Chapter 10

# Participatory Plant Quality Breeding: An Ancient Art Revisited by Knowledge Sharing. The Portuguese Experience

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Additional information is available at the end of the chapter

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## 1. Introduction

### 1.1. Participatory plant breeding and on-farm conservation

Since the first domestications of wild plants about 12.000 years ago, farmers have been responsible for the development and conservation of thousands of crop landraces in hundreds of species [1]. Farmers put aside, for the next generation, a part of the harvested seed. Depending on the crop and the farmer, selection is carried out to obtain a crop answering better to the wishes of the growers and communities [2].

Following [3] definition: "a landrace is a dynamic population(s) of a cultivated plant that has historical origin, distinct identity and lacks formal crop improvement, as well as often being genetically diverse, locally adapted and associated with traditional farming systems".

Especially in more favorable environments, landraces are being replaced by modern varieties, which are less resilient to pests, diseases and abiotic stresses and thereby losing a valuable source of germplasm for meeting the future needs of sustainable agriculture in the context of climate change. However, landrace cultivation persists in less favorable environments [4]. This persistence is not due to increased productivity levels but because of their increased stability, accomplished through generations of natural and deliberate selection for favorable genes for resistance to biotic and abiotic stresses and intergenotypic competition and compensation [5]. They may also be kept by their dietary or nutritional value, taste, or for the price premium they attract because of high-quality traditional properties that compensate for lower yields [6]. This seems to be the case of the maize (*Zea mays* L. ssp. *mays*) landraces in Portugal that survived based on their quality traits such as technological capacity and aroma characteristics highly valued for bread production [7]. This bread making ability seems to depend on a range of particular traits not found on the available commercial hybrid varieties, and this is probably why maize landraces have not, in these regions, been totally replaced by hybrid varieties. As long as farmers themselves find it in their best interest to grow traditional varieties, both farmers and society as a whole will benefit at no extra cost to either partner [8].

As reviewed in [9], the food price crisis of 2008, sustained high prices, and more recent peaks observed in 2011 and 2012 have brought agriculture back onto global and national agendas. By 2050, global population is projected to increase by about one third, which will require a 70% increase in food production. To meet this need we should focus again on shifting the crop yield frontier, but also increasing production in more marginal environments through the increase of resistance to stress and improving competitiveness and sustainability. Traditional varieties continue to be fundamental in trying to achieve this global food security [6]. The erosion of these resources results in a severe threat to the world's long-term food security. Although often neglected, the urgent need to conserve and utilize landraces genetic resources as a safeguard against an unpredictable future is evident [10]. Farmers can contribute to this objective. The conservation and use of traditional varieties by farmers might be increased or at least sustained if more information on their good characteristics (adaptive, quality) is gather and disseminated among farmers and consumers and if the materials themselves are enhanced (breed). On-farm participatory breeding may have a significant and positive influence by encouraging farmers to adopt simple population improvement methodologies allowing them to do better with their own landraces [7].

### 1.2. Addressed problems and advantages

Conventional plant breeding (CPB), emerged in the early part of the 20$^{th}$ century, based on Darwin's theory of evolution through selection and the genetic mechanisms of evolution developed by Mendel and others [11, 12], has become increasingly isolated from the traditional plant breeding performed by farmers. The emphasis of this conventional breeding has typically been on developing modern varieties with high yield and geographically wide adaptation to optimal, relatively uniform growing environments [13, 14]. This contrasts with farmer breeding and farmers' local varieties, which are usually assumed to have narrow geographical adaptation to marginal, relatively variable growing environments, and high yield stability in those environments from year to year [1]. Modern agriculture, conventional breeding and the liberal use of high inputs has resulted in the loss of genetic diversity and the stagnation of yields in cereals in less favorable areas [5].

With the development of modern sustainable low-input agriculture in industrialized countries, for economic and environmental reasons, emphasis has recently been placed on local adaptation, on preservation of genetic diversity and on quality. This has resulted largely from the increasing awareness of the limits to conventional breeding as a consequence of increasing scarcity and decreasing quality of production resources in the low stress environments of modern agriculture [15]. Also the awareness that future increases in productivity may depend on increasing yields in high stress environments and on the maintenance of the available genetic variation, has motivated emphasis on specific or narrow adaptation and on genetic diversity conservation [15]. Conventional plant breeding has been successful in favourable environments, or in those which can be made favourable (e.g. by the use of inputs), but is less successful in traditional low-input or organic farming systems with higher stress growing conditions especially in small-scale farms.

Under this scenario, participatory research approaches have emerged as a relevant and necessary response to the problem of conserving genetic diversity also in industrialized countries [16]. Participatory plant breeding (PPB) programs are arising world-wide to meet the needs of farmers in low-input and organic environments that are normally overlooked by conventional crop breeders.

Several scientists, as reviewed by [17] discriminate among different types or modes of PPB, which are not necessarily mutually exclusive. However we agree with [18] on that the definition of PPB does not imply pre-assigned roles, or a given amount of collaborative work, nor imply that farmers and breeding institutions are the only partners. Experience in practicing PPB tells us that a true PPB program is a dynamic process with permanent collaboration, where both the roles of partners and the extent and the manner in which they collaborate, change with time. As farmers become progressively more empowered the type or mode of participation also evolves.

In PPB programs, farmers are invited to interact with professional breeders in their own farm and intervene at different stages of the breeding program, such as the generation of diversity, selection and seed multiplication. PPB helps farmers and breeders to communicate more efficiently with each other so that breeders can use their knowledge of biological theory, statistical design and analysis, to help the farmers' selection and access to a wide range of genetic diversity. Farmers can use their knowledge of their crops and environments and learn simple population genetics methodologies that will help them to progress more rapidly and efficiently in their seed selection. This collaboration should lead to varieties that better meet farmers' needs and conditions and conserve crop genetic diversity *in situ*, thus contributing to sustainable agriculture [15]. PPB exploits the potential gains of breeding for specific adaptation through decentralized selection, defined as selection in the target environment, and is the ultimate conceptual consequence of a positive interpretation of genotype x environment interactions [19].

Conventional plant breeding has aimed at pure lines and increasingly use of hybrids, resulting in a decrease of genetic diversity in conventional varieties. Also genetic diversity at the regional level is decreasing with few varieties grown over large areas [20]. In PPB, biodiversity is maintained or increased because, besides the use of heterogeneous populations with an inherent high level of diversity, different varieties are selected at different locations. With PPB, decision on which variety to release depend on the initial adoption by farmers; the process is demand-driven. This is expected to increase adoption rates and also reduce production risks, since the farmers gain knowledge of the variety's performance as part of the selection process [19].

The essential advantages of PPB over CPB involve: better targeting of local environmental conditions, better definition of selection criteria important to the end-users, faster and higher adoption of improved cultivars by the farmer and increase/maintenance of genetic variability. PPB also gives voice to farmers and elevates local knowledge to the role of science [19].

Participative approaches to agricultural research and development are now extensively used throughout the world to help define and address the practical research needs of farmers. They have proved useful in solving practical problems in complex and diverse farming systems, characteristics typical of organic farming and low input systems. In the case of maize breeding, very effective PPB projects are reported all over the world. This is the case of the Andean region (Ecuador, Peru and Bolivia) [21], Brazil [22, 23], China [24], Ethiopia [25], Ghana [26], India [27, 28], Kenya [29], Mexico and Honduras [30], Nepal [31] and in Nigeria [32]. In Portugal, a very successful long running PPB project in maize (the VASO project, Vale do Sousa - Sousa Valley) is on going since 1984 [33]. This PPB project was developed to cover the needs of small maize farmers, with scarce land resources, in polycropping systems for human uses (bread production). This project has recently been enlarged and extended to other regions of the country and special attention is now given to quality traits such as nutritional and health beneficial quality aspects besides the already considered technological ability for bread production.

## 2. Maize participatory breeding in Portugal – VASO project

### 2.1. Historical prespective

Maize domestication resulted from a single event involving its wild progenitor teosinte (*Z. mays* subspecies *parviglumis*), introgression from other teosinte types and the segregation into two germplasmpools between which much hybridisation occurred (reviewed by [5]. Portugal, by its privileged historical and geographical position as an enter point of new species into Europe, was among the first European nations to adopt maize (*Zea mays* L.) in its agricultural systems, more than five centuries ago [34]. The idea of hybridization among different maize introductions all over the country, rather than a slow northward dispersion accompanied by selection for earliness from one only germplasm introduction is supported in the case of Portugal (and Spain). The Iberian maize germplasm display no close relationship with any American types, but sharing alleles with both Caribbean and North American flints [35, 36].

