Their great interest relies on the large diversity of varieties available, in their adaptive potential to low input and agroecological conditions and in their organoleptic and nutritional quality.

Limits: Usually they need some further adaptation to the current growing conditions; if they have been maintained *ex situ* during several decades, their diversity might be drastically reduced due to the small size of populations for maintenance and regeneration.

Example in DIVERSIFOOD: Tomato landrace evaluation (3.3, ITAB, RSP).

2.3. Mixtures of landraces or old varieties (or pure line varieties bred for organic farming)

While harbouring many interesting traits, landraces and old varieties might be too far from farmers' expectations to be grown individually. To this end, favourable traits from different origins might be combined by mixing a set of selected landraces, or old varieties, to design a "blend" with the expected morphological, adaptive and quality traits. However, interactions among plants (competition vs. complementarity or synergy) from different varieties are not predictable. It might be necessary to manage the mixture by applying mass selection during several generations. Mixing populations of self-pollinating species will lead to limited recombination among plants while mixing out-crossing populations will allow the creation of a new broad-based open pollinated population.

Interest: Increases drastically within field diversity therefore providing buffering capacity, resilience and adaptability. Complementarity among carefully chosen components can provide positive effects in the mixture (e.g. lodging resistance when mixing a short and solid straw variety with taller ones). The process is rapid and simple, and it can easily be achieved by farmers. Even for self-pollinating populations, a small rate of outcrossing (e.g. 1-10% such as in bread wheat) will allow the creation of a significant (and increasing over generations) number of new genotypes; the more different genotypes in the population, the more new genotypes may appear by crossing.

Limits: Mixing populations of self-pollinating species will lead to limited recombination among plants and therefore, although the mixture can be obtained in one generation, the emergence of new genotypes will take several generations. If one or several components are uncompetitive, they might disappear very quickly from the mixture.

Examples in DIVERSIFOOD: Buckwheat, bread wheat dynamic populations (INRA) and bread wheat (RSR).

2.4. Bi-parental crosses

Crossing two parents (landraces, old or recent varieties) allows combining their characteristics through recombination of their genomes. The more distant the parents, the more diversified the progeny. Mass selection within the cross progeny may be applied to select new phenotypes or to frame the outline of the variability.

Interest: Allows rapid and efficient recombination among parents' genomes and the creation of potentially interesting new genotypes combining advantageous traits from both parents. New phenotypes can even appear by crossing parents of similar phenotypes but with very distinct origins and therefore genotypes.

Limits: Crossing plants of self-pollinated species is feasible on-farm by farmers, although it is time consuming and requires dedicated skills.

Examples in DIVERSIFOOD: New bi-parental populations of bread wheat (INRA & RSP), and carrots (PSR).

2.5. Composite Cross Populations (CCP) / Mixtures of crosses

CCP consists in crossing several parents (any type of varieties) two by two during one or several consecutive generations. The more crossings, the more recombination and possibilities for new genotypes to be generated. In case of a small number of parents, all crosses among all parents can be realized ($n*(n-1)/2$ crosses with n parents), while if a larger set of parents is to be crossed, only $n/2$ crosses can be made, followed by crosses among the descendants for a certain number of generations. The objective is that all parents contribute equally to the population and with a maximum of recombination among them. This approach is used in self-pollinated crops and requires patient hand crossing work. In case of out-crossing species, plants will randomly mate if mixed and there is no need to limit to particular crosses. This will lead to a new broad-based open-pollinated population (see below). Mass selection in further generations may allow to frame the CCP or to detect particular new phenotypes of interest.

Interest: Create populations with a broad genetic basis (similar to mixtures) but with more diversity due to the numerous new genotypes obtained from the crosses. The adaptive potential is therefore larger as much more genotypes are present in the population and it provides a large potential for mass selection in the subsequent generations due to the increasing number of new genotypes.

Limits: It is very heavy to achieve even with a limited number of parents. It can be more difficult to design CCP than bi-parental crosses since interactions among several genotypes are more complex to anticipate.

Examples in DIVERSIFOOD: Buckwheat, bread wheat (INRA), lupin (FiBL), bread wheat (ORC and RSR).

2.6. Open-pollinated population

In an out-crossing population (see above), plants are highly heterozygous if within-population genetic diversity is sufficient, i.e. population size is sufficient (at least several thousands) and selection is not too strong. New open-pollinated populations can be created by mixing several landraces, old or recent varieties (bred under organic conditions) of an out-crossing species.

Interest: It is easy to create new populations as it does not require careful handling of crosses. It allows to combine many different parents in one or several steps.

Limits: It might be difficult to apply efficient mass selection since most often the male parents of the subsequent generation are not known and selection is made only on the female parents. However, selection can also be applied on the male parent before pollination (e.g. by detasseling of less interesting plants). Optimization of the within-population diversity is not straightforward.

Example in DIVERSIFOOD: Maize (ITQB & IPC).

3. ANALYSING EXAMPLES OF NEW POPULATIONS CREATED WITHIN DIVERSIFOOD T3.1

3.1. Development of different composite crosses populations of white lupin (*Lupinus albus* L.) (FiBL)

White lupin cultivars have no sufficient tolerance to *Colletotrichum* to allow cultivation in Switzerland. Based on large scale screening of genetic resources (2014-2018, see WP2.3) parental lines were selected and crossed in order to increase genetic diversity that allow for selection of populations or lines with higher disease tolerance.

In 2017, 32 single and double crosses were performed to combine different resistance sources, resistance x vigour, resistance x earliness. Unfortunately we lost most F1 seeds due to insect attack. Crosses continue in 2018.

The F2 seeds obtained from F1 plants (from 10 different crosses of 14 parents in 2015) grown in the field in 2016 under severe *Colletotrichum* infestation were bulked to create 2 different composite crosses that were grown again under severe *Colletotrichum* infestation:

- CCP1: 5-6 seeds from each cross,
- CCP2: available seed per F1 plant (more tolerant plants had more seed).

