DIVERSIFOOD

Embedding crop diversity and networking for local high-quality food systems

Grant agreement n°: **633571**

H2020 - Research and Innovation Action

D2.3

A guide to participatory experiments with underutilised genetic resources

Due date: M48 – 28th February 2019

Actual submission date: 28th February 2019

Project start date: March 1st, 2015 **Duration:** 48 months

Workpackage concerned: WP2

Concerned workpackage leader: Ambrogio Costanzo (ORC)

Lead Beneficiary: ORC, ITAB

Dissemination level:

- X **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

A guide to participatory experiments with underutilised genetic resources

Ambrogio Costanzo[1] and Estelle Serpolay[2]

Ambrogio.c@organicresearchcentre.com, Estelle.serpolay@itab.asso.fr

With contributions from Veronique Chable (INRA), Sara Bosi (UNIBO), Camille Vindras (ITAB), and all DIVERSIFOOD partners involved in WP2

[1] The Organic Research Centre, UK, www.organicresearchcentre.com

[2] Institut Technique de l'Agriculture Biologique, France, www.itab.asso.fr

Contents

Introduction

Throughout history, thousands of plant species have been domesticated and used in agriculture. DIVERSIFOOD has worked to build a knowledge basis, aimed to provide a central source of information, on a wide range of crop genetic resources that are currently underutilised and/or could form the basis of new cropping approaches to respond to both climate change and social changes in food requirements and uses. This work started from revising existing knowledge and agreeing a working definition of what underutilised crops are: *Plant genetic resources with limited current use and potential to improve and diversify cropping systems and supply chains in a given context.*

DIVERSIFOOD has undertaken a series of parallel experiments, all testing the same underlying hypothesis expressed in the "working definition": that reintroducing genetic resources with a status of underutilisation can trigger benefits in provisioning agroecosystem services and supporting local, high-quality value chain, in the overall framework of agroecological systems. These experiments were conceived as an exploration of genetic resources in the context of specific, local agro-environmental or market challenges and opportunities, often in link with farmer initiatives.

The evaluation of underutilised genetic resources in DIVERSIFOOD has triggered at least two levels of innovation. First, it attempted the introduction of a diversity of species in an agricultural context characterised by standardisation of cropping systems and supply chains and a shrinking species and genetic diversity. A second, more methodological level, is to distribute the evaluation in a diversity of farming environment and communities that can build added value on underutilised crops. The combination of these two levels of innovation has created a steep learning curve for all the actors involved.

Our wish is that more communities will want to engage in this learning curve and share the practice of distributing a diversity of genetic resources and embedding their evaluation in sustainable cropping systems and supply chains. This booklet incorporates the experience of the DIVERSIFOOD trials to evaluate underutilised crops and the lessons learned on how to plan and conduct such trials, including practical consideration and tips to facilitate their execution out of the controlled conditions of experimental stations while still obtaining detailed and relevant information.

Sourcing seeds for diverse crops and food systems

One of the most important points to implement the evaluation and/or breeding of underutilised crops ("outsider" species, "forgotten" species and/or neglected germplasm of common species) for organic farming, is to access seeds and varieties. Which genetic resources to start with? Where to find them? Here, we propose a practical approach to bring diversity back to life in a multitude of farming contexts.

A 'sleeping' treasure to mobilise

To test and develop efficiently adapted, diversified varieties for underutilised crops, a large panel of initial genetic diversity is required. This panel may not be easily accessible when addressing underutilised species: modern varieties are non-existent, or they exist but have been created for very specific contexts, traditional landraces or populations are not cultivated anymore or they are in very small number and quantities. The wide genetic diversity is stored in gene banks. Landraces and old varieties have been preserved ex-situ thanks to the diversity conservation policies started in the 1970s. The number of accessions stored in gene banks is huge. Apart from Arabidopsis, there are more than 1.3 million of accessions from different species in the Eurisco Network (mostly Europe). This is certainly the starting point for deploying more genetic diversity in agriculture.

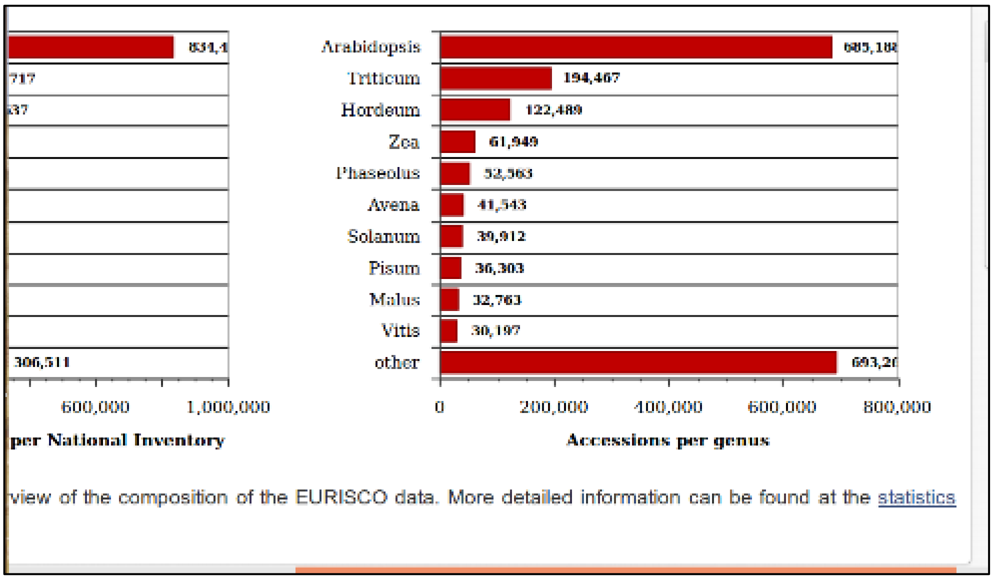

Fig 1. Number of accessions per botanical genus, available on the Eurisco Genebak platform (https://eurisco.ipk-gatersleben.de/apex/f?p=103:1:0:)

State of the art of the accessions

There are important considerations when starting to work with gene bank accessions. Being aware of these points is essential to drive the strategies to bring underutilised crops back into breeding and cultivation.

- **Very limited knowledge** is available for the accessions in the gene banks (often, only passport data: date and place of collection, type of variety - landrace, breeding material...): nearly everything must be "discovered" when bringing the accessions back in to cultivation.
- **The accessions are genetically very homogeneous** because of the way they are preserved in gene banks: very small quantities of seeds are reproduced at each cycle and subjected to "conservative selection" that eliminates plants that would not be considered exactly in the target phenotype. This can drastically reduce the genetic and phenotypic heterogeneity of what would have been diverse landraces.
- **Very few seeds are available** per accessions, and in contrast the number of accessions is huge.

How to 'awaken' crop genetic diversity in three steps

Before starting breeding underutilised crops from gene bank accessions, we propose a three-steps process to consolidate the efficient deployment (or mobilisation) of underutilised crops and varieties.