After its introduction in the 16$^{th}$ century, maize spread rapidly throughout the country leading to an agricultural revolution, enhancing the rural population's standard of living. Numerous landraces (open pollinated varieties, OPV) have been developed during the centuries of cultivation, adapted to specific regional growing conditions as well as farmer's needs.

However, after World War II, Portugal was one of the first European countries to test the American maize hybrids which initially were not well accepted by the Portuguese farmers due to several handicaps such as late maturity or kernel type, not fitted for food or poly-cropping systems. Nevertheless, several breeding stations were established within Portugal at that time, from North to South, in the cities of Braga (NUMI), Porto, Viseu, Elvas and Tavira, releasing adapted hybrid varieties based on inbreds developed from Portuguese and American germplasm. This was the case specially of NUMI and the breeding station at Porto with their famous white flint double-crosses being the preferred seed by the Portuguese farmers, during the 60's and 70's. In fact, during that period of time the commercial yellow dent hybrids from the international seed companies never reached the same level of preference by our farmers, who needed a type of plant with a cycle that could fit their poly-cropping system and with a type of quality kernel for human consumption and not for feed. Accordingly, an enormous decrease in the number of cultivated landraces occurred by replacement with hybrid varieties. Due to this, a growing concern that numerous Portuguese maize landraces may have been lost forever started to be felt since the late 1970's. This awareness of genetic erosion led Silas Pego to initiate collection missions for maize in 1975. In the following years a more in-depth collection supported by FAO/IBPGR covered the entire country in successive missions. These materials gave rise to the first long-term cold storage facilities, the precursors of the present Portuguese Plant Germplasm Bank (BPGV), with one of the best European maize germplasm collections (ca 3000 landraces accessions) [37].

After Portugal and Spain entered the European Community, in 1986, a new political reality took place with a consequent change in our agriculture policy, smashing down the traditional small farming characterized by a poly-cropping, quality oriented and sustainable agriculture. In two decades these small farmers were pushed to bankruptcy. Also later on, during the 1980's, the scientific community became aware of the importance of the genetic resources co-evolution and the need for *in-situ* / on-farm conservation. The main question now was, how to restore this sustainable and quality oriented agricultural system in Portugal, and bring it back to business? Why not to apply some science to those genetic resources that had been selected by our traditional farmers during the last four centuries?

To provide an incentive for *in-situ* conservation of traditional maize landraces Silas Pego had the idea of engaging local farmers and their seeds in a participatory maize breeding program. By doing this, his goals were not only to conserve, but also to improve the social well-being of this rural community by increasing farmers' income through rising yields from some of their own seeds. To bring that idea to practice he led, in 1984, a detailed survey on farmer's maize fields at «Vale do Sousa» Region (Sousa Valley Region) in the Northwest of Portugal. The collected materials were the starting point of a PPB project, with simultaneous on-farm breeding and on-farm conservation objectives (VASO- ''Vale do Sousa''- project). This project aimed to answer the needs of small farmers (e.g., yield, bread making quality, ability for polycropping systems) with scarce land availability due to a high demographic density, where the American agriculture model did not fit and the multinationals had no adequate market to operate. From our previous knowledge of the small farming reality of the mountainous North of our country we knew that a project oriented to one crop integrated within a system should be oriented under a general developmental framework, understood and accepted by the farmer. This would require a different philosophic approach that we coined as *integrant philosophy* in opposition to the *productivist philosophy*. While in this last model the plant breeder occupies the center of decision, in the *integrant philosophic approach* is the farmer who occupies that position. Furthermore, we needed to link our philosophy with the basic formula of any production system: energy + raw material+ science = final product. This commanded our decisions, preference for renewable energy instead of fossil, local genetic resources instead of exotic germplasm, and an adequate breeding methodology accessible to the farmer's understanding and participation. Besides, a set of parallel decisions came along, as:

**1.** start with the farmer's genetic resources,

**2.** move to the farmer's place and develop the project at his own plots,

**3.** work side-by-side and let the farmer be the decision maker,

**4.** respect the agricultural system and the farmer's preferences and

**5.** always be ready to share knowledge and enthusiasm.

If we could get at least some of these achievements, we needed to be sure that they had to be reached at the farmer's speed and under his own constraints. This model also embraced a quality oriented perspective (human food) where the quality factor must be financially valorized in order to sustain the agricultural system.

To implement this project several main decisions had to be made, such as the choice of the location to represent the region, the farmer to work with side-by-side, the germplasm source, and the breeding and management methodologies to apply.

### 2.2. General procedures

In this section, and taking the VASO project as a model, a generalised description of the most important decisions to be made on a maize PPB project implementation is presented.

As in any collaborative approach all the plans and decisions have to be made between all the involved partners and with that purpose breeders need to meet with local farmers and discuss selection strategies to understand farmer's objectives and constraints. Following this, four main decisions must be taken to implement correctly the participatory breeding approach:

#### *2.2.1. Farmers, breeders and environmental choices*

The selected farmer's fields must be located in a traditional production area where the crop to be breed is important and where support from local authorities/farmers associations is guaranteed. Farms should represent the different soil and climatic conditions under which the crop is grown, the different size of farms and farm types.

In the case of the VASO project, the Sousa Valley was chosen after the results of the 1984 survey and taking in account several factors:

**a.** At that time only 15% of the Portuguese farmers were using hybrid seeds. However, in the fertile Sousa Valley this percent was higher - 25%. This meant that even in this region 75% of the farmers were still using their own regional varieties of maize. This created a perfect situation for developing alternative production systems.

**b.** That region was known as a source of the best inbred lines which were in the basis of one most successful double cross national hybrids – HP21.

**c.** A previous sociological survey that had been carried out by a small team of scientists had raised a general and thorough picture of the local rural society and its organized structures. Out of it came the information of a private farmers' association (CGAVS) – Center for Agriculture Management of the Sousa Valley – that was open to collaborate and was offering its headquarter facilities as a basis for the maize breeder.

**d.** The region was among the national most populated areas, with good soil quality and plenty of water supplies.

**e.** Both the communities of the closest town Lousada, and the Agriculture Cooperative of another close town Paredes, were willing to follow the project.

**f.** After a national contest promoted by the Portuguese Ministry of Agriculture, it came out that the national winner, with a long cycle yellow dent single cross hybrid, was located in the Sousa Valley. To run a maize breeding project in a place where our national champion was located, certainly was a natural challenge.

Choosing the right person to work with is also a major decision on participatory approaches, where the work is carried out side-by-side and the power of decision shared. A high farmer initial acceptance of this type of approach and enthusiasm for joining this kind of projects are the best guarantee of success. Nevertheless, a careful respect of the breeder for the local traditional agriculture is also crucial.

The farmer' selection in the VASO project was made having into account both a previous information obtained in the mentioned sociological survey, and from direct contacts within the farmers' organization CGAVS. From this collected information, the most willing and also contradictory people were chosen. In its beginning only 3 farmers were directly involved.

In the process of choosing the right person, it is very important, as highlighted by [18], to clarify

**i.** what plant breeding can offer and how long it can take;

**ii.** what sort of commitment in land, time and labor is required from the farmer;

**iii.** what are the risks for the farmer and how these can be compensated for (in-kind compensation vs. money); and

**iv.** what overall benefits farmers can expect if everything goes well.

These farmers will need to be trained/updated with practical examples of how selection could be improved. The plant breeder will have to take into account the selection objectives of the farmer and the farmer will learn simple population genetics methodologies that will help him to progress more rapidly and efficiently in their seed selection.

In a PPB program it is very important to maintain contacts with farmers beyond and besides specific scientific activities. These 'courtesy' visits are not only instrumental in building and maintain good human relationships between scientists and farmers by bridging gaps, but are an incredibly valuable reciprocal source of information [18].

In the VASO project the initial enthusiasm of some of the contacted farmers that still today collaborate with the project was fundamental. Also the support of a local elite farmers' association (CGAVS) which agreed to be part of the project was very beneficial to the success of the project.