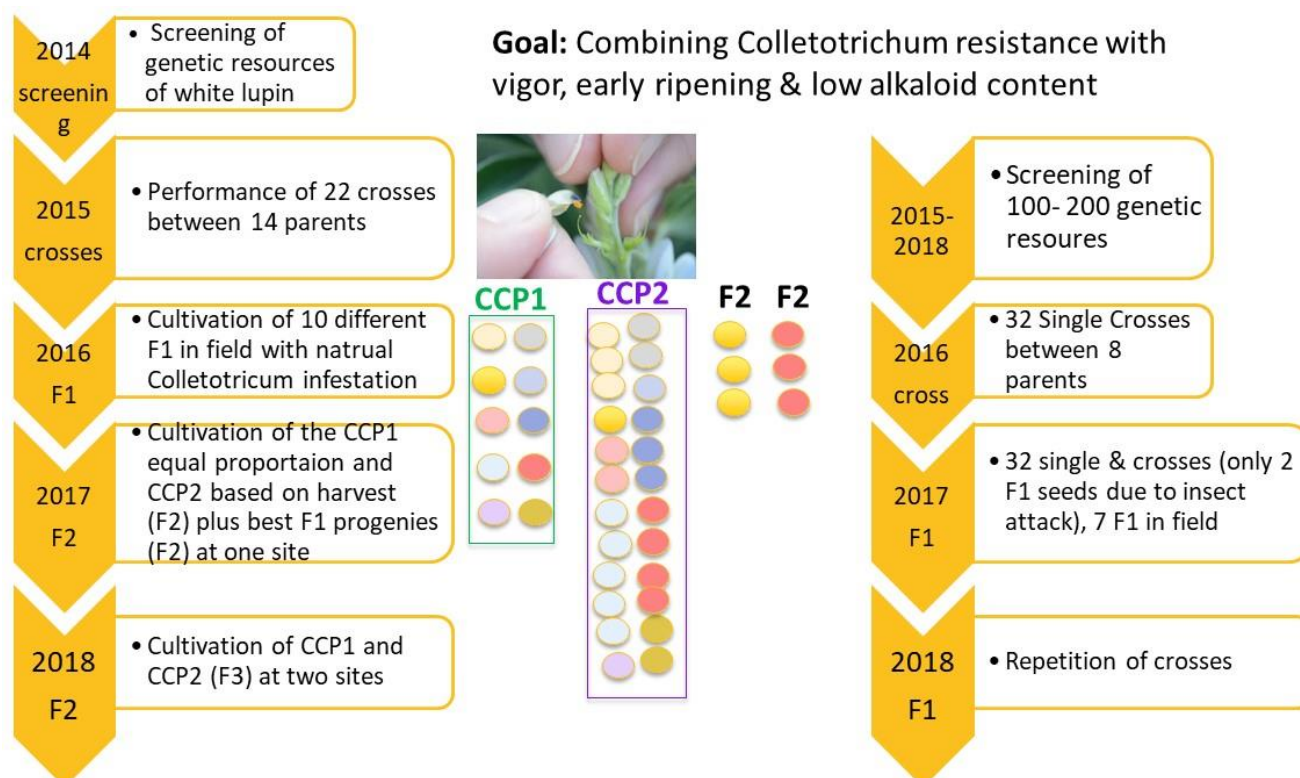


Figure 1 : Creation of FiBL's composite cross populations of white lupin since the beginning of the project.

Main results:

Although predominantly self-pollinating, considerable amount of outcrossing can occur in the field. This results in off-types and bitter seed during seed multiplication.

On the one hand this allows selection of out-crossed healthy plants and on the other hand makes pollination protection necessary to maintain lines or populations.

Level of resistance is quite rare, most genetic resources have no superior resistance level. Best accessions came from Turkey, Azores, Ethiopia, and Greece.

Seed derived from own multiplication have high level of seed born *Colletotrichum* as detected by qPCR.

Therefore, multiplication of CCP will cause high selection pressure, as highly infected plant will not produce any seed. We will then evaluate the potential of CCP methods to create relevant diversity for *Colletotrichum* resistance breeding.

3.2. Identification of maize germplasm with superior quality traits for new hybrid or synthetic populations' development (ITQB-NOVA)

To select genetically more dissimilar maize accessions with superior quality to create relevant diversity through crosses under the DIVERSIFOOD project, phenotypic data (quality traits) and genotypic data (molecular markers) were jointly analysed.

The quality data was measured on maize flour from 32 potential parental maize open-pollinated populations (EU-SOLIBAM, single year field trial), and from 134 maize inbred lines (FCT-MOXI, 2 years of field trials in a randomized complete block design, with two replicates).

The genotypic data was obtained genotyping the aforementioned open-pollinated populations and inbred lines with 20 and 57 microsatellite markers, respectively. The quality traits assessed in both cases were compounds related to nutritional value of maize flour (such as protein and fibre content), flour technological and rheological properties (such as flour viscosity parameters), potential bioactive compounds (such as carotenoids and tocopherols content) and aroma-related compounds (volatile aldehydes). Afterward, the phenotypic information was used to rank the different accessions in what concerns aroma, antioxidants content and rheological ability and the genotypic information was used to calculate the genetic distances among them. Following the IPC partner indication, white and yellow/orange germplasms were separately ranked. The phenotypic rank combined with the genetic distance information was then used to select, from the most promising sources of particular quality traits, the pairs of accessions that were genetically more distant.

The quality improvement of Portuguese maize germplasm for its use on the development of more nutritious baking food products should consider increasing their limited antioxidant compounds content and decreasing their high flour viscosity and particular volatile aldehydes that may contribute to a reduced "breadability" and the production of off-flavours in food products. Taking this into account the rank was used to select the maize open-pollinated populations and inbred lines to be used as parental lines in crossing schemes to increase (tocopherols and total free phenolic acids content) or decrease (overall viscosity values or volatile aldehydes content) particular quality traits. The top-ranking accessions for each of those subsets were identified using the information from Figure 2 (genetic distances among maize open-pollinated populations) or Figure 3 (genetic distances among maize inbred lines). The

recommended crosses were given taking into consideration the genetic distances between accessions by selecting the accessions genetically more distance or avoiding crosses between the most similar genetic accessions.

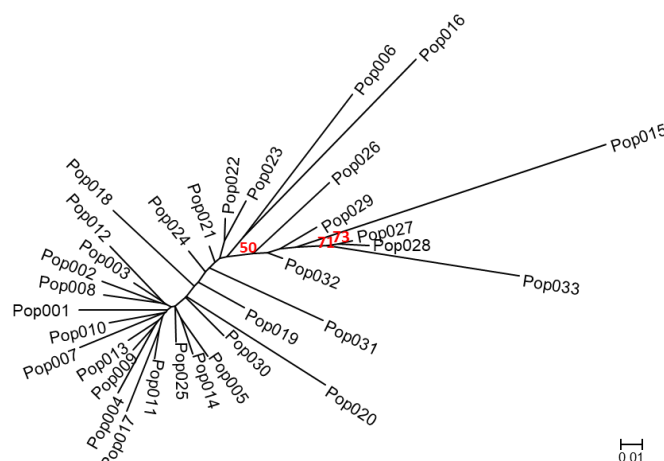


Figure 2 : Fitch-Margoliash's tree based on Cavalli-Sforza-Edwards' chord distances between 33 maize populations [including the BS22 American used as an outer group]. Bootstrap support values higher than 50% over 1,000 replicates are given above the branches.