1. **A monograph of the crop** should be produced first. This monograph will gather information about the history of the crop and its selection. This part is important for non-local crops in order to start clarifying the **agronomy requirements**. Knowing the story of the crops' selection is also very important to **understand specific issues and needs for breeding**, e.g. to select varieties or accessions developed compatibly with organic farming principles.
2. **A large panel of accessions** from diverse origins should then be gathered. Different gene banks and databases are available. The monograph can help **sort and choose** a panel of accessions according to the few criteria available (country of origin, sometimes region and place, type of variety - landrace, old variety, breeding material... - etc.). Here are some databases to find accessions:
   - http://www.ecpgr.cgiar.org/ for Europe (sharing the information of the different national databases).
   - https://www.ars-grin.gov/ for the United States of America.
   - https://www.genesys-pgr.org/ that give access to different databases worldwide.
3. **First cycles of multiplication and observation** of the samples should be organised before starting performance evaluation and/or breeding programs. The number of seeds shared by the gene banks is very small, and they need to be carefully multiplied before starting a real evaluation of the resources. The years of multiplication (one to four, depending on the initial number of seeds and on the success of the multiplication) can be used for first observations and comparisons (especially phenotypic observations, see "Phenotypic description" below), that will guide the next steps of the breeding programs. This multiplication work can be done on-farm in some cases, whereas in other cases it is advisable that a research team, or gardeners, or a botanical garden, or any other partner with time and facilities available, takes charge of the multiplication. This initial partnership is the occasion to create links between different types of partners and draw together the program from the beginning.

What next? The DIVERSIFOOD experience on Rivet Wheat in France

After the implementation of these three steps, the testing and breeding of the accessions can start and new diversified populations adapted to organic agriculture and high-quality products can be created following different ways according to the objective, organisation, target market. In DIVERSIFOOD, ITAB and INRA developed a method to create diversified populations based on common traits of interest and experimented it for Rivet Wheat.

After two years of multiplication of a collection of about 200 accessions, the list of phenotypic traits observed was proposed to different farmers involved in different networks, asking them what the features of a diversified and personalised population for them would be. From their answers, the research team created different populations by mixing all the accessions corresponding to the criteria of interest asked by the farmers (fig. 2).

Examples of farmers' preferences are a specific range of height, high soil cover, floury or vitreous grain, early ripening. Farmers often combined two, three or four different traits. Here is the main innovation compared to the conventional "ideotype" concept: the fact that some traits are needed to be homogeneous does not preclude that all other traits can be heterogeneous in the resulting population, which therefore becomes, at once, a crop and a basis for evolutionary breeding and further farmers' selection. After having distributed the populations to all the interested farmers, 17 accessions were not included in any population, so a specific population of "orphan accessions" was created.

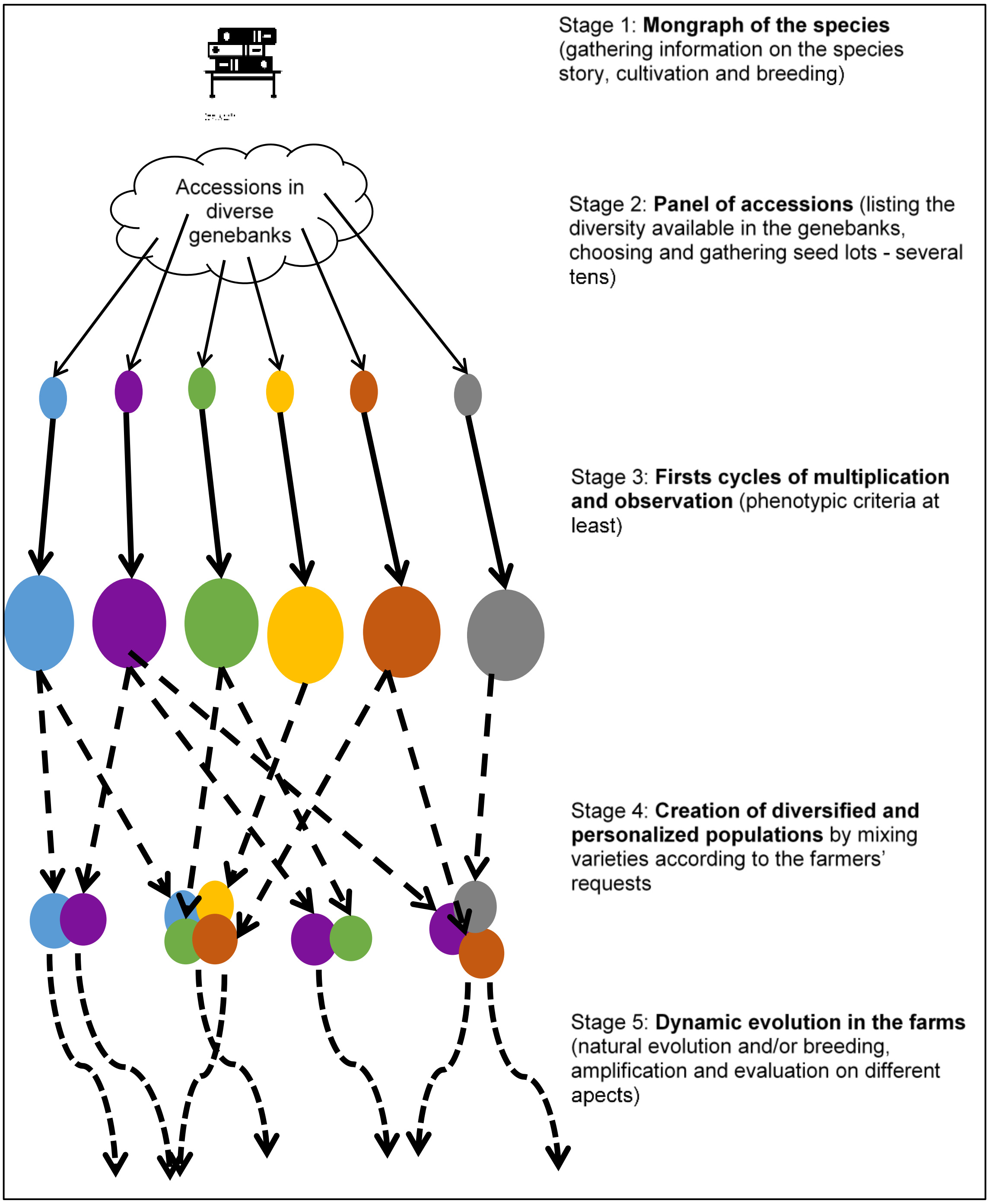

Fig. 2. Scheme of the re-diversification process - "from genebanks sleeping diversity to dynamic on farm diversity"

Fig. 3. Example of stage 3: multiplication of hundreds of accessions (DIVERSIFOOD, March 2018, Britany, France) (Credits: Estelle Serpolay)

Fig. 4. Example of Stage 4: creation and distribution of diversified populations to farmers (DIVERSIFOOD, October 2018, Poitou, France) (Credits: Emma Flipon)

Performance evaluation

In DIVERSIFOOD, a series of parallel experiments with underutilised genetic resources has been carried out on the basis of a common underlying hypothesis expressed in the "working definition": that *reintroducing genetic resources with a status of underutilisation can trigger benefits in provisioning agroecosystem services and supporting local, high-quality value chain, in the overall vision of agroecological systems and circular economy*. As far as testing underutilised crops is concerned, the focus is therefore on **crop performance**, *i.e.* **the capacity and effectiveness to provide multiple *services***. Experiences on plant genetic resources either have a very specific focus (e.g. resistance to a specific disease) or assume that yield on its own is a proxy of overall performance.

It is worth attempting to conceptualise how to assess performance addressing multiple services, as multiple services at the same time are expected from a sustainable crop in a sustainable food system. We encourage having a threefold focus and create information on (i) agroecosystem performance; (ii) productive performance, and (iii) quality performance. To improve the relevance and usefulness of the performance information, in order to enable predictions, it is essential to link the actual performance with its potential predictors: (i) the crop phenotype, in terms of morphology and phenology, and (ii) the crop growing environment.