*2.2.2. Starting germplasm and variability generation*

According to the VASO philosophy, the project should start by using local landraces as its genetic resources, selected by the breeder as the most representative of the local farming. A survey was made by the breeder in 1984 during the summer time along the maize fields of that region in a close look for particular plant phenotypes and ear size. Further, at harvest time, several sets of store houses ("*sequeiros*") were visited and farmers contacted. From this first survey two regional varieties were selected: "Pigarro", a white flint type FAO 300 cycle with strong fasciation expression, used in the best soils for human consumption, and "Amarelo miúdo" ("Amiúdo"), a yellow flint type FAO 200, adapted to the poorest soils with low ph, water stress and aluminum toxicity, but also with quality for bread production. Afterward, the VASO project was also conserving additional landraces such as "Basto", "Aljezur", "Aljezudo", "Castro Verde", "Verdial de Aperrela" and "Verdial de Cete". In parallel with the landraces approach, a synthetic population, Fandango, was also included [38].

So, as highlighted from the VASO project, one of the prerequisites for the implementation of a PPB project is the existence of local adapted germplasm. In this way the farmer's selection pursued over several centuries (quality preferences) will be respected and the environmental adaptation already achieved either for the soil or climate will be assured [33].

The inclusion of high quality parents is of particular importance when considering the quality objectives of the population. Quality is difficult for farmers to access if they grow a crop for the commercial market, and is not necessarily improved by natural selection [39].

When needed, genetic diversity can be generated by crossing genetically diverse and adapted local cultivars as starting genetic base. Other foreign materials (exotic germplasm) can also be added after this point to overcome any limitation typical from the local cultivars (as disease susceptibility).

*2.2.3. Breeding methodologies*

Technically the process is similar to conventional breeding, with three main differences. Trials will be grown in farmers' fields rather than on-station, covering a range of target environments and using farmer's agronomic practices and levels of inputs. Selection will be conducted jointly by breeders and farmers (and other end-users when appropriate), so that farmers participate in all key decisions. This process can be independently implemented at a large number of locations [19].

The goal of any plant breeding is to develop a plant population composed of phenotypes that meet farmers' criteria, and that farmers will, therefore, adopt and maintain. So selection criteria must consider farmers objectives like quality traits, such as flavour or nutritional value, pest and disease resistance and enhanced capacity to survive in highly changeable environment typical of low input/organic farming systems (yield stability) through genetic diversity maintenance or enhancement.

The choice of the breeding methods cannot be made without considering whether and how farmers are handling genetic diversity. An issue related to the choice of the breeding method is how much breeding material farmers can handle. The choice of the breeding method also depends on the desired genetic structure of the final product, i.e. pure lines, mixtures, hybrids or open pollinated varieties [18].

Common mass selection, usually performed by all farmers, consists on the identification of superior individuals in the form of plants from a population, and in the case of crops like maize, the bulking of seed to form the seed stock for the next generation [40]. It requires relatively little effort compared with other selection methods and, practiced season after season with the same seed stock, has the potential to maintain or even improve a crop population, depending upon the heritabilities of the selected traits, GxE for the trait, the proportion of the population selected, and gene flow in the form of pollen or seeds into the population [15]. PPB is a cyclic process where the best selections will be used in further cycles of recombination and selection or selection will just give rise to experimental cultivars, to be tested again on the farmers' fields.

Within the VASO project a tacit agreement was made between the breeder and the farmer involved. While the breeder would apply his breeding methodologies, the farmer would continue a parallel program with their own mass selection criteria, starting with the same initial populations. With this agreement the breeder had to accept low-input and intercropping characteristics, as well as to accept and respect the local farmer as the decision maker. On the other hand the farmer was able to compare the effectiveness of the two breeding systems allowing him to base his decisions on solid grounds. Due to the choice of locally adapted germplasm, diversity and quality were considered as selection priorities.

In relation to the selection approach followed by the VASO project the breeder initially opted by the *S2 lines recurrent selection*, due to its potential for favoring a good amount of additive gene action. This methodology worked very promisingly with the "Pigarro" germplasm, but not so good with "Amiúdo". In fact, while we could observe a surprisingly low inbreeding depression when we tested the S2 lines of "Pigarro", a different situation came out from the S2 lines of "Amiúdo". Here, the inbreeding depression was so high that we had to move to the *S1 lines recurrent selection methodology*. On the other hand, farmers were advised to use improved mass selection approaches, such as with a two parental control (*stratified mass selection*), where selection takes place not only after harvesting, at the storage facilities (ear traits), but also during crop development in the field such as in a cross pollinating species before pollen shedding, for the male parent selection by detasselling all undesirable plants, and before harvesting, for the female parent selection (ear size, root and stalk quality and indirectly pest tolerance; [33]). As the time was passing by, the Common Agriculture Policy (CAP) from the EU, which favored the big farming oriented for feed production, pressed the small farming to bankruptcy. As a consequence, our VASO project also suffered a sudden lack of governmental support leaving the breeder without any support to adequately pursue the mentioned methodologies, both requiring a big amount of hand pollinations. Again, the breeding methodology had to be changed, now for *stratified mass selection* to all the other landraces in the project.

The improvement program included yield, lodging performance, pest and disease tolerance, and indirectly, adaptation to climate changes.

During the VASO project, some pre-breeding methodologies were developed such as the HUNTERS (High, Uniformity, aNgle, Tassel, Ear, Root lodging and Stalk lodging) or the Overlapping Index [7, 38]. These are nowadays very useful on our PPB maize landrace selection on-farm.

In all cases in the VASO project, the traditional poly-cropping technology was followed: surrounded by vineyards, the plots were usually planted with three main crops: maize *(Zea mays L.)*, beans *(Phaseolus vulgaris L.)* and a forage crop *(Lolium perene L.)*. The first two simultaneously planted along the same row in May and the forage later on in July between the rows. This organic system has three main sustainable advantages:

1. a probable symbiosis between a *Leguminosae* (beans) and *Rhyzobium sp*, as a natural source of nitrogen for the *graminea* (maize),
2. any possible plant damage or failure along the row could be compensated in favor of the other crop to which was allowed more sunlight and
3. after harvesting the maize crop in September the soil was already covered with a forage layer that not only functions as a protection against erosion but also as a source of 3-4 cuts of forage for animal feeding during the winter.

*2.2.4. Seed management and dissemination*

Since the beginning of the VASO Project, phenotypic data were collected and seed of each selection cycle, either from phenotypic recurrent selection or from S2 recurrent selection, was kept at 4ºC in our national Plant Germplasm Bank (BPGV) cold storage facilities [38].

Several maize OPVs were selected within this project with the joint collaboration of the breeder Silas Pego and the farmers.

The seed however has not yet been nationally distributed /commercialized due to the lack of appropriate legislation allowing the certification of OPV with a certain level of heterogeneity. These aspects will be further developed under the PPB success evaluation and the seed dissemination and ownership sections.

### 2.3. PPB Vaso project success evaluation

During more than a quarter of a century of continuous participatory on-farm maize breeding, some *ups* and *downs* came along, mainly influenced by political fluctuations that affected both the governmental support and the market prices of its quality oriented output. In 2001, the mayor of the town of Lousada presented the situation of the VASO project governmental funds cutting to his general assembly that unanimously decided to substitute our Ministry of Agriculture institution (DRAEDM) as the sponsors of this long term PPB project.

In spite of these *ups* and *downs,* our main achievements can be centered in three areas:

1. breeding output,
2. technological improvement and
3. seed diffusion.

#### *2.3.1. Breeding output*

All the outputs from this project are improved OPVs. "Pigarro" is the one which received our main investment in breeding, with several cycles of *S2 lines recurrent selection* and repeated cycles of *stratified mass selection*. Its yield level is now between 8 and 9 t/ha, and is the type of seed that better fits the high quality standards of our most famous maize bread – *Broa de Avintes*. "Amiúdo" went to some cycles of *S1 lines recurrent selection* and fits the conditions of tolerance to soil (low ph and Al) and water stress. As a yellow vitreous flint and small kernel size, this quality enables this variety to be used both for maize bread as well as for the specific market of feed for carrier-pigeons and pigeon breeding. "Fandango", an ear size champion (at the Sousa Valley best ear contest), had its original FAO700 cycle reduced to FAO600 and a competing yield over 10 t/ ha. A set of other improved varieties like "Aljezur" FAO 400 yellow flint, "Aljezudo" FAO 300 yellow flint, "Castro Verde" FAO 600 yellow flint, and some others, complete a minor output that can be improved in future attempts.