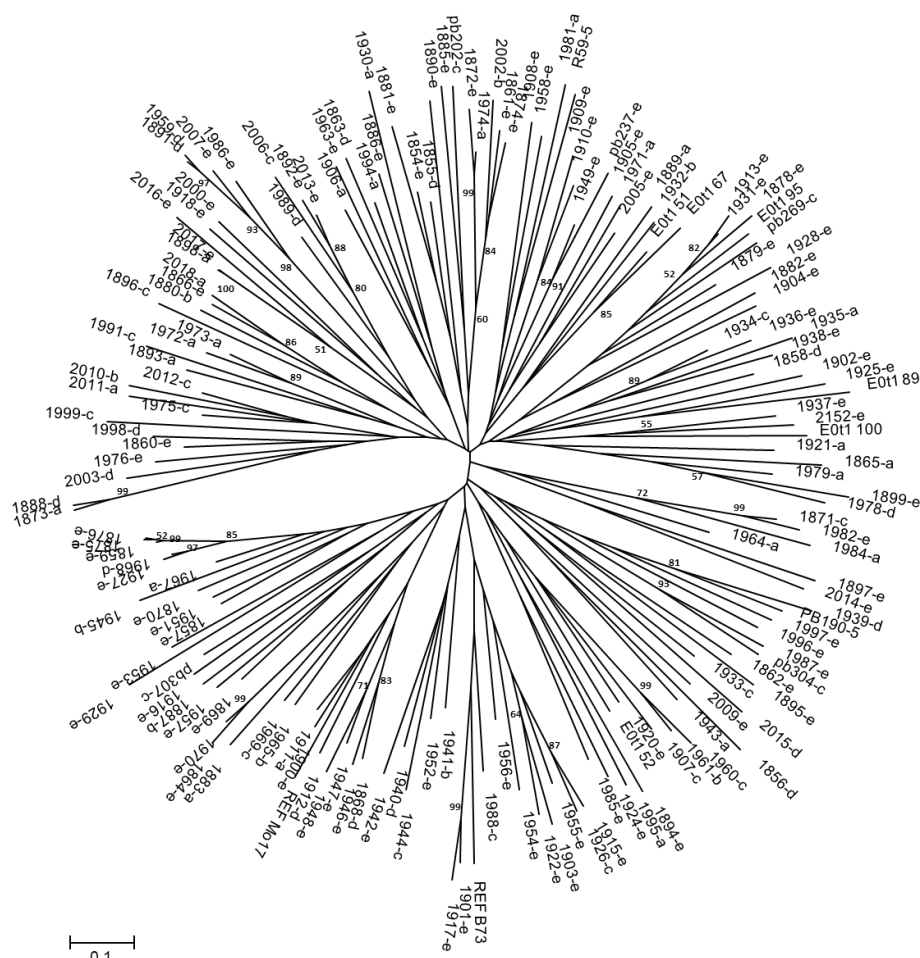


Figure 3 : Neighbour-joining unrooted phylogenetic tree based on the log-transformed proportion-of-shared-alleles distance between 163 maize inbred lines. Bootstrap support values higher than 50% over 1,000 replicates are given near the branches.

Examples of recommend crosses for some of those subsets can be found compiled in Figure 4 to Figure 6, for maize open-pollinated populations, and in Table 1 to Table 3, for maize inbred lines.

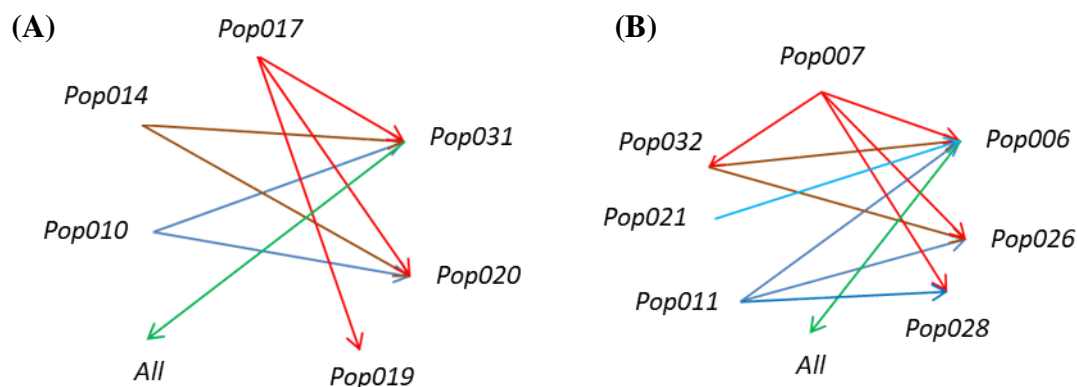


Figure 4 : Schematic representation of the preferred crosses between maize open-pollinated populations for new hybrid or synthetic populations' development with increased tocopherols and total free phenolics content: (A) maize open-pollinated populations with white kernels; (B) maize open-pollinated populations with yellow/orange kernels.

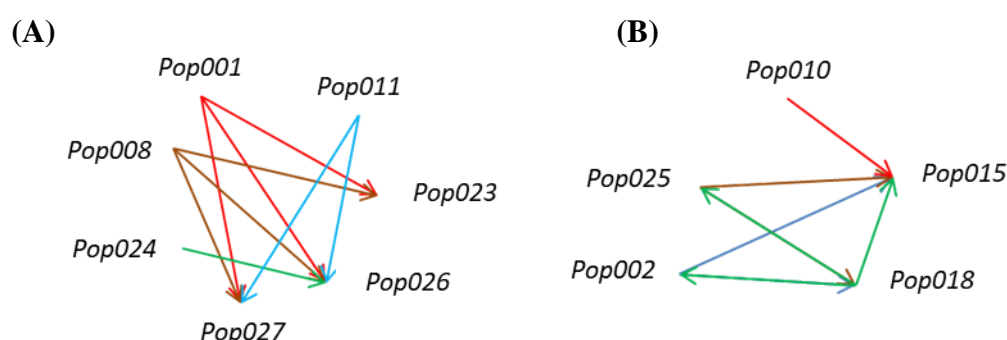


Figure 5 : Schematic representation of the preferred crosses between maize open-pollinated populations for new hybrid or synthetic populations' development with decreased volatile aldehydes content: (A) maize open-pollinated populations with white kernels; (B) maize open-pollinated populations with yellow/orange kernels.