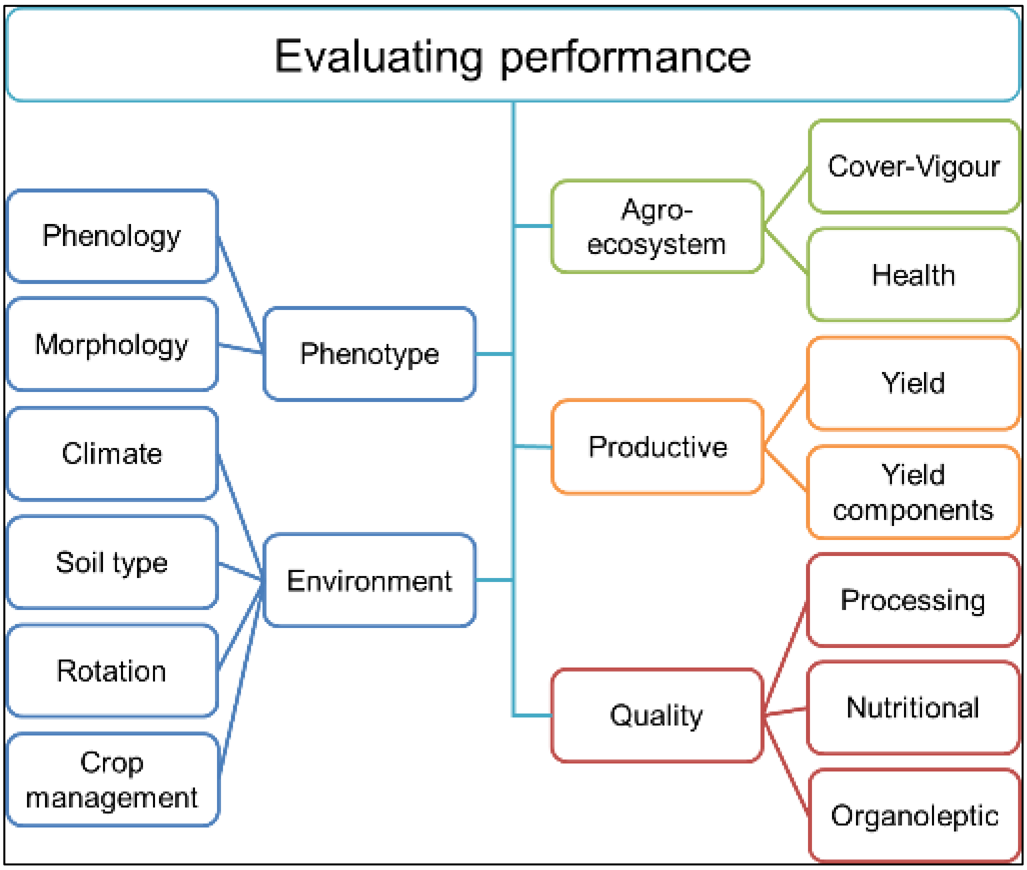

Fig. 6. The different aspects of crop performance evaluation (right-hand side) and examples of the key predictors of performance that is essential to record (left-hand side)

Examples of phenotypic description

Morphology

In a participatory framework, the key aspect is to decide which are the most important morphological traits, considering that these could be relevant to two distinct dimensions. First, morphology is relevant to the identification of the accession. Shapes, colours. Second, morphology is relevant as performance predictor. Height, growth habits, canopy architecture can be associated to the adaptation of a plant to a specific environment or management. The initial monograph about the target species can inform on which are the most important morphological traits to target the description.

Why it is important, and what we have found

As far as crop descriptors are concerned, two main remarks emerged. First, certain traits reappear that, during modern breeding, were lost. The wide diversity also included undesirable traits, that have been bred against and might also have played a role in the abandonment of certain phenotypes. Second, single genetic resources show considerable within-crop phenotypic diversity, which can either be part of their genetic structure, being them landraces or OPVs or composite cross populations, or result from intentional or even accidental mixtures, as observed in certain entries of rivet wheat which included considerable amounts of bread wheat.

Example from DIVERSIFOOD: rediscovering forgotten brassica types

In Brittany, an area particularly favourable to all forms of Brassica crops, farmers of a seed association (Kaol kozh) wished to observe a collection from INRA genebank including ancient varieties from small companies which does not exist anymore. These companies had worked for vegetable growers during the mid-20th Century for areas of vegetable production mainly all around the city of Rennes ("Les ceintures vertes").

Phenotypic descriptors were simplified for the first step of observation: earliness, length of the cycle, type of product, evaluation of the quality. When the product resulted to be of interest, farmers organised themselves to share the multiplication of the accessions from remaining seeds. If not selected for multiplication, seeds were tested for another season of experimentation with different crop period, aiming the same very basic observations before multiplication (Fig. 6).

Di Jesi cauliflower offers a very delicate, sweet flavour and beautiful curd surrounded by light green leaves: a very attractive plant. Since their discovery in the HRI genebank (UK), several accessions have been observed for a group of specific traits - besides the usual criteria used for cauliflowers at the same period of production, for example:

- the curd showing a special grain and a pale-yellow colour, with two kinds of shape (a pyramidal fractal organisation like Romanesco type or a smooth fractal organisation as on the picture)
- the vegetative part of the plant showing its "spoon-shaped" leaves: the farmers aim at conserving this light green colour with a specific form of leaves covering carefully the curd until maturity (Fig. 6).

Fig. 6. (1) Grosse côte de beurre" is a cabbage which looks like a chard, in which petiole and rib are consumed; (2) a cabbage which is a pointed Milan type, (3) Di Jesi cauliflower (credits: Véronique Chable).

Phenology

Crop growth cycle, and the timings of development stages, referred to as "phenology", are perhaps the most important drivers of environmental adaptation. This is what makes crops requirements synchronised with resources availability, driven in turn by climatic patterns, and this is what can keep vulnerable stages of crop development away from pests and pathogens aggressive stages in their own life cycles, for example. Describing crop phenology is essential also as a reference for any other assessment: for example, it is pointless to assess the severity of foliar diseases without knowing at which growth stage the crop is.

The main way to describe phenology is focusing on key development stages (e.g. flowering or ripening), and record when they happen. However, recording e.g. the flowering date of ten wheat accessions growing in a trial would require visiting the trial very often to capture when flowering really happens for every variety. Also, having the date of flowering, as such, can be a far too detailed information considering that it can change drastically from year to year.

Numeric phenological scales are an essential tool to describe crop cycles. These scales associate the succession of growth stages with a succession of increasing numbers. There are several growth scales available, some very species-specific. We encourage using the **BBCH decimal growth scale**, originally created for cereals, which describes crop development from seed to post-harvest with numbers from 1 to 99, and is applicable to every

possible plant, including wild plants and trees[1]. Using growth scales, especially the BBCH one, has several advantages for comparative trials:

- you may be able to visit the trial just once around flowering, and therefore be unable to capture dates of all accessions. Associating each accession with the number corresponding to its growth stage that day, **you can still obtain the exact ranking of "earliness of flowering"**, which is the most important information;
- you can **assess the within-crop diversity** of, e.g., a heterogeneous population, monitoring a certain number of individual plants;
- you can better predict when a specific growth stage is going to happen, therefore **plan management operations** accordingly.
- you can easily **describe the growth cycles of coexisting plants**, as e.g. the crop and the most dangerous weed species, the cereal and the legumes growing in an intercropping, the herbaceous crop and the overlying trees in an agroforestry system.

Agro-ecosystem performance

*How does the crop perform in the field during its growth cycle? **Agro-ecosystem performance** represents those services who ensure that the crop develops as independently as possible from external inputs and contributes to the sustainability of the cropping system it is part of. For example, knowing whether a crop can compete effectively against weeds or not allows to predict whether it is suitable for an organic, weedy environment.*

Agroecosystem performance can be evaluated under different angles, but we suggest to make sure two main "packages" of indicators are considered:

- the **cover and vigour**, representing how much, and how fast the crop can occupy the space and appropriate resources, therefore having a competitive advantage over weeds;
- the **health**, representing how well the crop can withstand the effects of diseases and pests.