Yield trials for evaluation at the farmer's place, were the most difficult task to carry out in a way to fit the levels of statistical significance. Nevertheless for "Fandango" and "Pigarro" OPVs these trials were already established, and for some of the other VASO OPVs are now under way. In the already established evaluation trails we should take into account the original specific objectives defined for the farmers' and breeders' selections. For the "Pigarro" *stratified mass selection* the farmer aimed at obtaining bigger size ears while maintaining the flint and white type of kernel. The breeder with its alternative *S2 lines recurrent selection* method aimed at increasing favorable alleles both for yield, ear placement and stalk quality. For the "Fandango" *stratified mass selection* the farmer was looking for big ear size maximization and the breeder for yield maximization. Evaluation field trials for selection gain on these two OPVs were established in several locations in Portugal and in the case of "Fandango", also in the USA [38, 41]. The statistical analysis indicated that *stratified mass selection* in "Pigarro" lead to an increase in days to silk and anthesis, ear diameter, kernel row number and fasciation. On the contrary, ear length decreased significantly [38]. Molecular SSR marker data, from [42], on three selection cycles (C0-1984, C9-1993 and C20-2004), revealed that no effective loss of genetic diversity has occurred during the selective adaptation to the farmer's needs and the regional growing conditions. Variation among selection cycles represented only 7% of the total molecular variation, indicating that a great proportion of the genetic diversity is maintained in each selection cycle. Genetic diversity has not been reduced from the "Pigarro" bred before 1984 to those examples improved after 2004, but the genetic diversity maintained is not exactly the same. Mass selection seems to be an effective way to conserve diversity on-farm, and interesting phenotypic improvements were achieved as the bigger ears farmers' objective [42].

On the other hand, the response to the "Pigarro" *recurrent selection by S2 lines* indicated that after three cycles of selection, days to silk, uniformity and the cob/ear ratio increased significantly, but also without a significant yield increase [38].

The "Fandango" *stratified farmers mass selection* evaluation performed in Portugal revealed that the ear length and the thousand kernel weight decreased significantly and simultaneously plant and ear height increased significantly [41], accomplishing the bigger ears farmers' objective. These traits had no significant changes during the selection cycles performed by the breeder. Additionally, days to silk had a significant increase during selection. For yield no significant changes were observed during selection when all the evaluation locations were considered. Nevertheless, when considering only the trials performed at the location where the PPB took place (Lousada), a significant yield increase was recorded especially during breeders selection cycles (3.09% gain per cycle per year), being less pronounce during farmers selection cycles (only 0.63% gain per cycle per year). The lack of significant progress in yield for both "Pigarro" and "Fandango" can be explained by low selection intensity due to the exclusion of stalk lodged plants in the basic units of selection [38, 41] what must be taken into consideration in future selections for yield increase.

Both selection methods used in "Pigarro" and "Fandango" different phases of selection, suggest that *stratified mass selection* is better than *S2 lines recurrent selection* due to the following reasons: *Stratified mass selection* is a cheaper methodology, technically more accessible to farmers, with one cycle of selection completed each summer, without reducing the conserved genetic diversity [41].

The evidence of genetic diversity maintenance by this PPB project with simultaneous phenotypic improvement, fundaments the preservation of these on-farm selection programs where threatened landraces of great interest for future use in breeding programs and for developing new farming systems are preserved.

*2.3.2. Tecnological improvement*

A modified planting system was developed in such a way that two rows of beans (20 cm apart) were planted between two consecutive rows of maize (130 cm apart). The difference to the original technology was that one row of maize was eliminated, but the seed density was maintained by doubling the plant density along each row. On one hand, this higher plant density was compensated by the larger space between rows that the plants could take advantage from. On the other hand, the now separated rows of beans, due to its fast growing, rapidly covered the soil avoiding weeds. This new technology, besides being weed control efficient, facilitates harvest of beans in July and the following planting of the forage crop over that empty space between rows of maize. Additionally, it had a final positive effect in September at the harvest of maize, because the soil was already protected against the erosion from the rainy season that was coming in. Moreover, during fall and winter, 3 or 4 cuts of forage could be made for animal feeding.

Another technological improvement was discovered by the farmer. In a certain morning of June, when the maize was in its four to five leaves stage, the farmer was observing an intense flight of birds over the maize field. With a closer look he noticed that birds were catching the larvae of the pest *Agrotis segetum* L. He then realized that the soil was still humid from his irrigation the afternoon before. Our conclusion became obvious: the larvae were superficial because the soil was still cold, but go underground when the sun heats. We realized that a sustainable insect control tool was available and a light superficial irrigation became usual in each afternoon.

### *2.3.3.Seed difusion*

The diffusion of the improved seeds from this VASO project, while limited to the Sousa Valley area, has been very easy, as expected. Farmers are always ready to share seeds and it happens frequently. However, a private initiative from a local farmers' cooperative (Cooperativa Agricola de Paredes), in the early 90's, with a regional contest for maize - *The maize best ear of the Sousa Valley* turned to be the best diffuser of our seeds since they became quickly champions of ear size. Among our improved varieties the long cycle "Fandango" became a real champion beating, year after year, the best commercial hybrids in ear size. One of our collaborative farmers, *Francisco Meireles,* became the most rewarded Portuguese farmer, with more than 50 trophies. This has contributed to the recognition of the farmer by the community, but also attracted new farmers and new germplasm to this PPB project that in this way could be identified and preserved on-farm by the same approach [38].

## 3. Participatory plant quality breeding in Portugal

Presently, in particular Portuguese regions, known by their high quality maize bread, farmers keep on cultivating their traditional landraces. Traditionally selected landraces are mainly white kernel flint types, demonstrating quality over yield and maintaining genetic diversity to increase adaptability to a large variety of edaphic/climatic conditions, such as drought or aluminium toxicity [37].

Maize is definitely a deep-rooted crop in the Portuguese rural tradition and the available genetic variability of its landraces offers a superb challenge for breeding for special quality traits. Since the on going PPB project at Sousa Valley (VASO) revealed promising breeding results, our objective is to get further support to maintain the actual project and extend it to other maize landraces production areas as a way to increase the use value of this traditional germplasm and by doing so promote *in-situ/on-farm* conservation and halt the serious genetic diversity erosion. The inclusion of organic breeding objectives in the actual PPB project is also being considered to add value to these improved landraces since the low input sustainable farming system typical of the traditional production systems is already very similar to the organic farming directives. In this more recent PPB approach, molecular and detailed quality data will be used in order to increase the effectiveness of selection when appropriate.

### 3.1. New material and farmers prospection

With the idea of establishing an on-farm conservation project, with farmers' engagement through participatory breeding approaches, to halt the genetic erosion by improving those landraces and increasing their market value, members of our team engaged in a field expedition to the Central region of Portugal to collect enduring landraces [7]. In this region farmers grow maize landraces, known for their good maize bread quality, in association with common bean local varieties, in a traditional intercropping practice. The collected landraces represent important sources of genes and gene combinations not yet available for crop quality breeding programs and due to their intrinsic quality traits (that promoted their maintenance in cultivation) are the best candidates for expanding the already existing participatory breeding program (VASO) to other regions with more emphasis on quality breeding. Around 50 different (yellow and white) maize landraces were collected, characterized using pre-breeding approaches and conserved in cold storage [7]. These landraces, together with other landraces that were subsequently collected from the surrounding regions, represented the basis for the PPB net existing in the country. During this expedition several associated crop (beans, rye and pumpkin) landraces were also collected. The collected bean landraces are also now being characterized at agronomical, genetic diversity and quality level to select the best material to implement also participatory breeding approaches.