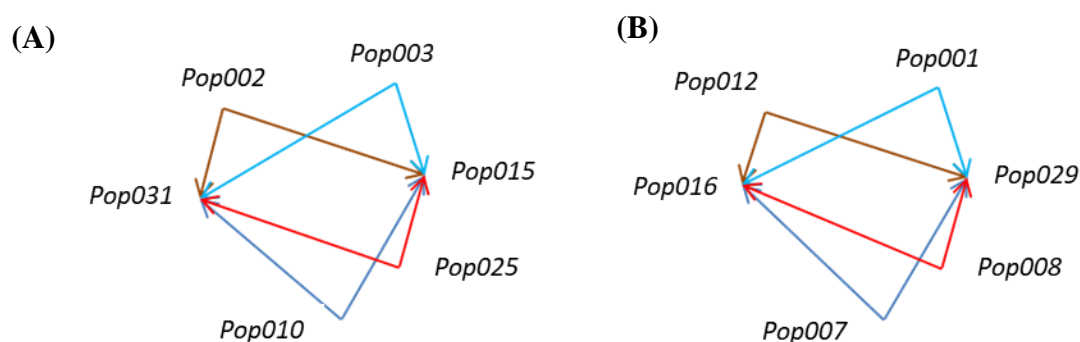


Figure 6 : Schematic representation of the preferred crosses between maize open-pollinated populations for new hybrid or synthetic populations' development with decreased overall viscosity values: (A) maize open-pollinated populations with white kernels; (B) maize open-pollinated populations with yellow/orange kernels.

Regarding white maize inbred lines, 10 lines (Table 1) showed the most promising levels of tocopherols and total free phenolics content; 6 lines (Table 2) showed the most promising levels of volatile aldehydes content; and 11 lines showed the most promising levels for the overall viscosity values (Table 3) and should be the ones selected for future crossing schemes. The recommended crosses were given taking into consideration the genetic distances between lines by avoiding crosses between the most similar genetic accessions.

Table 1: Potential maize inbred lines with a white kernel to be used as parental lines to increase the levels of tocopherols and total free phenolics.

Optimal source Inbred line	Do not use in the same cross with
1896-c	1896-c; 1880-b; 1866-e
1880-b	
1887-b	
1872-e	1974-a; 1872-e
1895-e	2005-e; 1971-a
1971-a	
1859-e	
1875-e	1859-e; 1875-e; 1876-e
1876-e	
1881-e	

Table 2 : Potential maize inbred lines with a white kernel to be used as parental lines to decrease the levels of volatile aldehydes.

Optimal source Inbred line	Do not use in the same cross with
1896-c	1896-c; 1973-a
2003-d	2003-d; 1873-a
1873-a	
1861-e	
1981-a	1861-e; 1872-e
1872-e	

Table 3 : Potential maize inbred lines with a white kernel to be used as parental lines to decrease overall viscosity values.

Optimal source Inbred line	Do not use in the same cross with
1864-e	1887-b; 1864-e; 1859-e; 1875-e; 1876-e; 1883-a
1974-a	1874-e; 1974-a; pb202-c; 1881-e
1971-a	1981-a; 1971-a
1972-a	1972-a; 1976-e; 1873-a; 1880-b; 1896-c
1976-e	
1873-a	
1880-b	
1896-c	
1982-e	1982-e; 2014-e; 1897-e
2014-e	
1897-e	

The ultimate assessment of the quality and genetic diversity of the new hybrids or synthetic maize populations developed under the DIVERSIFOOD project is still ongoing. These crosses were developed by the IPC partner and tested under field conditions during last growing season. ITQB partner will measure the molecular diversity and overall quality of the seed of these newly created populations during the next months. With this combined approach considering the molecular characterization, we expect to avoid a drastic reduction of diversity by crossing only genetically distant plants and by considering quality traits that are important for the nutritional and technological ability ("breadability") of these plant breeding populations. This will contribute to a more efficient and effective development of higher quality genetically diverse new maize germplasm with a positive contribution for maize quality breeding.

3.3. Tomato participatory breeding (RSP-ITAB)

A national network of tomato participatory breeding is being built in France with multi-local experiments to explore specific questions. The multi-actor group of research develops an iterative process and we present here the evolution of the questioning of the group concerning diversity for breeding for tomatoes. This group has tried to better understand how quality can be managed according to the type of selection and how it can be associated to diversity. First of all, they aimed to elucidate the main factors driving quality by comparing F1 hybrids (widely used even in organic production) and traditional landraces.

During 2 years (2015 and 2016), the question investigated by the group was to assess the differences between 2 breeding strategies (F1 hybrid varieties and populations) for tomatoes within the Coeur de boeuf type. Trials were set up on several farms. In 2015, 6 population-varieties and 2 HF1 were observed on 4 farms (in Drôme, Aude and Lot-et-Garonne) and in 2016, 8 population-varieties and 2 HF1 were evaluated on 5 farms (in Drôme, Aude, Lot-et-Garonne and Finistère).

The objective was to observe the genetic effects ("variety" effect) and environmental effects ("farm" effect) on the behaviour of hybrid varieties and populations and to detect any difference between these two types of varieties. One of the initial hypotheses was that hybrid F1 varieties of heart-shaped tomatoes would be less appreciated by consumers in terms of organoleptic qualities compared to the population varieties. One of the aims of these trials was therefore to investigate the nutritional and sensory characteristics to detect potential differences among hybrid heart-type varieties and original population-varieties.

Measures of agronomic variables (number and weight of marketable and non-marketable fruits, precocity and vigor) and organoleptic (hedonic tasting tests, nappings) were carried out. In 2016, nutritional analyses were also subcontracted to a laboratory on samples of 6 tomato varieties from each farm.

At the production level, F1 hybrid varieties tend to show higher yields than population varieties only in 2 of the 5 farms (see **Erreur ! Source du renvoi introuvable.**). After analysis, it appeared that the difference in production between F1 hybrids and populations was larger in fields with a high production potential, while it was limited or non-existent in less productive farms. With regard to their organoleptic qualities, F1

hybrid varieties showed an overall tendency to be less popular than population varieties in sensory tasting tests. In general, the most productive varieties were less appreciated by taste.

Finally, nutritional analyses showed that the concentrations of nutrients analysed seemed strongly linked to the environment (terroir, climate, practices of the different farmers). Indeed, **Erreur ! Source du renvoi introuvable.** shows that the results tend to cluster according to the trial location rather than the variety in the principal component analysis performed on these data.