Why it is important, and what we have found

The overall outcome of DIVERSIFOOD experiments is that agroecosystem performance of a same underutilised genetic resource can vary greatly depending on where it is grown and must therefore be looked at a very local scale. This reinforces the importance of deploying and testing genetic resources in multiple farms rather than in centralised research stations.

The "Cover/Vigour" package

Assessing and monitoring the growth and development of a crop can provide an enormous wealth of data. However, it can be such a laborious work that it is often neglected. There are simple ways and indicators that, if captured with an adequate framework, can provide the essential information to describe the environmental fitness of genetic resources. For example, in many experiments crop cover seem to be the strongest, and more consistent, variable associated with crop yield. Cover of a crop incorporates information on how well the crop was established, how much solar radiation it is able to capture, how well it can compete against weeds.

To assess cover it is important to look at the canopy from above, identify a reference frame (e.g. four rows of a cereal crop, or a 50cm quadrate, and ask yourself the question "how much of the space is actually occupied by the crop?". A grid reference guide can help setting up the eye against fixed values and correcting against the different possible appearance (e.g. patchiness) of the canopy. An example is provided in Fig. 3. Percentage

[1] BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4

data are difficult to estimate correctly but are very useful to elaborate with sums, averages and statistical analysis. However, having a reference scale can enable you to attribute scores (1 to 9) if it is considered easier for data capture.

Tips: *take orthogonal pictures, making sure there is no direct sunlight, and assess the cover directly on the pictures once back on a computer.*

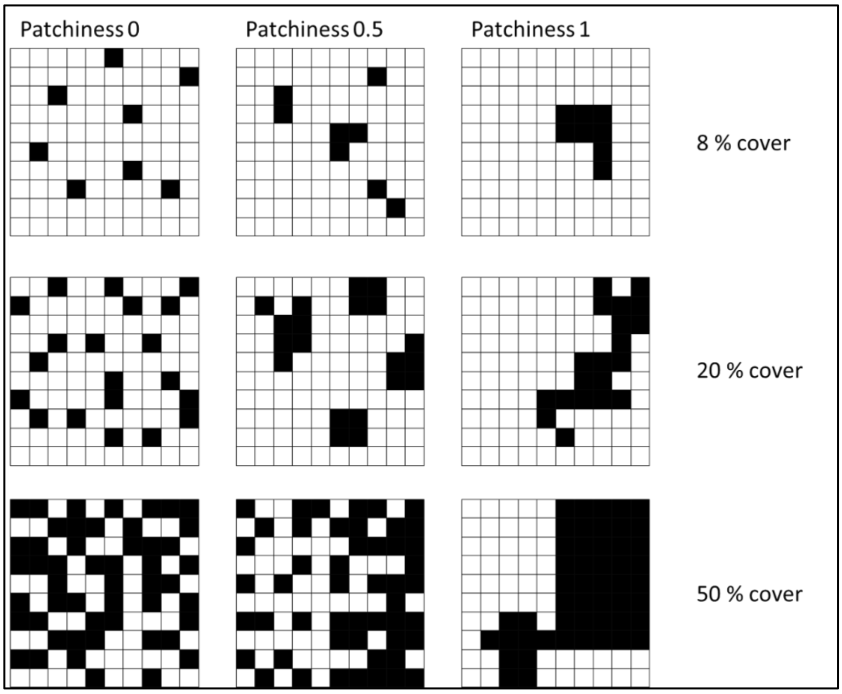

Fig. 7. Example of a visual grid to help estimating ground cover. (Credits: Ambrogio Costanzo, ORC)

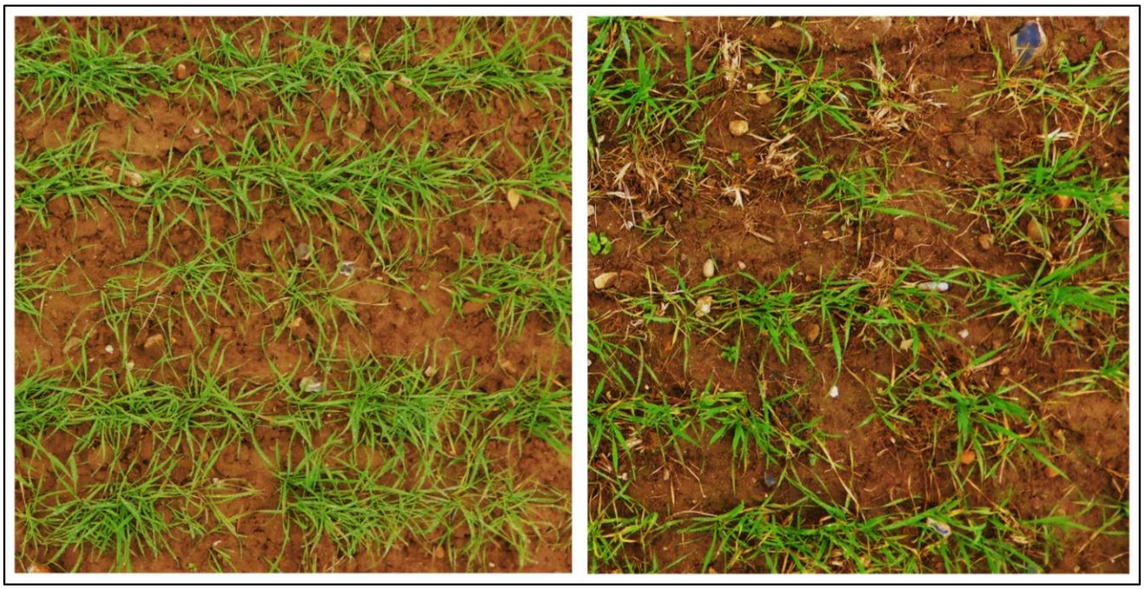

Fig. 8. Orthogonal pictures of two winter wheat plots during late tillering. Plots are sown with the same seed rate and inter-row distance. Can you estimate wheat cover? (Credits: ORC)

Fig. 9. "Add the weeds": orthogonal picture of a spring wheat plot at the onset of stem extension. At least two weed species are present: fat hen *(Chenopodium album)* and meadowgrass *(Poa sp).* Can you estimate or score the cover of the crop and the two main weeds? (Credits: ORC)

Software and apps that can automate calculation of the cover through colour analysis are available, but we are convinced that no software is as reliable as the human eye. For example, in fig. 8 above only crop and bare soil are visible, but in fig. 9, quite typical of an organic crop, there is crop (wheat), bare soil, and at least two different species of weeds. In this case, the best procedure is assessing the cover of the crop and the cover of the weeds, ideally by weed species or relevant groupings, recording data on a table as Table 1.

Remember: different plant species canopies overlap: the total sum of cover can be higher than 100%.

Tab. 1. Example of cover assessment data collection.

Position →	1	2	3	mean
Crop cover	25%	50%	40%	*38.3%*
Broad-leaved weeds	30%	15%	20%	*21.7%*
Grass weeds	10%	10%	15%	*11.7%*
Bare soil	50%	40%	30%	*40.0%*
Total weed cover	*40%*	*25%*	*35%*	*33.3%*

Cover is a bidimensional representation of the above-ground crop canopy. Many farmers are perhaps more familiar with vigour, but it might be hard to measure it in an as univocal way as cover, unless biomass samplings are performed, which may be overambitious to perform with a high number of accessions or far from an experimental station. However, vigour is nothing more than a three-dimensional assessment of how much space is occupied by the crop, and its visual assessment can be organised in a smart and useful way. As for cover, the key starting point is establishing a reference scale, as the one prepared by ITAB for winter wheat (Fig 6). The important aspect is to "imagine" with the farmers involved what the "maximum vigour" should look like for the target crop in the local condition and system, attribute to this the maximum score and then score the accessions in the field accordingly.