During this expedition, the first steps on contacting new farmers to extend the original PPB net were also given and the most enthusiastic farmers meet during those days, are now involved in the participatory research. VASO still continues nowadays and it is the best inspiration for those intending to start their PPB programs. For this reason, VASO initial actors have been invited to give their testimony to the new associated farmers within the actual extended PPB net. This action boosted the program giving new perspectives to the new farmers, and new paths to our program. Some meetings have been prepared to have farmers' perception (e.g. know what are the kernel preferences for maize bread), or where researchers present their achievements to the network, with time for discussion (e.g. soil, agronomic traits, genetic diversity and quality). Along this process an identification of the farmers' profile was made, their motivations and interests (e.g. germplasm development, trials) in order to fully engage them with the project. Even though the majority of farmers has no background on basic statistics (e.g. replication), they do their empirical research. For this reason appreciate the contact with a "practical" academia, where both look in the same direction and where the arena is at their farmers fields. This action demands an intensive networking regarding motivation and science, where future perspectives are discussed, and results depart from farmers' fields.

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### 3.2. Quality breeding objectives

In the national Portuguese panorama we are now more concerned with the landraces still in cultivation and the quality that prevented their replacement by the generalized hybrid use. These materials represent the actual enduring and surviving genotypes and so constitute the best candidates for a quality breeding program. Additionally, these materials should be allowed to pursue a natural evolution under *in situ* conservation and farmers must be rewarded for their contribution to halt the current and continuing loss of plant diversity. The only way to achieve this is to promote a sustainable use of plant diversity, where conservation responsibilities and benefits will be shared with farmers [7]. A participatory approach seems to be the most logical solution. We try to identify ways of supporting farmers in the maintenance of traditional varieties and crop genetic diversity by performing better with their own seeds improved.

Portuguese maize landraces have been preserved on-farm, due to particular quality traits not found on their competing modern hybrid varieties. These landraces are mainly flint type open pollinated varieties (OPV) with technological ability for the production of the traditional maize leavened bread called "broa" that still plays an important economic and social role on Central and Northern rural communities of the country [7].

Due to this, we decided to start studying in more detail the technological ability for bread production of maize landraces as the major quality trait to breed for. However, later on, it has been described that other quality traits, such a flavor or aromas were also contributing to the consumers preferences for bread obtained from traditional maize landraces in detriment of maize hybrid varieties bread [43]. Volatile components responsible for the aroma were then also included into our detailed study. Presently and due to consumers higher concern about the quality of their food and how their diet can influence their well being also antioxidant compounds and their bioactivity are also being analyzed on the flour and bread made from our traditional maize varieties.

Our objective is that our improved varieties will be attractive to consumers, processing industry and farmers, answering health and environmental public concerns and increasing sustainability of farming systems.

#### *3.2.1. Technological ability for bread production*

The introduction of maize in the Iberian Peninsula during the fifteenth century produced important changes in agriculture and in the diet of the people. Maize has become highly integrated into Portuguese agriculture and diet, and and appears as the major cereal for bread making in the middle of 19th century. The bread produced at that time was "broa" where maize flour meal was mixed with wheat or rye. Presently, Portuguese rural areas continue to produce "broa", mainly in the northwest parts of the country in a wide variety of recipes, some with protected geographical identification and traditional methods of baking. The quality of "broa" is the result of empirical knowledge that is very closely related to the quality of the maize, kernel processing, blending flours and baking procedures, including fermentation and baking.

There are many traditional recipes to prepare “broa”, but the traditional process involves adding maize flour (sieved whole meal flour ranging between 50% and 80%), hot water, wheat and rye flours, yeast and leavened dough from the late “broa” (acting as sourdough). After mixing, resting and proofing, the dough is baked in a wood-fired oven. This empirical process leads to an ethnic product highly accepted for its distinctive sensory characteristics.

The process begins with blanching maize flour in water boiled followed by kneading. Blanching is important to obtain high consistency dough’s because in the absence or reduced amount of gluten the dough rheological properties are provided by the starch gelatinization [43]. The addition of sourdough is another important aspect in the preparation of “broa”, and its microbial diversity has been characterized [44] with mainly lactic acid bacteria *Lactobacillus* (*brevis, bulgaris plantarum*) being present in addition to yeast (*Saccharomyces cerevisiae*).

In terms of maize physical characteristics, kernel size and shape, weight and density, degree of stress cracks and resistance to milling and compression have all been linked to hardness and this is the primary cause for the large differences in rheological properties of flours which have subsequent effects on processing.

The majority of commercial hybrid maize varieties that are currently grown in Portugal are dent type, but also some flour types can be found, characterized by a soft or floury endosperm respectively. However, in the traditional varieties and landraces predominates the flint type with the hardest kernels, resulting from the presence of a large and continuous volume of horny (vitreous) endosperm. Maize flint type has harder endosperm than dent types, resulting in different viscosity profiles. Flours from maize flint grain have lower peak viscosity and lower retrogradation than dent types [45, 46].

White maize is the preferred choice by northwest rural populations, probably due to cultural and historical reasons that have created food habits. Indeed in the 18th century, when the maize was the main cereal used for bread making, white bread was the most appreciated, symbolizing wealth and prestige [47], in this context white maize flour was the most suitable for blending with wheat flours. However, it is important to understand if there are differences between the rheological behavior of maize flour from white and yellow grains. With this aim, we analyzed a collection of maize OPVs and found no significant correlation between the Colour Chromameter b* - yellow/blue index and viscosity parameters of flours [34]. From the nutritional standpoint, the white grain has the disadvantage that it is devoid of carotenoids, which are important antioxidants for health. Nevertheless, our preliminary results indicate high amounts of other nutritional compounds such as tocopherols.

Kernel processing into milling is an important quality factor for the production of “broa” because it determines the performance of the flour. Dry-milling process is used for the production of maize meal used for bread making and whole grain is processed traditionally in stone wheel mills, moved by water or wind, and nowadays frequently by electricity.

The native starch can be damaged to a greater or lesser extent thereby influencing the flour water absorption capacity and enzymatic attack, especially $\alpha$-amylase. The type of grinding may also affect ash flour content, which interferes with the evolution of pH during the fermentation step. The grinding mills driven by water occurs at a slower rate, flours obtained with this process have lower ash content, lower proportion of damaged starch, and higher maximum viscosity than obtained in electrical mills [46].

In addition to the "broa" sensory specificities and the need to diversify baking products to fulfill the consumers' appreciation range of traditional breads, there are also reasons related to nutrition disorders that promote the study of maize quality for bread making.

The high indices of chronic diseases, such as obesity and diabetes, increase the demand for the development of breads with starch that is slowly digestible or partially resistant to the digestive process namely resistant starch [48]. "Broa" revealed a greater resistant starch content than the wheat bread [49]. Differences in starch digestibility or type of dietary fiber, the typical fermentation and bread volume also contributes for lower glycemic index of "broa" when compared with wheat bread [49]. Gluten enteropathy (coeliac disease) is another serious chronic disease, caused by an inappropriate immune response to dietary wheat gluten or similar proteins of barley or rye. Maize is a gluten-free cereal, thus suitable to produce foods addressed to celiac patients. The acquired knowledge on "broa" (made from composite maize–rye–wheat flour) is important for facing the challenges of producing gluten-free bread that usually exhibits compact crumb texture and low specific volume [43]. Baking assays were performed and demonstrated that bread making technology could be satisfactorily applied to produce gluten-free "broa" [43].

Strategies to further improve maize kernel quality for "broa" production considering flour rheological properties and nutrients are under intense investigation, mainly focused on viscosity profiles, protein content, carotenoids and tocopherols.

Management of large number of accessions implies adoption of rapid and non destructive tests for efficiently screening quality traits, consequently research on Near Infrared Spectroscopy (NIR) models to estimate maize kernels quality is under progress and it will be of extreme importance as a fast and inexpensive way to support quality PPB.

Moreover, the selection parameters adopted in quality PPB should reflect "broa" consumers' preference and therefore "broa" bread sensory analyzes with consumer panel are being implemented and the data obtained will be used to improve the screening quality maize tests for bread making.

#### *3.2.2.Aroma*

Aroma strongly influences food quality and therefore consumer's preferences and acceptability for the products. Sweet, sour, salty, bitter and umami tastes, olfactory responses, oral sensory sensations related with astringency, coolness and pressure, contribute to food aroma [50]. Since olfactory responses involve a huge number of descriptors to distinguish hundreds of different odors, it is not surprising that most of the work developed in aroma research has been related with volatile compounds analysis [51].