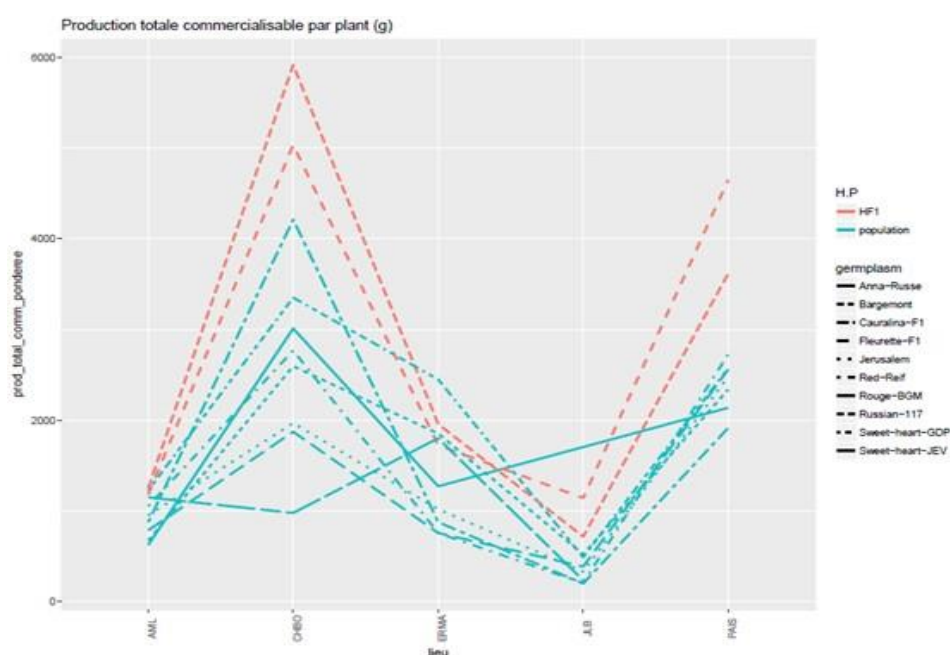


Figure 7 : Total marketable production (grams per plant) of the different observed varieties in 2016 and for each trial. F1 hybrids are coloured in red and population-varieties are in blue. Names of the different farmers along the abscissa axis are: AML : Anne-Marie Laverny (Lot-et-Garonne); CHBO : Christian Boué (Lot-et-Garonne); ERMA : Éric Marchand (Drôme); JLB : Jean-Luc Brault (Aude); PAIS : organic trials platform (Finistère)).

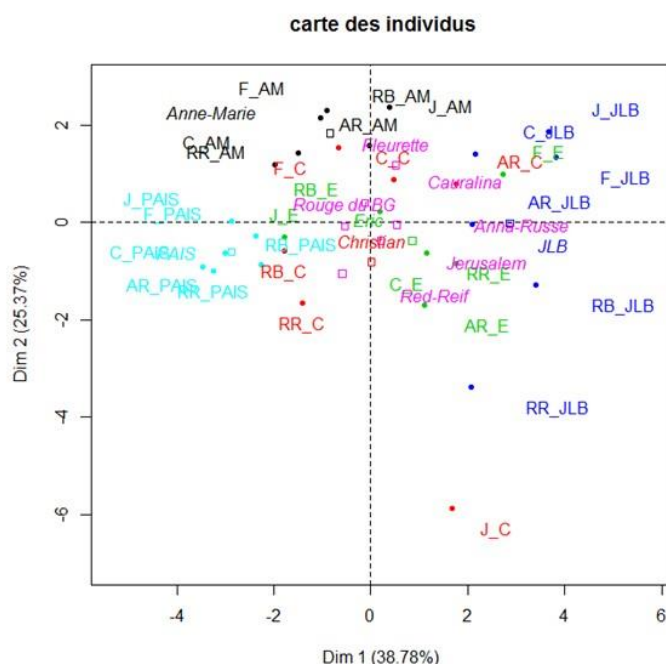


Figure 8: Biplot of the principal component analysis of nutritional measures in 2016. Results are coloured according to the trial (black: Anne-Marie Laverny (Lot-et-Garonne); light blue: PAIS (Finistère); dark blue: Jean-Luc Brault (Aude); green: Éric Marchand (Drôme) and red: Christian Boué (Lot-et-Garonne)).

In 2017, the trials focused on the effect of agricultural practices on the behavior of tomato varieties, in addition to observe genetic and environmental effects. In fact, during previous trials, differences in yield were observed on a farm depending on the type of practice implemented (trellising versus growing on grids). In 2017, 9 tomato varieties (including a hybrid F1 variety and 8 population-varieties) were tested on 3 farms according to 2 practices per farm. Analysis of the trials are still in progress during 2018.

In 2018, it was decided to initiate plant breeding activities in this group of farmers and to focus on populations with an evolutionary capacity. According to the previous results, quality was found to be under the influence of environment and practices. Farmers should adapt populations according to their practices. Hybrids do not have this capacity so they were abandoned. The focus was on the adaptative capacity of different varieties, that have been grown in different environments for several years. As an example, different people cultivate “Rose de Berne”, so different versions (“progenies”) of this variety exist and will be exchanged among participants to study their ability to grow in other environments.

Thus, this example shows how a group of farmers involved in tomato breeding has evolved the issue of assessing the potential of various methods to create diversity relevant to their objectives (F1 hybrid vs population), towards the issue of managing adaptation to their specific environments, the key to answer to their objective of good quality.

3.4. Evolutionary – Participatory Breeding generates wheat populations adapted for organic agriculture in Italy (Rete Semi Rurali, RSR)

The same bread wheat EP, named SOLIBAM (Ceccarelli et al. 2010), evolved for seven growing seasons (2011-2017) at sites in Tuscany (To) and Sicily (Si), resulting in two EPs, SOL_FL and SOL_LR respectively. The EP evolving in Sicily was moved to Abruzzo after three years, where it was called SOL_RO. From year 5 (2015), we conducted comparative trials for three years in each of the two main regions of adaptation (To, Si), and at two further locations in Molise (Mo) and Piedmont (Pi). In addition to the three EPs, the trials included a farmer's positive selection within SOL_FL named SOL_FLS, three other EPs obtained in the UK, three variety mixtures, three local varieties and one modern variety developed for low-input and draught tolerance as control (Table 4).

Table 4: Materials used

ENTRY	Name	ABBREVIATION	Nature
1	SOLIBAM Floriddia	SOL_FL	Population
2	SOLIBAM LiRosi	SOL_LR	Population
3	SOLIBAM Rosati	SOL_RO	Population
4	SOLIBAM Selection Rosario	SOL_FLS	Population
5	Cobra A	C_A	Population
6	Cobra Y	C_Y	Population
7	Cobra Q	C_Q	Population
8	PIEMONTE TENERO MIX)	P_MIX	Mixture Old Varieties Piemonte
9	VECCHIE VARIETA' MIX	OV_MIX	Mixture Old Varieties
10	VARIETA' MODERNE MIX	MV_MIX	Mixture Modern Varieties
11	Maiorca	Maiorca	Local Variety from Sicily
12	Andriolo	Andriolo	Local Variety from Tuscany
13	Gentilrosso	Gentilrosso	Local Variety from Tuscany
14	Emese	Emese	Modern Variety for Organic

Field trials followed a row-column design in two replications with optimized randomization. A spatial analysis, adapted from Singh (2003), was run to generate Best Linear Unbiased Estimates (BLUEs). Genotype x Environment and Genotype x Trait Interactions were then derived using GGE biplot in R.

Adaptation and Stability

Current and future climatic similarities between each “Evolution” location and the rest of the region was investigated using the Climate Analogues approach, whereby the climate of a chosen location is compared to that of a *search range*. The desktop Analogues R package developed at CIAT, Colombia (Ramírez-Villegas, et al. 2011) was used to obtain climate similarity rasters, which were rendered into maps using GIS software QGIS.