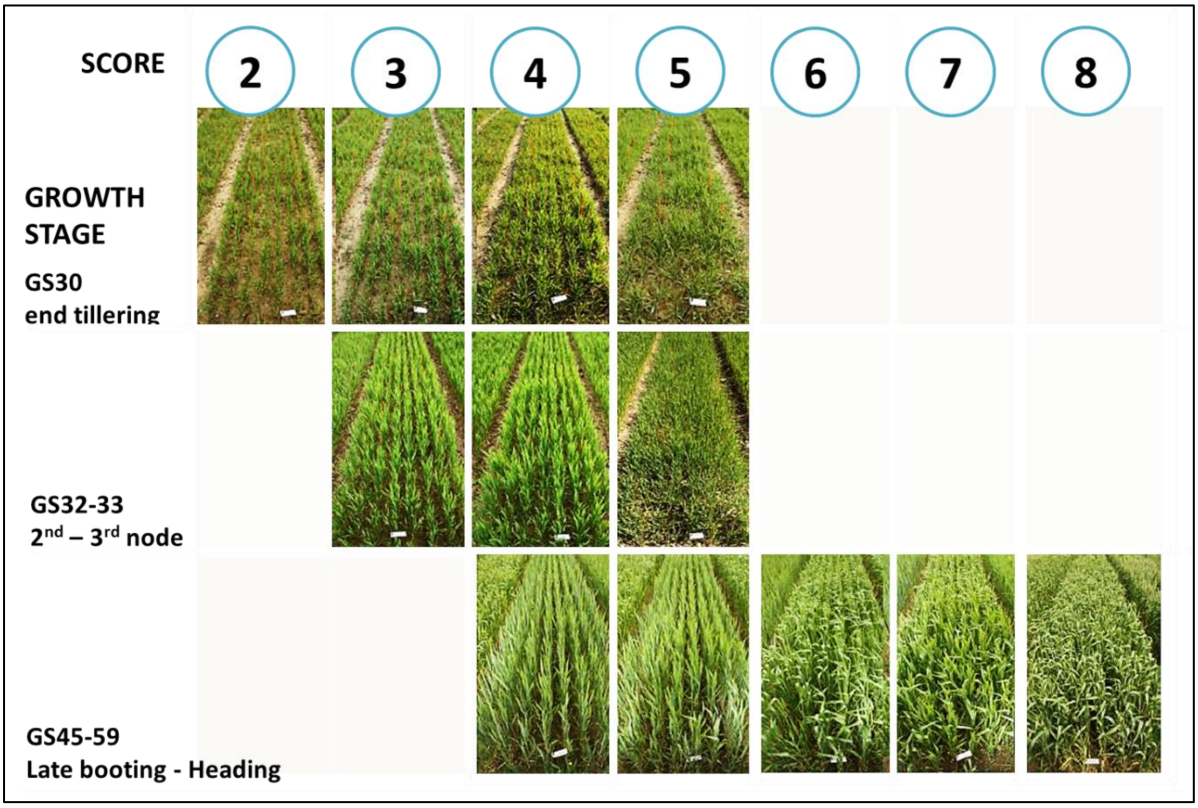

Fig. 10. Massot et al. 2014[2], modif. (Credits: ITAB)

Fig. 11. Two landraces of rivet wheat photographed in the same field in the same day. Could you score their vigour based on the cereal visual guide? (Credits: ORC)

[2] Massot et al. 2014. *Guide de notation de la couverture du sol par le blé tendre.* Institut Technique de l'Agriculture Biologique (ITAB) http://www.itab.asso.fr/itab/varietes-gc-pot.php

Fig. 12. Two landraces of buckwheat photographed in the same field in the same day, same distance and angle of the camera related to the plot. Could you try to score their vigour based on the cereal visual guide? (Credits: ORC)

The "Health" package

Susceptibility to diseases is another key aspect of crops environmental adaptation that needs to be assessed and quantified. However, some important aspects need to be considered to ensure reliable information.

Diseases have their own life cycle, interacting with the crops' life cycle. Knowing whether a pathogen is soil-borne, wind-borne or seed-borne can help understand the whole cropping system where the crop is grown and understand its weaknesses, i.e. the phases in which disease reproduction and spread are favoured instead of hampered. This is relevant to the overall management of genetic diversity and can be especially important when new material is introduced in an environment. "Outsider crops" can introduce in an environment "outsider diseases". Poor quality seeds can introduce pathogens in the soil. On the other hand, reproducing seeds in a non-long enough rotation can expose to the risk of build-up of pathogens, as e.g. for cereals grown immediately, or shortly after, other cereals.

How to assess diseases. It is important to target critical stages of the crop's growth cycle where specific diseases can appear and be harmful to the crop. As an example, for winter cereals, booting (BBCH GS41-49) to anthesis (BBCH GS61-69) are critical stages for foliar diseases, especially when invading the flag leaf, which carries most of the photosynthesis during the production stages. In the stages of late milk – early dough ripening (BBCH GS 75-83) it is important to focus on the ears to detect signs of Fusarium head blight, which may produce mycotoxins and as well be carried to the next season if the seed is resown. Ideally, diseases assessments should not be done once, but repeated over time to be sure to capture the dynamic of diseases development. This is especially important in comparative trials with accessions with different phenological cycles. Measuring diseases is one of the most challenging tasks in crop science, and this is not the place for a thorough guide. However, whether the assessment is done with specific quantitative methods or through visual scorings, it is important to remember that there are three variables involved: (i) the number of plants affected by disease lesions, (ii) the extent of these lesions on the affected plant, (iii) the "disease severity", which is the combination of the previous two.

Multiple diseases can coexist. Ascertaining resistance or susceptibility to a specific disease would ideally require a bespoke experiment where the crop is kept away from every other pathogen and inoculated with the target pathogen. When working in farm environment, however, this is far from being the case: multiple diseases coexist on the same crop, even on the same leaf, they can be difficult to visually distinguish from one another and they can also compete against one another. This makes it hard to make conclusions about resistance to a specific disease. When working with comparative trials, the spread of e.g. wind-borne diseases can be further complicated by the diversity of accessions growing near one another. There are two main corrections possible to help collect reliable information: (i) have a replicated trial, (ii) record the "health" of the crop, which can be an indicator of disease-free plot or leaf surface, and then record the diseases symptoms observed.

Fig. 13: Diseases on an emmer leaf (left-hand picture): yellow rust and leaf spot are coexisting and overlapping, and only a small fraction of the leaf is still disease-free. Bean rust (*Uromyces fabae*) infection on field bean: early (centre) and severe (right) symptoms in two plants photographed in the same field in the same day. What can we say about 'diseases resistance'? (credits: Ambrogio Costanzo, ORC)

Productive performance

*How much, and in which way does the crop produce? **Productive performance** represents the multiple dimensions of yield. There are aspects common to every crop species, like the production per surface unit and its stability, and aspects that are more species-specific. For example, a broccoli accessions can be preferable compared to another one with similar yield if it can ensure a longer harvest season.*

Why it is important, and what we have found

In DIVERSIFOOD, yield of underutilised crops gave contradictory results. In some cases, yield can be a serious limiting factor for underutilised genetic resources: in cases where the tested material can be either low-yielding or difficult to harvest. In many other cases, it can instead be a relief for marginal conditions where mainstream crops cannot be a successful option: species like einkorn, emmer or rivet wheat can thrive where their commonly grown closest relatives (e.g. durum or bread wheat) are not a viable option. This is one of the key benefits expected from underutilised crops: that they can be a valuable option for areas that would perhaps be abandoned if only relying on widely available seeds. However, it is essential to correctly assess the productive performance to know in which conditions, and with which genetic resources, this happens.