Volatile compounds in plants and foods are produced during harvesting and processing, by enzymatic degradation [52]. The type of volatile compounds depends on plant species, genotype, plant part and environmental growing conditions [53]. Alcohols, aldehydes, ketones, hydrocarbons and terpenic compounds are among the main volatile compounds responsible for foods' aroma and most of them are present in trace amounts, which difficult the task of aroma analysis [50]. Drying, handling, milling and storage conditions may affect aroma of foods [50].

Solid Phase Micro Extraction (SPME) combined with Gas Chromatography-Mass Spectrometry (GC-MS) is now the most used technique for the analysis of volatile compounds [52]. Aroma volatile compounds of different maize types and preparations have been studied by SPME-GC-MS. Characteristic odor of dimethyl sulfide has been associated with sweet maize aroma. Other important compounds include 1-hydroxy-2-propanone, 2-hydroxy-3-butanone and 2,3-butanediol. Higher concentrations of such volatile compounds were reported in canned maize, when comparing canned, frozen and fresh maize [50, 54]. In popcorn, 6-acetyltetrahydropyridine, 2-acetyl-1-pyrroline and 2-propionyl-1-pirroline were described as the most important aroma compounds [54]. In maize tortilla and taco shell it was possible to identify an aroma component not previously identified, 2-aminoacetophenone [54].

Information about aroma volatile compounds in Portuguese maize and maize bread, until now, is scarce. In order to characterize these compounds in this national germplasm and respective food products (bread), we studied a collection of 51 Portuguese maize landraces representing the starting material of the present national participatory maize breeding project. Solid Phase Micro Extraction (SPME) combined with gas chromatography and mass spectrometry was used for the analysis. Volatiles were identified based on comparison with mass spectra in reference libraries NIST 21.LIB and Willey 229.LIB and by the Linear Retention Index (LRI). Aldehydes (hexanal, heptanal, nonanal, 2-nonenal (E), and decanal) were identified as main volatile compounds responsible for maize flour aroma being Hexanal the most representative aldehyde compound on the analyzed flour.

The analysis of the aroma volatile compounds released from traditional Portuguese bread ("broa") made from selected maize varieties is under way using the same conditions of analysis.

*3.2.2. Phenolic compounds*

Phenolic compounds are secondary metabolites produced by plants as protection against fungi, herbivores, UV radiation and oxidative cell injury, revealing also important functions in several aspects of plant life as growth, pigmentation and reproduction [55].

Phenolic compounds can contribute, with other dietary components such as vitamins C, E and carotenoids to the human protection against oxidative stress caused by an excess of reactive oxygen species (ROS) [55, 56]. Their antioxidant activity contributes to the inhibition of oxidative mechanisms underlying several degenerative diseases such as diabetes, cardiovascular diseases, and cancer [57, 58]. Besides their health promoting effect phenolic acids present in maize samples, for example, may contribute indirectly to flavor quality trough inhibition of lipid oxidation [50].

Phenolic compounds can be classified into two major classes, flavonoids and non-flavonoids. Phenolic acids (hydroxybenzoic acids, hydroxycinnamic acids) and flavonoids correspond to soluble compounds (easily extracted with polar solvents such as ethanol, methanol and mixtures with water) which can be separated and identified by High Performance Liquid Chromatography (HPLC) and detected in the UV-Vis and by Mass Spectrometry (MS) [59].

Some studies have been conducted in order to characterize maize polyphenolic content, and vanillic, *p*-coumaric, ferulic, protocatechuic acids, derivatives of hesperitin, quercetin and anthocyanins like cyanidin-3-glucoside and pelargonidin-3-glucoside [60] were identified as the most important ones. The actual knowledge about phenolic compounds bioaccessibility and bioavailability [61], contributing to the protective effect in biological systems, is still scarce.

In common beans (*Phaseolus vulgaris* L.) phenolic compounds (phenolic acids and flavonoids) were mostly described in the seed coat and at lower amounts in cotyledons [58, 62, 63]. Compounds, such as *p*-hydroxybenzoic, vanillic, caffeic, syringic, coumaric, ferulic and synapic acids as well as flavonoids such as quercetin, kaempferol, daidzein, genistein, *p*-coumestrol and anthocyanins like delphinidin and cyaniding were already identified in common beans [63]. It is widely accepted that thermal processing (boiling or steaming treatment) affects phenolic compounds content and antioxidant activity values [64, 65].

In relation to the Portuguese maize and beans germplasm, and to our knowledge, no information on the phenolic compounds as ever been published. So our initial goal was to characterize the flour composition of 51 Portuguese maize landraces and of 32 different varieties of Portuguese beans.

Spectrophotometric assays were performed to determine total phenolic and total flavonoids content in the samples. For maize, total phenolic content ranged from 100.30 ± 4.81 to 206.83 ± 9.55 mg of gallic acid equivalents/ 100g DW (dry weight) and total flavonoids content ranged between 0.69 ± 0.07 and 17.01 ± 0.52 mg of catechin equivalents/ 100g DW. For beans, the total phenolic content ranged between 1.00 ± 0.02 and 6.83 ± 0.31 mg of gallic acid equivalents/g and total flavonoids content ranged between 0.09 ± 0.00 and 2.50 ± 0.01 mg of catechin equivalents/g.

With the main objective of identifying soluble free, soluble conjugated and insoluble phenolic compounds in maize flour by HPLC, acidic and alkaline hydrolysis were performed. The phenolic fraction which presented higher amount of compounds corresponded to the insoluble. Using HPLC with diode array detector (DAD) it was possible to identify *p*-coumaric and ferulic acids as well as aldehydes such as vanillin and syringaldehyde. In bean's extracts, phenolic acids such as caffeic acid and flavonoids such as catechin, quercetin-3-O-rutinosideand kaempferol-3-O-glucoside were identified.

Studies of the antioxidant activity by Oxygen Radical Absorbance Capacity (ORAC) have also started and were already performed for some maize flour extracts. Values obtained range from 364.30-1223.55 μmol Trolox Equivalents Antioxidant Capacity (TEAC)/100g. In bean extracts, values obtained were between 28.99 ± 2.09 and 189.12 ± 10.20 μmol TEAC/g. These ORAC values showed a strong positive correlation with total phenolic content (R=0.9087) and total flavonoids content (R=0.9171), evaluated by colorimetric methods. The results obtained, until now, revealed a great variability of polyphenolic content and antioxidant activity in the samples analyzed anticipating a high potential for quality breeding within these materials.

Future studies for phenolic compounds' identification and quantification by HPLC-DAD and LC-MS/MS will be performed in raw and processed maize, whole beans seeds and beans fractions (seed coat and cotyledons obtained after beans soaking) submitted to acidic, enzymatic and alkaline hydrolysis. Those studies will allow recognition of the digestion impact on maize and beans' phenolic composition and represent very interesting information to provide to the consumer. This information may increase the crops market value and should be taken into consideration on future participatory breeding selection.

### 3.3. Development of molecular tools for assisting quality selection

Genetics, particularly molecular genetics, provides further information on patterns of diversity distribution and allows the investigation of the relation of observed diversity with environment, social and cultural factors, providing means to reconcile farmer's classification schemes with genetic distinctiveness. It also helps determine whether there is a wide enough genetic base for future improvement of the *in-situ* materials, or whether there is sufficient diversity to provide system resilience [6]. It can also underpin the identification of ways of supporting the maintenance of traditional varieties, such as in supporting protected geographical identification of certain plant or crop product.

Presently, in our extended PPB project we are conjugating the identification of agronomic and specific quality traits with molecular characterization so as to exploit efficiently the local diversity and produce varieties that are superior in marginal environments, but have a broad genetic base and a high quality level. Nevertheless, in Portugal, molecular breeding is still given its first steps.

In this section we will summarize the development of molecular tools to assist the implementation of participatory breeding program focusing on maize improvement for producing high quality bread. One of the key elements for the implementation of a successful breeding program is the existence of decision supporting tools. Different molecular markers are being tested in order to create new decision supporting tools. Among the different classes of molecular markers, we started initially to use simple sequence repeat (SSR or microsatellite) markers that have proven to be the marker of choice for a variety of applications, particularly in breeding [66]. We are now starting to use also single nucleotide polymorphisms (SNPs) molecular markers that are more abundant in the genome and amenable to automation for high-throughput genotyping [67].