The Suitable growing areas for each of the two SOLIBAM wheat EPs, based on current climatic similarity projections is shown in Figure 9.

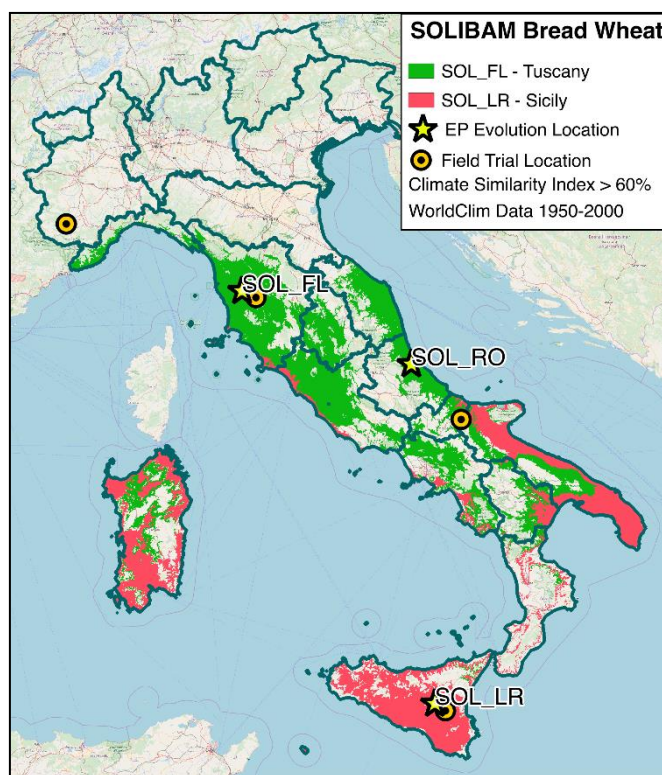
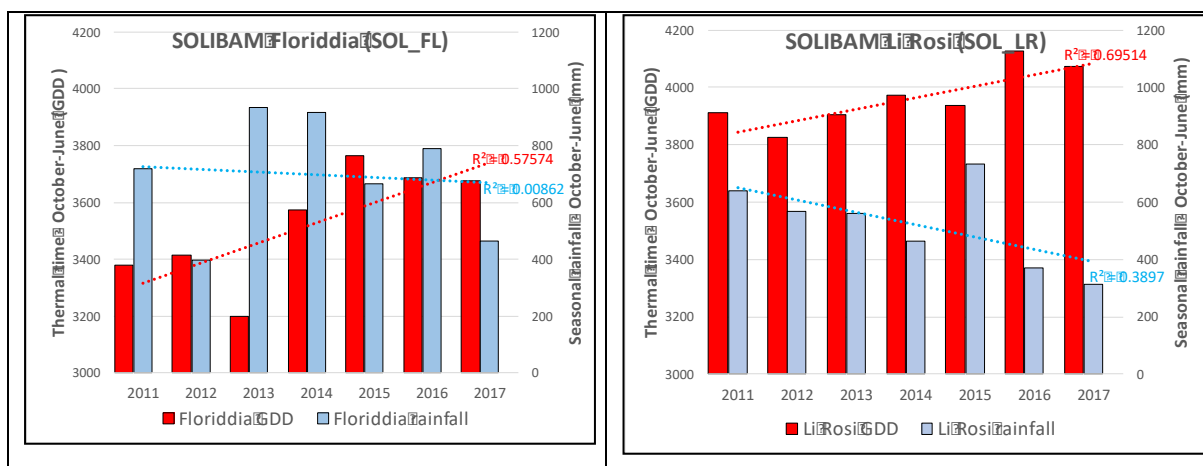


Figure 9: Field trials locations, EP “Evolution” locations with climatic similarity projections for SOL_FL and SOL_LR (Climatically homogeneous zones for the two EPs, SOL_FL and SOL_LR - WorldClim data 1950-2000; GCM Models’ ensemble for Climate Change Scenario RCP 8.5 2020-2050; Credit: CCAFS/CIAT- Colombia: www.ccafs-analogues.org).

Seasonal weather patterns show inter-annual variability for the Tuscan site (Table 5- left), and a clear trend for dryer and hotter growing seasons in Sicily (Table 5 - right) potentially leading to a much stricter natural selection process than observed in the case of Tuscany.

Table 5: Monthly temperatures and precipitation for EP “Evolution” locations in Tuscany and Sicily.



After years grown in the same locations, the three SOLIBAM EPs submitted to natural selection (SOL_FL, SOL_LR, SOL_RO) were specifically adapted to the regions where they have evolved. This was accompanied, by a high level of yield stability in the environment in which each population evolved, and by a high instability across environments (Figure 10).

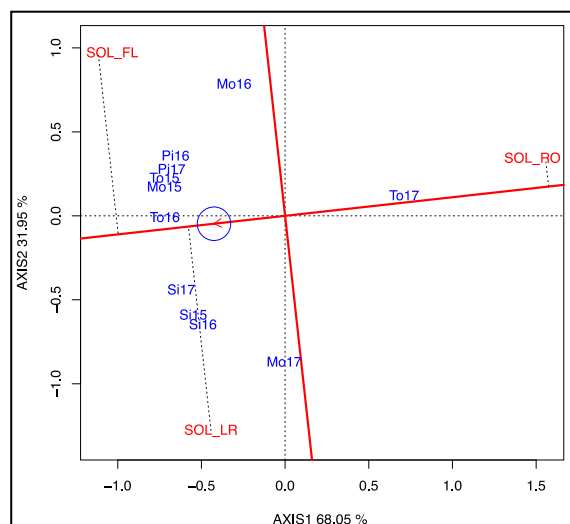


Figure 10: Specific Adaptation and Stability of Evolutionary Populations

During the three years, more than 350 farmers evaluated the populations and varieties tested, the preference went to some of the highest yielding EPs and varieties, such as SOL_FLS, SOL_FL, Andriolo and Maiorca, but also for low yielding mixtures, such as the mixture of old varieties (OV_MIX), because they were very tall (Figure 11).