How to harvest the crop at a trial scale vs. at a commercial scale

Correctly assessing the yield of different accessions growing in small plots can be a tricky operation, yet it is often the main criterion upon which selection is made. For instance, cereal plots are generally harvested with a plot combine harvester. However, plants laying on the external rows and on the top and bottom areas of a plot are subjected to less within-crop competition, and can therefore yield more, than those in the centre of the plot. When the whole plot is harvested, this "plot border effect" leads to overestimate the yield and, since different 'varieties' can behave differently and have different neighbouring populations, even the yield ranking can be misleading. Also, the way plots are managed can be quite different from the way a field is managed. Recent research[3] shows that plot-scale estimates are not directly transferable to field-scale, especially in organic and low-input systems. Therefore, it is good practice not to only rely on "plot yields" in these cases and spare some time to assess key yield components.

What are the most informative aspects of production?

The main indicator for every crop is generally the amount of product harvested by unit of surface, which can be misleading when working with small plots. Several additional variables can be captured to have more relevant and useful information, depending on the crop species. Overall, two main aspects can be explored: yield components and period and partitioning of harvest.

Yield components in cereals

Yield components refer of those structures of the crop that directly relate to the yield. Fer example, in small-grain cereals the yield is linked to four main components that are determined in different successive growth stages and, can also tell the development story of the crop, the stresses it has endured and how it dealt with them. These are, in chronological order, (i) ears density (number of ears per m^2); (ii) number of spikelets per ear; (iii) number of grains per ear; (iv) grains weight. We strongly recommend to at least collect ears density in cereal plots, as it may be a more reliable information than grain yield. Assessing yield components is also a very powerful diagnosis of crop adaptation and, although laborious, it can be facilitated by some tips.

Yield components as diagnostic traits of the process of yield formation can be analysed for every crop, with obvious adaptation to the physiology and morphology of the target species. The advice of a crop scientists experienced with the target crop can be useful in identifying the most important yield components and their information content.

Yield partitioning in vegetable crops

Productive performance does not only mean yield. There are crops, especially vegetables, where fresh product is harvested, where there are additional aspects to consider. For crops like broccoli, the **length of the harvest season** is a crucial aspect in determining the suitability for a production system, as important as total yield if not more. Yield of fresh product is generally divided into first class, second class and unmarketable, which are important indicators of product acceptance in different supply chains. For example, many big retailers have requirement of product homogeneity and size/weight (defining the "first class"), whereas direct marketing and some retailers accept, or may even value, what would be "second class" in other markets.

These aspects cross the boundaries with quality. However, they are included as production performance indicators because of their link with crop productive processes and yield components and because, after all, it is during harvest that they must be assessed. Assessment of productive performance in these cases requires a discussion among farmers and other stakeholders in the potential supply chain to identify based on which

[3] **Kravchenko AN**, Snapp SS, Robertson GP (2017) Field-scale experiments reveal persistent yield gaps in low-input and organic cropping systems. PNAS, vol 114:921

criteria the harvest can be partitioned. This could help better understand the requirements from the market side, and the potential from the production side, in supply chains where the transfer of information is not always linear and straightforward and could also trigger novel opportunities for market of new or better products.

Tab. 3. Yield component in small grain cereals.

	When and how to measure	Keep it simple, avoid mistakes	What does it tell about crop adaptation
Ears density	From flowering time on, count the number of ears in the middle of the plot: – on a 1m long row, if the crop is sown in rows (Fig 10). – on a given surface (at least 50*50cm) if the crop is broadcast sown	If the crop is sown in rows, **record the exact distance between rows,** because this will be needed to correctly calculate the density per m^2. **Repeat the count at least twice** in each plot	Low ear density means that the crop might have suffered in establishing and capturing the essential resources needed in the early stages, and not be adapted to the local environment
Number of spikelets	Collect 10 random ears per plot and count all the spikelets. A picture can help (Fig 11).	**Do not just measure ear length:** it does not link with spikelets number as these can be very dense or far from one another. You can still count spikelets from a picture.	Large ears with high number of spikelets indicate a better capture of nutrients at the right time (around the onset of stem extension) than small ears (but it depends on ears density too)
N. of grains per ear	Thresh 10 random ears and count the number of grains	**You can estimate the number of empty spikelets** (they will appear much smaller than the others and generally at the bottom of the ear, therefore obtaining the percentage of fertile spikelets	High number of empty spikelets indicates that the crop suffered important stress during the booting to flowering stage. For example, heat or frost can hamper fecundation
Grain weight	From the harvested grain, collect at least three samples of 100 grains and weigh them. From the average, calculate the thousand grains weight	Grain weight varies a lot according to humidity: **grains should be oven-dried at 100°C** until constant weight, and weighed immediately after taking them out of the oven, as they take up moisture very quickly	Very small and/or light grains indicate suboptimal grain filling due to drought or diseases affecting crop ripening. Thousands grain weight is also important for quality and for calculation of seed rates for subsequent sowing

Fig. 14. Ears density: the most important yield component in small-grain cereals. Count ears on a 1m long line in a cereal sown in rows (left) or on a 50*50cm quadrate in a broadcast sown cereal (right) (Credits: ORC).

Fig. 15. Take pictures of randomly sampled winter wheat ears, to assess their features (e.g. dimensions, colour, health, number of spikelets) at a later time. It is useful to include in the picture a size reference (in this case an A5 notebook) to have an indication of ears length, and a post-it with the ID of where the ears came from. How many information can you extract from these pictures? How does the wheat on the left-hand (ID 202) compares to the one on the right-hand (ID 210)? (Credits: Tegan Gilmore, ORC)

Quality performance

Quality can be evaluated under the different angles of (i) processing quality, (ii) nutritional and nutraceutical quality, (iii) organoleptic quality. These dimensions of produce quality are the gateway to develop successful value chains around underutilised crops. A diversity of crops triggers a diversity of products that needs adaptation in both the processing and the methods and concepts to assess their quality.

Basic and processing-related quality

Processing quality has to be evaluated according to the process and to the final product. For some products and some varieties, it is quite simple because the usual tools of processing can be used. For other varieties and/or species, however, it is necessary to adapt the usual processing to the raw material, or to create a new processing adapted to this unusual raw material. Therefore, a specific evaluation of the process has to be created, and it is important that it is created by the different actors involved in the process (different steps and different people using the process).

As an example, in the former EU project Solibam, a French group of farmers-bakers and bakers evaluated different varieties of bread wheat for natural bread-making. An evaluation grid already exists for classical bread-making evaluation, but it is not adapted to natural bread-making (the steps and skills are sometimes different from the classical bread-making process). Therefore, the group of practitioners created a specific grid to evaluate natural bread-making, adapting the standard grid. This grid is now used by several French bakers and farmers-bakers in research projects to evaluate different varieties for natural bread-making.

Fig. 16. Bread making test of different winter wheat modern and historic varieties and CCPs from different locations at a London bakery. Grains were provided with anonymous codes (credits: E5 Bakery)

Nutritional properties and active compounds

The nutritional and health benefits of underutilised crop are, in the wide audience, one of the most widespread claims supporting an increase of genetic diversity in sustainable agriculture. Starting from the assumption that the most important transition in food systems towards a better health is ensuring diversified and balanced diets, when testing underutilised crops, it is important to be aware of which active compounds can trigger health benefits and how these compounds can be measured. For example, the typical bitter and spicy taste of broccoli is due to glucosinolates: secondary metabolites that have positive effects on health and are anticarcinogenic, and whose content in the product is highly dependent on the genotype and on the environment[4].