Molecular markers are being used to achieve two main research objectives. First we are using molecular markers to evaluate the progress obtained in conserving or increasing diversity through participatory breeding, as already described in the section 2.3 of PPB success evaluation [42]. The genetic diversity of the newly introduced maize and beans landraces into the participatory plant breeding net is now also routinely characterized, with 20 to 22 SSR uniformly distributed throughout the maize and bean genomes respectively. This method enables us to check if sufficient diversity is present to allow selection and to select the most promising landraces in order to increase the genetic diversity by crossing genetically distant landraces. These studies have also allowed us to compare the genetic diversity with quality clustering of landraces [34]. In detail, 46 traditional maize landraces collected from known high quality maize bread Portuguese regions, plus six participatory improved maize OPVs from the VASO project, were analyzed for eight different parameters related with their technological ability for bread production, and 13 SSR markers. It was possible to classify these OPVs into three distinct clusters based on the quality traits. Nevertheless, no clear clustering based on genetic distances was observed despite the high levels of genetic diversity presented by these Portuguese landraces [34]. Based on the existence of diversity at molecular level and high quality, the Portuguese maize landraces conserved on-farm represent valuable germplasm with high potential for bread quality improvement [34]. This study also provided important information for the selection of landraces to keep under the extended PPB project.

Second, we are developing genetic studies to identify the genes responsible for our quality traits of interest and subsequently develop molecular markers that target those genes and that can be useful for marker assisted selection (MAS). Quality parameters for bread making, such as technological, nutritional and organoleptic traits, are generally characterized by a continuous variation. This continuous variation suggests the influence of several genes, and because of that, it is difficult to grasp by breeders and farmers. It is expected that several of the maize bread quality parameters show quantitative inheritance. The identification of molecular markers that are linked to the controlling genes will be very helpful for the indirect selection through MAS of these complex quality traits. Marker-assisted selection is a powerful tool for the indirect selection of difficult traits at an early stage, before production of the next generation, thus speeding up the process of conventional plant breeding and facilitating the improvement of traits that cannot be improved easily by conventional methods (reviewed by [67]). The identification and location of genes controlling quantitative traits through Quantitative Trait Loci (QTL) analysis has already been successful undertaken on maize nutritional quality [68, 69, 70].

In our genetic studies we started to use a marker-trait association analysis based on biparental populations, where only a few target traits can be mapped within each population. It was possible by using this approach to identify several genomic regions responsible for ear fasciation related traits, widely present in the Portuguese maize landraces (on going research). Nevertheless, to be able to use this information for indirect selection of fasciated phenotypes, several runs of MAS and population development would be needed to narrow down the genomic regions. This method is time-consuming, but very powerful for the genes with large effect and the alleles with low frequency [71].

Another approach to identify molecular markers for using in MAS is association mapping based on linkage disequilibrium (LD). The availability of high-throughput genotyping technology, together with advances in DNA sequencing and the development of statistical methodology appropriate for genome wide mapping analysis in the presence of considerable population structure, in species such as maize, contributed to an increased interest in LD association mapping [72]. This is the approach that we are now following for the quality genetic studies.

Unlike conventional biparental mapping populations, the natural populations used on this type of linkage analysis, are the products of many cycles of recombination and have the potential to show enhanced resolution of QTLs. Success depends on population size, control of population structure and the degree of LD in the population. LD levels vary both within and between species [73]. With this approach, marker–trait association is only expected when a QTL is tightly linked to the marker, because the accumulated recombination events occurring during the development of the lines will prevent the detection of any marker–trait loose association. In maize, the application of this approach has demonstrated the association between several candidate genes and kernel composition traits, starch pasting properties and amylose levels [74].

Using SSR markers, the genetic diversity among inbred lines derived from the Portuguese germplasm collection was evaluated and compared with worldwide maize inbreds representatives [36]. The Portuguese inbred lines have maintained a level of genetic diversity similar to the foreign lines. Moreover, it was concluded that they are derivatives of miscellaneous populations, showing high genetic diversity and consequently representing a potential valuable source of interesting genes to introduce into modern cultivars [36], and a valuable germplasm for association studies.

Until now, no LD analysis or association studies were undertaken on the group of inbred lines of Portuguese origin, neither the identification of genes/QTLs controlling bread making ability. Presently, in order to address this gap, the collection of Portuguese maize inbred lines, derived mainly from Portuguese landraces, is being genotyped using microsatellites to detect population structure and to study LD.

Currently, the national efforts are focused on the study of the genetic control and the environmental effect on the antioxidant and aroma compounds as well as the bread making ability. This study applies an association mapping approach using the previously characterized inbred lines that differ for endosperm types and colors. QTL associated candidate genes will be identify on the basis of positional information of the recently maize cloned genes (reviewed by [75]). Candidate genes will be validated on the enduring landraces [7] and modern improved OPVs (VASO project) that are also being characterized at genetic, nutritional, organoleptic (aroma volatiles) composition and antioxidants bioactivity. Specific molecular markers tightly linked to the identified QTLs will be identified or developed to provide breeders and farmers user-friendly markers to select for superior genotypes for quality maize bread. Additionally, it will allow the exploration of maize local resources and natural quality diversity in the reinvention of traditional maize to produce modern high quality bread with potential health benefits.

### 3.4. Testing of higher quality experimental cultivars

Nowadays, the most promising maize populations at agronomic, molecular and quality level, collected during the 2005 expedition, are being evaluated and selected under a participatory approach in 13 different locations. This field research has been done in articulation with the original VASO project locations and improved populations, and now is under the supervision of the ESAC researchers. The association of the farmers' perception with the newly available molecular and quality data can be extremely valuable to aggregate or separate populations, creating possible pools with heterosis that will be very useful to generate new populations or inbred lines. All the molecular and quality evaluations performed on these materials are being developed by researchers at ITQB/UNL and INIAV.

After a detailed characterization of the agronomic, genetic, nutritional, organoleptic and technological quality traits of 41 initial maize OPV (from the collecting expeditions plus VASO project), the most interesting materials were selected for the development of hybrid populations with specific quality traits and maintaining genetic diversity. Hybrid populations can contribute to yield improvement and to avoid the collapse of some interesting germplasm. Dialel tests of the best materials are providing indications regarding heterosis among the chosen germplasm. These new populations are now under field evaluations. New synthetic populations with increased precocity are also being developed and are based upon the most superior Portuguese maize OPVs at agronomic level plus some American populations. These synthetic populations are also under field trial evaluation/selection at different farmers fields. Molecular and quality evaluations will follow on all these new developed materials to sustain their improvement.

## 4. Seed dessimination and ownership

The potential advantages of PPB, such as the faster dissemination of new varieties, higher adoption and increased biodiversity within the crop, can only be achieved if the seed of the new varieties is available in sufficient amounts to all the farmers' community [19]. Although the varieties developed through PPB will have specific adaptation to certain environmental conditions, it is likely that they will also perform well on farms that share similar climates and soil types. It is unlikely that they will spread as far as varieties specifically targeted to have wide adaptation in higher input systems [76], but it is possible that they will benefit many farmers in neighboring areas. Genetically variable materials, such as OPV and synthetics, make more likely their usefulness to farmers in environments that differ from the original selection environment [77].

The global community, through the Convention on Biological Diversity and the International Treaty on Plant Genetic Resources for Food and Agriculture, has recognized the contribution of farmers to the maintenance of genetic resources. Given the actual and potential future impact of PPB, this contribution will increasingly include new PPB' varieties. [78]. Such varieties need recognition and protection.