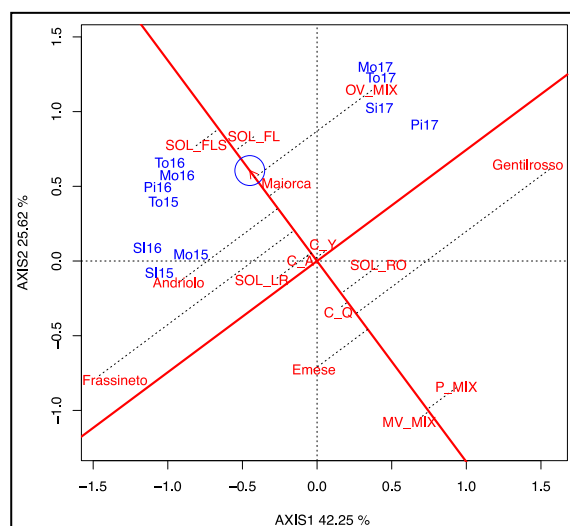


Figure 11: Mean and Stability of farmer preference

Conclusion

Crop varieties specifically bred for organic agriculture are lacking, imposing limits on the uptake and efficiency of organic production systems (Wolfe et al. 2008). EP Breeding can provide organic farmers with a low-cost technology, which in the present scenario of nearly complete absence of public breeding programs specifically addressing the needs of organic agriculture, is capable of generating populations

specifically adapted to both the physical and agronomic conditions of organic farmers. Evolutionary Populations of crops can adapt to climate change and different agro-ecological environments, increasing farmers' resilience and enhancing on-farm biodiversity.

A precondition for the successful deployment of EPs is that the seeds is produced in the same region in which it will be utilized, with farmers playing central roles as both seed users and producers. There is evidence that specific adaptation of bread wheat populations can occur within a relatively short period of time, when these are grown within climatically homogeneous areas (Dawson, et al. 2012).

We propose that the geo-climatic delineation of these environments can be used to inform the establishment of EP seed networks, enhancing favourable Genotype X Environment Interactions in the short term, whilst offering a clear perspective on climatic trends over the longer term for the region.

The EU Commission's implementing decision (2014/150/EU) providing for certain derogations for the marketing of populations of wheat, barley, oats and maize, has provided the legal framework for the marketing of SOL_FL and SOL_LR bread wheat EP seed through decentralized seed systems in Italy in 2017. This is the first time that non-distinct, non-uniform and non-stable cereal materials have been sold as certified seed since the institution of the EU Common Catalogue.

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3.5. Comparison of phenotypic diversity of 2 populations (CCP and Dynamic populations) (ITAB-INRA)

ITAB-INRA with a group of farmers in the West of France have initiated the evaluation and comparison of the phenotypic diversity of two bread wheat populations, created with two different breeding strategies (and with the same six parents): a dynamic population and a Composite Cross Population (descent of crosses 2x2) (Figure 12 & Figure 13). From 2015, the two populations have been multiplied in two different sites (FM and GS).

In 2017, phenotypic diversity was evaluated for both populations in the two sites, on 60 plants and spikes per population. On site FM, the farmer realized a selection, which was also observed.

List of the criteria observed to follow the structure of the diversity within each population:

- Plant: base of ligule, base of spike, total height, spike length;
- Spike: shape, color, “black”, awns, glumes hairiness, sterile spikelets, fertile spikelets, number of grains, TKW

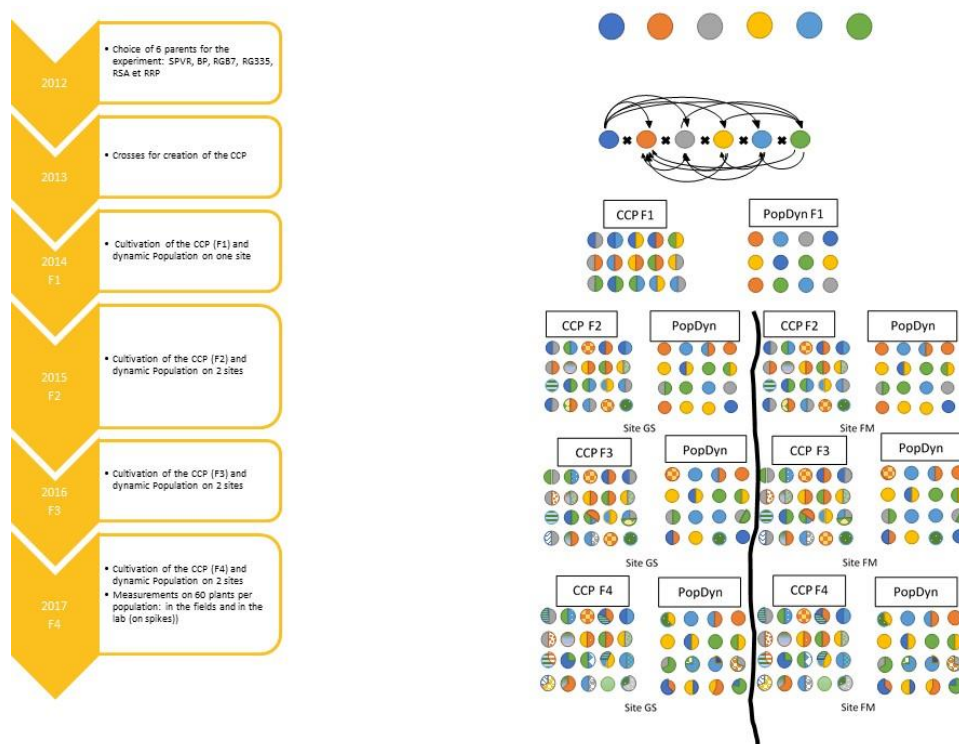


Figure 12: Evaluation and comparison of the phenotypic diversity of two bread wheat populations, created with two different breeding strategies (and with the same six parents): a dynamic population and a Composite Cross Population (descent of crosses 2x2)

From the results, each type of population had its “own design”, which seemed slightly affected by the environment of cultivation (Figure 12). In terms of diversity, at GS, the CCP was slightly more diverse than the Dynamic population, contrarily to FM site. CCP’s diversity level seemed more sensitive to the environment (0.65 at GS and 0.60 at FM), while Dynamic population’s diversity level seemed stable across environment, even if slightly different (specific to the environment). There was an interaction between the type of population and the environment for the structure of diversity.

Conclusion: Both populations (CPP and dynamic population) have revealed different pathways of evolution through generations within two geographical areas. Now, farmers will apply their own selection within both populations. Then, we will follow the evolution of selected and unselected population in both sites and to observe which kind of initial diversity will fit better to farmers’ selection.