As far as cereals are concerned, several researches confirmed that whole grains and whole-grain-based products, in the context of a balanced diet, can have a protective effect on humans and the ability to enhance health beyond the simple provision of energy and nutrients. These important functions are due to macro-, micro-nutrients and phytochemicals present in whole wheat grain. Determination of active compounds requires laboratory analyses (tab. 3). It is essential to be aware of which material the analysis is made on (grain, whole flour, final product) and which further processing this material is undergoing: most phytochemicals are accumulated in the bran and germ fractions of the kernel and can therefor be lost during refining processes.

Tab. 3. Value and determination methods of main active compounds in cereal grains

	Nutritional and nutraceutical value	Determination methods
Dietary fibre components	The American Association of Cereal Chemists defines dietary fibre (DF) as the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. From an analytical point of view, dietary fibre may be subdivided into two groups: soluble dietary fibre (SDF) and insoluble dietary fibre (IDF).	Dietary fibre determination is performed through an enzymatic-gravimetric procedure. Briefly, whole flour is subjected to sequential enzymatic digestion by heat-stable α-amylase, protease and amyloglucosidase to remove starch and protein. Sample solution is then filtered to obtain the insoluble dietary fibre (IDF) residue and the filtrate is treated with 95% heated ethanol to precipitate the soluble dietary fibre (SDF).
Phenolic compounds	Phenolic compounds are secondary metabolites that constitute the major group of phytochemicals found in plants. The most common phenolic compounds in whole grain cereals are phenolic acids and flavonoids. In wheat kernel they are mainly located in the outer layer of the kernel. The interest in phenolic compounds is due to their high antioxidant activity acting as radical scavengers. Moreover, many studies suggested they may have a role in the prevention of degenerative pathologies such as cancer and heart disease. Organically produced wheat is expected to accumulate higher concentrations of phenolic compounds with respect to conventionally grown varieties.	In wheat kernel, phenolic and flavonoid compounds occur in soluble (free) and insoluble (bound) forms, cross-linked with cell wall macromolecule. To recover the free phenolic compound, whole flour is treated with methanol 80%. The residue from the free phenolic extraction is subjected to alkaline and acid hydrolysis to recover the bound phenolic compounds. Free and bound polyphenol and flavonoid content are determined according to a colorimetric method, using respectively gallic acid and catechin as standard.

[4] Jeffery H et al. 2003. Variation in content of bioactive components in broccoli. Journal of Food Composition and Analysis 16 (2003) 323–330

Organoleptic quality

Measuring **organoleptic quality** needs finding a balance between **subjectivity and objectivity**. The best approach is to involve a panel of experts in an interactive process. According to the objectives of the experimentation (or the stage of breeding process), you can involve experienced people and use different types of tests (see the table below). Involving experienced people brings more objectivity but is very expensive, as it requires panels of experts that must be trained and meet regularly, whereas involving consumers (even if they are used to eat the tested product) through the so-called "**hedonic tests**" will induce subjectivity, but is a very cost-effective option. A balance can be found with the "**napping method**". This method combines descriptive and hedonic approaches and can involve both non-experts and experts. It was tested in the former SOLIBAM European project for organoleptic evaluation of bread and broccoli and has then been applied within DIVERSIFOOD for bread and tomatoes.

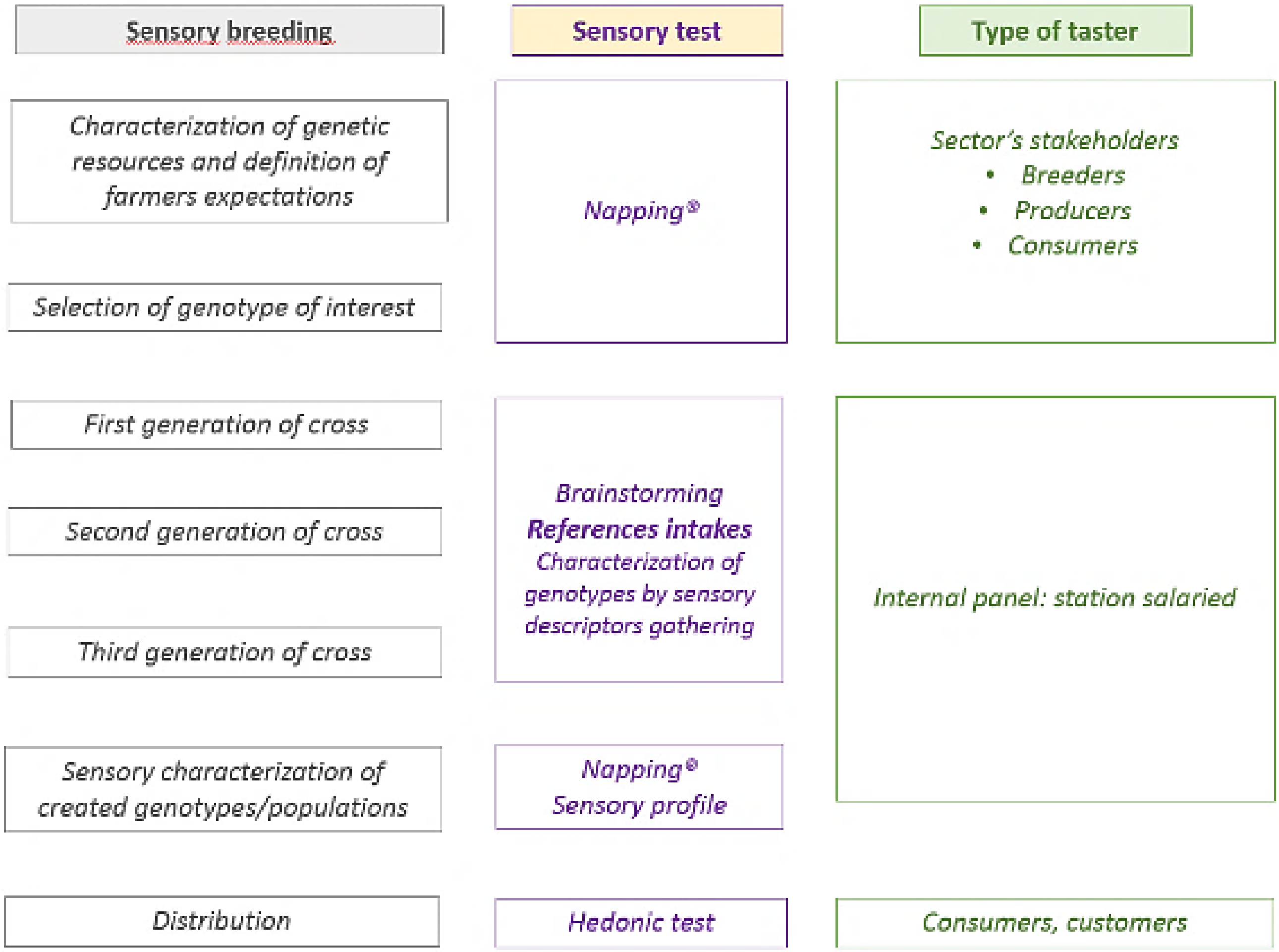

Fig. 17. Choosing the appropriate sensory test according to the stage of the breeding process and the specific targets (Credits: Camille Vindras, ITAB)

Focus on the Napping method

Napping is an original way to measure sensory perception based on categorisation and similarity. The tasters are required to position the different samples on a "tablecloth" with a defined size. Each position is transformed in coordinates by the data analyst and a specific statistical analysis is then carried out with an R package to draw conclusions from the whole panel of tasters.