According to European Union regulations, farmers are allowed to reproduce non-certified seeds for themselves, but they are not able to sell them. Generally, only varieties that are officially registered and listed, after meeting DUS and VCU requirements, can be multiplied by the formal seed system [79]. Formal seed systems were put in place in Europe, in the mid-19$^{th}$ century, as a result of the development of specialized plant breeding products and to create transparency in a seed market where variety names were rapidly proliferating [80]. Current variety registration for commercial purposes requires that the new variety be distinct from all the varieties of common knowledge, uniform in its essential characteristics and highly stable after repeated multiplication (DUS= Distinctness, Uniformity and Stability,

[80]). In addition, testing for cultivation and use values (VCU) was introduced as a requirement for commercial release, in order for farmers to have an independent assessment of the yield, quality and value of the grain [6]. These last ones are the real concerns for farmers, but in practice this evaluation is based mostly on quantitative Weld criteria such as grain yield, maturity time, standing ability and disease resistance, which are easy to measure. Less attention is given to aspects such as storability, cooking quality and by-product use, which may determine the overall value of the variety, especially for small farmers. To this extent, standard VCU tests do not easily reflect the more complex requirements of small-scale farmers and this has been one of the problems of official variety release in meeting the needs of such farmers [79].

This formal system is unfriendly for farmers' varieties such as landraces and new varieties developed through PPB, leaving these varieties outside the legal market of seeds [81]. These varieties are less likely to meet the stringent DUS and VCU criteria because they lack uniformity and rarely perform well across the majority of test sites. Nevertheless, the European Union has recently approved a special treatment for the so called "Conservation Varieties" by which landraces and varieties adapted to local and regional conditions and threatened by genetic erosion can be registered for commercialization under certain conditions (Directive 2008/62/EC from 20 June 2008). The special treatment consists, of

1. a certain degree of flexibility in the level of uniformity that is required, and
2. an exemption from official examination if the applicant can provide sufficient information about the variety through other means such as unofficial tests and knowledge from practical experiences [6].

The varieties obtained in our project can be registered as conservation varieties. In the registration process, the varieties have to be characterized for a minimum number of morphological traits and only less than 10% of the plants can be out of type. Attention is given to the region where the variety is traditionally used as a crop and to where it is naturally adapted. The registration is obtained if description of the varieties, denomination, results from non-official trials, knowledge associated with sow, multiplications and use, and other information is provided to the genetic resources authorities. This information is then evaluated by the national entity (DGADR in the Portuguese case). In the national catalogue of varieties (CNV) the respective location of origin is indicated, and the regions of seed production can be identified besides the seed origin. Seeds are submitted to sampling and quality standards. Storage must be done in close packages and producer labels are required. The conservation varieties may be marketed only in their regions of origin or in additional regions, as long as these regions are comparable, regarding semi-natural and natural habitats, to the region of origin of this variety. The maximum quantity of seed per specie, allowed for commercialization purposes, is 10% of the seeds used annually in the country, if this condition does not exceed the total amount of seed needed to sow 100 ha. In the case of the Portuguese maize varieties, the maximum allowed is 0.3% of the seeds used in the country during a growing season, but limited to 100 ha. This means that conservation varieties, if we consider the maximum of 100 ha, represent 0,073% of the Portuguese maize area (100 ha/137 413 ha) and can represent 10 000€ (20 kg of seed/ha x 5 €/Kg of seed x 100 ha) in Portugal. This data indicate that this

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germplasm should be used preferably in marginal areas under PPB project. It also indicates that maize PPB projects should be integrated in the food market preferably in those where the direct output is not the seed itself, but for example, the bread that has a higher market value. Nevertheless, in the process of registration of conservation varieties, the PPB farmer alone will not be able to provide all types of unofficial tests and information needed. So supportive associations should step forward and help on this registration process.

In Portugal, such an association has been created in 2010, the ZEA+ association, where a cluster of maize researchers and participatory breeding farmers are joined together. This association was the logical step to fill in the gap at the logistical level to deal with all the information that had been collected at etnobotanical, agronomical, genetic, and molecular and food quality by the national maize cluster of research. This association main objective is the study and promotion of the conservation and valuation of agricultural genetic resources in a perspective of rural development, emphasizing the link with the urban communities. In this way, this association is dedicated to traditional landraces, including conservation and autochthones varieties. It can provide logistical or managing support to germplasm improvement through participatory plant breeding where its associates collaborate. The Zea+ association could contribute to successfully market conservation varieties and to help establish some kind of small seed enterprise for farmers, in order to have a clean source of seed from those varieties. It should also support the registration of improved varieties already validated with field and molecular marker data. This would allow the reinvestment of the potential royalties in science, to provide more information to farmers and researchers.

Besides seed dissemination, it is also necessary to consider the maintenance of the genetic gains achieved. If the improved material is not managed in a systematic way, it may be diluted by physical contamination or out-crossing and thus dissolve back into the local population. The benefits achieved by PPB may then be lost, leaving no secure point of reference to return to in the future [79]. Consequently, this responsibility should be vested by a farmers/researchers association established to produce and market the seed.

This registration possibility does not mean that the process of selection cannot continue, rather than at certain intervals a reasonably defined 'milestone' is set up along the road of improvement [79]. Further enhancement of productivity and stability is achieved through practicing "non-stop selection" within landraces across the marginal production environments, to exploit the useful adaptive variation constantly released by the genome.

## 5. Future prospectives and market development

At a time when a team of young scientists is taking care of this PPB project, which is reaching it maturation, we can foresee a new future for the Portuguese small farming. Its quality oriented purpose for food, its sustainability and environment friend signature, will be an important piece to bring our sustainable small farming system back to its feet.

In the medium/long term, we expect that the abandoned northern agricultural systems will survive due to the local abundance of water. In these environments maize will play an important role in the production of quality food. In this case, the national germplasm will play an important role in the recovery of our small farming system. Participatory plant breeding is the tool necessary to take advantage of our rich maize germplasm collected in the 70's and preserved in our Portuguese Plant Gene Bank (BPGV), or in same cases still present at our farmers fields. Without such an investment in breeding, to raise their yielding capability to reasonable levels, our genetic resources will remain in a useless tomb or vanish definitely from our farm land.

In scientific terms, we foresee that with this new multidisciplinary team of young scientists new findings will came along, especially at understanding the genetics of important agronomical and quality traits that will translate into improved high quality varieties. The role of this quality oriented varieties, either under open pollination or hybrid form, will fit in a new agricultural system oriented to quality tourism, where maize will represent only a piece, as important as it may be, within this system. Entertainment, like the traditional "desfolhadas" (harvest festivities), historical, architecture, archeological and cultural attractions, together with the combined restoration of old water mills and cob stores, all of this complemented with folkloric music will complete the system.

The potential fixation of our farmers, consequence of the economic recovery, will benefit the most the environment (water management, soil conservation, genetic resources preservation, and the control of forest fires). Farmers will always be the breeder's best allies and the best curators of our genetic resources. New genetic and analytical tools are now available that can help the traditional plant breeding methodologies. Sustainable, quality oriented, and environmental friendly agriculture still has a role to play in countries like Portugal and beyond.

Nevertheless this extended PPB project was only possible due to funding obtained through several national (from Fundação para a Ciência e a Tecnologia, Portugal, POCI/AGR/57994/2004, PTDC/AGR-AAM/70845/2006, PTDC/AGR-ALI/099285/2008) and international (FP7 program, SOLIBAM project) research projects of limited duration. Its survival depends on finding sustainable ways of self-support that may be obtained by higher marketing of improve quality varieties, maintaining genetic diversity, with increased market value, more attractive to the final consumer.

So, further supportive actions to market creation and market promotion should be taken. As adapted from [6] to our national reality, partnerships should be built or strengthen through the organization of meetings involving market-chain stakeholders to discuss how to change market potential. Niche markets for traditional landraces raw materials or traditional landraces food products (maize bread) should be further exploit. This is the case of the gluten intolerant market, to which the 100% maize bread can be an attractive alternative. Also, the general market should be aimed with media advertisement campaigns to improve consumer awareness of important nutritional or ecological-friendly traits from traditional landraces, for example, using the summary of the research project activities. On the same level, an eco-labeling of products (such as the maize bread) obtained from traditional landraces as a commitment to the preservation of biodiversity, could call the attention of consumers. As already highlighted above, the ecological practices of traditional production systems, where traditional landraces are maintained, should be promoted. Agrobiodiversity ecotourism could be one way of doing so, because it publicizes the diversity of cultivated plants and the associated cultural practices by involving activities as farm and market visits, participation in agricultural activities and food (bread) preparation, food tasting and attending feasts or celebrations associated with agricultural practices. Finally and as a last resource, farmers who provide environmental services, such as conservation functions should be compensated. A governmental direct support could be provided to farmers who cultivated traditional varieties targeted for protection.

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