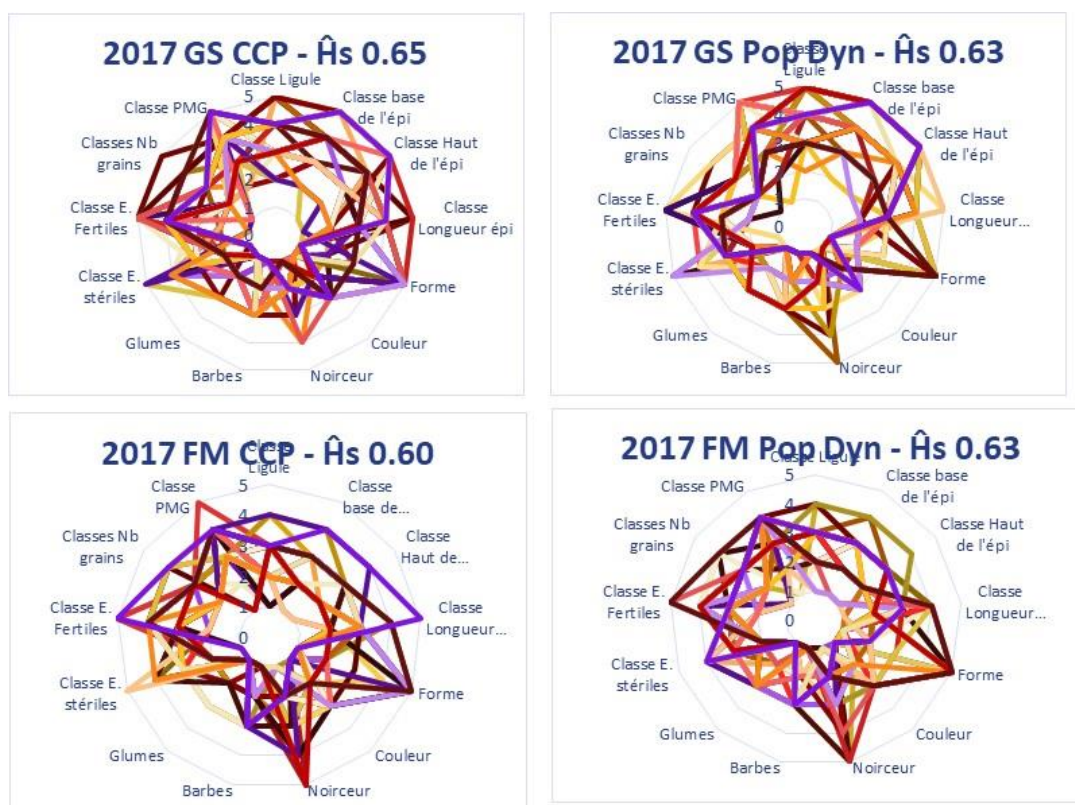


Figure 13 : Profiles of the different individuals observed and average phenotypic diversity across all traits observed (according Nei's index - \hat{H}_s).

3.6. Evaluation of new faba bean gene-pool (CSIC)

Faba bean genepools previously derived from two breeding approaches, selfing vs. open-pollination, and with different responses to the pollination environment have been used. The trials were conducted in the farmer's field for a second growing season, to explore on farm evolution of the performance of the gene-pool cultivated under a bi-crop system (strips intercropping vs. monoculture), faba bean-spelt.

Faba bean gene-pools' performance has been evaluated for the following three trait super-categories: plant architecture, floral traits related to pollinators, and seed production patterns. Climatic indicators were also recorded. Data was grouped according to different criteria and combinations: (a) cropping system and (b) breeding scheme origin and c) the response to pollinators (gene-pools). ANOVAs and multivariate approaches were used to explore differences in agronomic and morphological traits among cropping and breeding systems and gene-pools. Data from each year were analysed separately because year-to-year heterogeneity.

The first season results on the evaluation and comparison of the interest of the different cropping and breeding systems showed that intercropping and open pollination could lead to higher performances in terms of seed yield than the monoculture and self-pollination approach. Intercropping management and open-pollination breeding scheme interactively increase number and seed weight suggesting that intercropping intensifies the positive effect of outcrossing breeding scheme (breeding in presence of pollinators) in yield. In relation to the relevant traits, both intercropping management and open-pollination breeding approaches result in genepools with greater number of

seeds per plant distributed on more secondary reproductive stems and larger leaflet size and rounded shape.

First season results have been published in the Proceedings of the 19th Organic World Congress. Scientific Conference “Innovative Research for Organic Agriculture 3.0” <http://orgprints.org/32350/>.

Conclusion: Although results of the second growing are still under analysis, they showed that previous effects are not confirmed. Second season preliminary results show yield advantages for monoculture and self-pollination. However, this effect is not general. Each gene-pool reacted differently as the breeding approach and cropping system changed. The work on faba bean has been only performed during two growing seasons, with contradictory results. More trials have to be undertaken to better understand the long-term impact of cropping and breeding systems. The third growing season is ongoing.

3.7. Effect of the type of variety used in binary crosses of bread wheat (INRA-RSP)

In 2006, INRA GQE-Le Moulon and RSP started a participatory breeding project on bread wheat. RSP coordinated the programme with its members, organized in local community seed banks. As a source for on farm selection, a large number of crosses have been made using different types of parents: landraces (LR), old varieties (developed before the 60s, OV), modern organic varieties (MV). An experiment was set up within a previous European project, SOLIBAM (EU FP7), with the objectives of investigating (i) the impact of the parents used in crosses on the genetic diversity and on the phenotypic response of the progenies, (ii) the effect of the farms in which the populations derived from crosses have been cultivated and (iii) the impact of farmer's mass selection.

Methods: The populations (149 populations derived from 25 crosses made in 2005-2006), as well as their parents have been evaluated within a previous project, SOLIBAM (EU FP7). These populations came from 17 different farms, 29 had undergone mass selection and 28 were of cross type LR x OV, 28 were LR x LR, 15 were MV x MV and 33 were LR x MV. Phenotypic evaluation was done in three farms of RSP and at INRA GQE-LeMoulon. Genetic diversity of a subset of populations (65 populations) was assessed using the KASPAR method with 34 markers localized in candidate genes for flowering time, plant height and other adaptive traits and 48 “neutral” markers (15 to 25 individuals per population). The analysis of these data is on-going.

In addition, each year, new crosses designed by the farmers involved in the PPB project are realised in order to create new and original populations that will be selected in the network. In 2017, INRA- LeMoulon realised 20 new crosses (Figure 14) and RSP organized a training session on crosses and breeding.

Results: We found that two phenotypic characters (curve and spike weight) were influenced by the type of parents (LR, OV and MV) crossed, while all the others were influenced by the population effect. There were no unidirectional effects of mass selection and of the farm (soil and climate environment and agricultural practices) on phenotypic traits. Moreover, we found no effect of cross type on genetic diversity structure, but there was a strong differentiation between crosses and between populations within cross.

Conclusion: (1) Although crosses including one modern variety (MV) as parent tend to be more productive, there were many crosses based only on landraces (LR) and old varieties (OV) that were as productive indicating that significant performance can be obtained without resorting modern varieties. (2) The potential of diversification of bi-parental crosses including at least one LR or OV proved to be very high, as shown by the strong differentiation detected among populations derived from a given cross, both at the phenotypic and at the genetic level, and both due to natural and farmers mass selection.



Figure 14: Landraces or old varieties used as parents according to farmers' choice for hand-made crosses in a glasshouse at INRA-Le Moulon.