To ease the integration of sensory criteria during breeding process, a simple brainstorming, which can be achieved by means of weekly meeting, will bring taste references to judges and contribute to their training. At the beginning of the breeding process, napping can help the choice of genotype of interest. At the end of the process, it can help check if products of newly developed crops are different from the parent crop. It can also help highlight a genotype or an environmental effect, as well as the interaction between them.

Fig. 18. Example of a "tablecloth" for a Napping test of bread (Credits: Estelle Serpolay, ITAB)

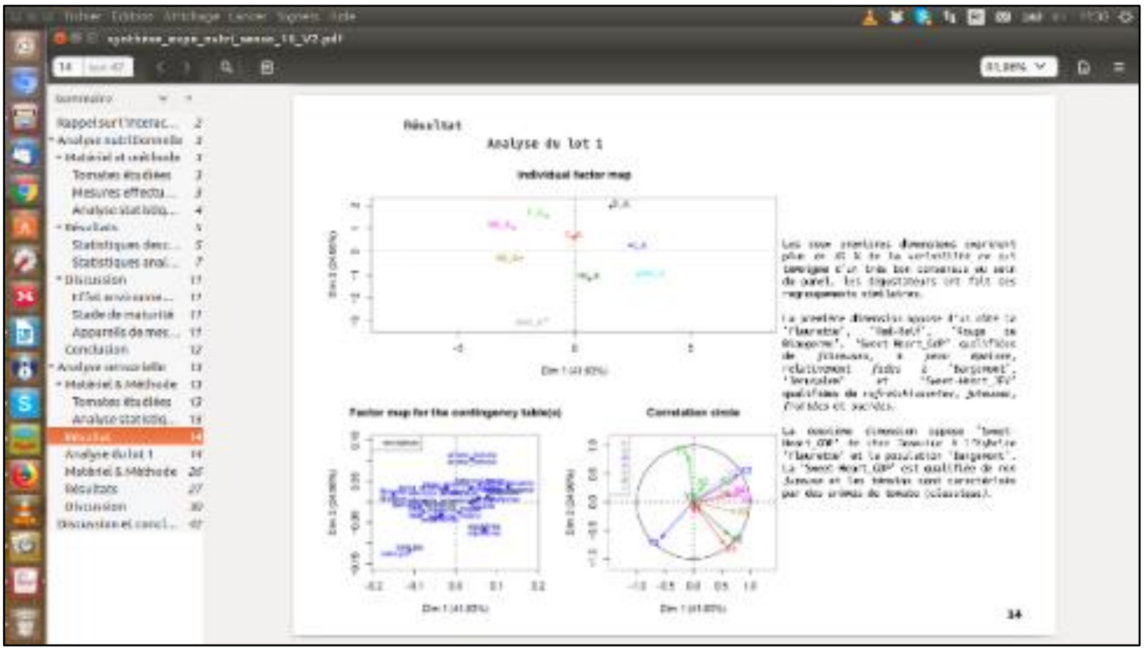

Fig. 19. Analysis of a napping test on Tomatoes (Credits: Camille Vindras, ITAB)

Practical and organisational aspects

Where to place the trials?

Any type of trial can be realized in an experimental station or on-farm. The important thing is that the field where the experimentation is located is accessible to the means and people required for sowing, assessing, maintenance and harvest. When the objective is to study and observe in depth the plants, or multiply the seeds, an area where a good level of control (irrigation if needed, hand-weeding, fencing) can be ensured is preferable. When assessing performance, instead, the area should ideally be representative of the environment where the crop would be grown commercially: holding the trial on an unutilised area of the farm or on the best field may not be the best option.

Save seeds

It is vital to always keep a backup of seeds: unpredictable events can occur and destroy the trial. The lower the amount of seeds available, the most important is to keep a backup. Beyond being lost, seeds can also be accidentally mixed-up. To address both these risks, seed multiplication should be kept separate from evaluation plots. If you have enough seeds, use separated strips in an adequate area for multiplication and randomised plots in an adequate area for the evaluation. If the seeds quantity is small, start with multiplication and plant description. If seeds are derived from in-situ, informal conservation, always look out for accidental mixtures or contamination with other species/varieties.

Collective organisation

The smaller the initial seed amount, the longer and more rigorous is the work. Cooperation between farmers and research centres are as important for the stage of amplification of the collection, as the collective organisation among practitioners. The sowing and maintenance of the collection can be done on a partner research station or on-farm with the means of the research station for example. As far as experimental machinery is concerned, the cost can be high, and it can only be taken in charge in the perspective of a collective organisation. For example, some farmer associations have bought a threshing machine that moves from one farm to another each year.

Put the trial in its context

When assessing performance, it is advisable to introduce a control in the experiments. How to select and include this control will depend on the type of species and varieties tested and on the question. For neglected germplasm of a common species in a given place, a mainstream commercial variety can be included in the experiment. When testing an underutilised species, instead, identifying a control may be difficult. In this case, it is worth considering that the target underutilised crop can reasonably be a possible alternative to a similar, commonly available crop. This latter will then be the control, directly included in the trial if possible, or monitored as closely as the trial at its nearest occurrence. For numerical performance variable, with or without a control variety, it is essential (and often overlooked) to identify a range between a minimum acceptable and a best achievable value, relevant to local conditions. This conceptual effort is the only way to enable understanding and substantiate conclusions or whether performance is “good” or “bad”.

Plan experimental design according to objectives and think about how to use the data

A complete technical guide can be found in DIVERSIFOOD Booklet #3[5].

[5] Goldringer I., Rivière P., 2018. Methods and Tools for decentralized on farm breeding. Booklet #3. DIVERSIFOOD Project.

Summary table of trials organisation based on initial seed amount

Seed amount	Means for cultivation				Objective of the trials	
	Plot size	**Sowing**	**Management**	**Harvest**	**Experiment design**	**Performance evaluation**
Very low (from few seeds to ~500g seeds for cereals)	From one line (one meter long) to about 5 m^2 for cereals and very small plots for vegetables	Sowing by hand	Hand weeding	By hand, experimental counter threshing machine	No experimental design if very few seeds (focus on amplification)	Prioritise seeds multiplication Possible evaluation of basic phenotypic traits (morphology and phenology)
Low (e.g. between 500g and 5kg for cereals)	Several square meters (from 3 to 100 for cereals), small plots for vegetables	Sowing with a hand sowing-machine (for vegetables and cereals) or experimental sowing machine (for cereals)	Hand weeding, mechanical weeding	By hand if small plots (and then experimental threshing machine for cereals), or with an experimental combine-harvester	Possible to start a randomised trial (see the DIVERSIFOOD booklet#3 for experimental designs)	Seed multiplication First performance evaluation: phenotype, interaction with the agro-system (production possible but not very reliable, some quality analysis - nutritional - according to the test)
Medium (e.g. between 5 kg and 50 kg of seeds of cereals)	Hundreds of square meters	Can be sown with farm machinery (for cereals from 15 kg of seeds)	Same maintenance as a production field	Experimental combine-harvester	Can start with trials in factorial designs or multi-location (see the DIVERSIFOOD booklet#3 for experimental designs)	Phenotype, environment, interaction with the agro-system, production, quality (nutritional, processing and organoleptic according to the final product suited)
Large (e.g. more than 50 kg of seeds of cereals)	Start testing at field scale	Can be sown with farm machinery	Same maintenance as a production field	Farm combine-harvester (think about cleaning the machine between different 'plots')	Production trails, multi-sites, everything possible	Complete performance evaluation: phenotype, environment, interaction with agro-system, production, quality